

**ESTIMATION OF ANAEROBIC CAPACITY:  
PRACTICAL LIMITATIONS AND PHYSIOLOGICAL ASSUMPTIONS**

**A thesis submitted in partial fulfilment of the requirements of the University of  
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## Abstract

High-intensity exercise necessitates simultaneous aerobic and anaerobic ATP resynthesis. Unfortunately, direct quantification of the finite amount of energy derived from the anaerobic energy system (i.e. anaerobic capacity; AnC) is challenging and not practical on a daily basis. Instead, there are two approaches to estimate AnC: the accumulated oxygen deficit (AOD) and the curvature constant of the power-duration relationship ( $W'$ ), both of which rely on several assumptions. First, determination of AOD requires an estimation of supramaximal oxygen demand, typically from the projection of the oxygen uptake ( $\dot{V}O_2$ )-power output relationship determined from various submaximal exercise intensities assuming a linear relationship. From a practical perspective, AOD is a time-consuming protocol. Secondly,  $W'$  is considered to represent anaerobic work capacity and correlates with AOD. Moreover, both AOD and  $W'$  are assumed to remain constant irrespective of pacing strategies and/or changes in oxygen availability. The overarching aim of this PhD was to investigate the assumptions and practical limitations surrounding AOD and  $W'$  as tests to estimate AnC. Study 1 investigated the linearity of the  $\dot{V}O_2$ -power output relationship during exercise below vs. above the lactate threshold. Ten cyclists completed consecutive 3 min bouts of exercise below and above the lactate threshold. The  $\dot{V}O_2$ -power output relationship remained constant up to intensities of  $\sim 95\%$  maximal  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ). Study 2 determined whether AOD remains constant during exercise to exhaustion at any supramaximal intensity. Twenty-one cyclists performed a constant work-rate (CWR) exercise bout to exhaustion at 105, 112.5, 120 and 127.5%  $\dot{V}O_{2max}$ . Compared to 112.5%  $\dot{V}O_{2max}$ , AOD was lower at 105 and 127%  $\dot{V}O_{2max}$ , but there were no differences between 112.5 and 120%  $\dot{V}O_{2max}$ . At 112.5%  $\dot{V}O_{2max}$ , the coefficient of variation of AOD was 8.7%. Study 3 determined whether AOD can be calculated in a single-day trial. Twenty cyclists performed CWR tests at 112.5%  $\dot{V}O_{2max}$  25 min after submaximal and maximal tests (single-day AOD), and after no prior exercise (traditional AOD). Single-day AOD was reduced by 17% compared to traditional AOD, suggesting that a single-day approach is untenable. Study 4 established the relationship between AOD and  $W'$ , and whether CWR and 3-min all-out (3AO) tests affected i) the strength of the relationship and ii) the magnitude of AOD and  $W'$ . Both measures were correlated during CWR ( $r = 0.654$ ) and 3AO ( $r = 0.664$ ) tests. However, AOD was greater in CWR tests, whereas  $W'$  was greater in 3AO tests. Study 5 determined whether AOD and  $W'$  were affected by hypoxia or hyperoxia during CWR and 3AO tests. AOD was determined during CWR and 3AO tests in 10 cyclists in hypoxia (15% oxygen), normoxia (21% oxygen) and hyperoxia (35% oxygen). There was no effect of environmental conditions on AOD, but CWR tests resulted in higher AOD than 3-min all-out tests. In contrast, there was no effect of environmental condition or pacing on  $W'$ . In conclusion, using 3 min stages to construct the  $\dot{V}O_2$ -power output relationship, the AOD reaches its maximum during a CWR test to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ , with a test-retest coefficient of variation of 8.7%. However, the magnitude of AOD is not consistent, as 3AO tests resulted in a reduced AOD. Moreover, using this protocol, two trials are needed to determine AOD. The magnitude of  $W'$  provides an indication of AnC, given that AOD and  $W'$  were strongly correlated.  $W'$  is a solid construct, and remains constant irrespective of pacing and changes in oxygen availability. Although  $W'$  is likely determined by other factors besides AnC, it appears to be a favourable option to estimate anaerobic energy production.

## List of Publications

### Papers

- Muniz-Pumares, D.; Godfrey, R., Pedlar, C., & Glaister, M. (2015). The effect of the oxygen uptake-power output relationship on the prediction of supramaximal oxygen demands. *J Sports Med Phys Fitness*. In Press
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### Further research publications

- Glaister, M., Pattison JR, Muniz-Pumares D, Patterson SD, Foley P. (2015). Effects of dietary nitrate, caffeine, and their combination on 20 km cycling time-trial performance. *J Strength Cond Res*, 29, 165-74.
- Glaister M; Muniz-Pumares D; Patterson SD; Foley P; McInnes G. (2015). Caffeine supplementation and peak anaerobic power output. *Eur J Sport Sci*, 15, 400-6.

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## List of abbreviations

[ ]: denotes muscle concentration

~: approximately

'O<sub>2</sub> eq': oxygen equivalents

<: less than

>: more than

≤: less than or equal to

≥: more than or equal to

°C: degrees Celsius

<sup>31</sup>P-MRS: phosphorus-31 magnetic resonance spectroscopy

ADP: adenosine diphosphate

AMP: adenosine monophosphate

AnC: anaerobic capacity

ANOVA: analysis of variance

AOD: accumulated oxygen deficit

AOD<sub>single</sub>: accumulated oxygen deficit determined in a single trial

AOD<sub>trad</sub>: accumulated oxygen deficit determined in an independent trial

ATP: adenosine 5'- triphosphate

ATP<sub>an</sub>: ATP derived from anaerobic energy sources

ATPase: adenosine triphosphatase

BLa: blood lactate concentration

CL: confidence limits

CK: creatine kinase

CO<sub>2</sub>: carbon dioxide

CP: critical power

CS: critical speed

CV: coefficient of variation

CWR: constant work-rate

D': "D-prime"; running equivalent of W'

dm: dry muscle

FiO<sub>2</sub>: fraction of inspired oxygen

H<sup>+</sup>: hydrogen ions

HHb: deoxygenated haemoglobin and myoglobin  
ICC: intraclass correlation coefficient  
kCal: kilocalories  
kJ: kilojoules  
La: lactate  
LDH: lactate dehydrogenase  
LT: lactate threshold  
MART: maximal anaerobic running test  
Mg<sup>2+</sup>: magnesium  
O<sub>2</sub>Hb: oxygenated haemoglobin and myoglobin  
PCr: phosphocreatine  
*P-d*: relationship between work rate and time to exhaustion  
pH: potential of hydrogen  
P<sub>i</sub>: inorganic phosphate  
P<sub>i</sub>O<sub>2</sub>: pressure of inspired oxygen  
rpm: revolutions per minute  
*SD*: standard deviation  
TSI: tissue saturation index  
TTE: time to exhaustion  
 $\dot{V}CO_2$ : carbon dioxide production  
 $\dot{V}O_2$ : pulmonary oxygen uptake  
 $\dot{V}O_{2max}$ : maximal oxygen uptake  
*W'*: "W-prime"; curvature constant of the hyperbolic work rate-duration relationship  
WAnT: Wingate anaerobic test  
 $\Delta$ : difference  
 $\tau$ : time constant, time to attain ~63% in an exponential curve

# **Chapter 1: Literature Review**

## 1.1. Introduction

As all living organisms on Earth, human beings require energy to function. The first law of thermodynamics states that energy cannot be created nor destroyed, but converted from one form to another. Humans eat plants and other animals to obtain energy that is used to maintain body functions. On a daily basis, however, there is a large variation in the energy demands that the body experiences. Whilst at rest or sleeping there is a low demand for energy; but physically demanding activities like walking or completing house tasks require a larger demand for energy. During strenuous, whole-body exercise, the energy demand in the exercising muscles can increase by a factor of one hundred compared to rest (Sahlin, Tonkonogi, & Söderlund, 1998). The body responds to these changes in the energy demands by continuing adjusting energy supply, thus there is a tight match between energy demand and supply.

Muscles and other tissues within the body use the hydrolysis of adenosine 5' triphosphate (ATP), an energy-rich nucleotide, to obtain the energy. Unfortunately, ATP has a high molar mass, so the body cannot store large amounts of it. As a consequence, ATP is constantly resynthesized in order to maintain a relatively constant muscular concentration of ATP ([ATP]). The resynthesis of ATP occurs through the coordination of different metabolic pathways. The simplest metabolic processes provide high rates of ATP resynthesis from substrates already available within the muscle cell, which unfortunately last only few seconds. At the same time, more complex (and therefore slower) metabolic pathways allow exercise to be sustained for longer periods, although the rate of ATP provision is compromised. These metabolic processes are typically grouped as being aerobic or anaerobic. The resynthesis of ATP using oxygen occurs via aerobic metabolism whilst anaerobic pathways do not require oxygen.

Aerobic and anaerobic energetic pathways differ in both their capacity and power. In this context, capacity is defined as the total work that can be produced from an energy system; whilst power is defined as highest metabolic rate achieved by the energy system (Heck, Schulz, & Bartmus, 2003). Green and Dawson (1993) defined anaerobic capacity (AnC) as a finite amount of energy that can be released from anaerobic energy systems. For the current PhD thesis, therefore, AnC is defined as the total work or energy derived from anaerobic metabolism. There are different limiting factors for aerobic and anaerobic energy production. On the one hand, anaerobic energy production yields high energy

rates, but it is limited in capacity. On the other hand, aerobic energy production is virtually unlimited in capacity, though it is limited in power (Greenhaff & Timmons, 1998).

During exercise, both the aerobic and anaerobic metabolic pathways coordinate to meet energy demands of the exercising muscles. For example, in a bout of high-intensity exercise, such as 800 m running, energy yield from anaerobic metabolism represents ~40% of the total energy demand (Duffield, Dawson, & Goodman, 2005; Gastin, 2001). As a consequence, AnC is recognised as an important factor for exercise performance (Brandon, 1995; Burnley & Jones, 2007; Craig & Norton, 2001; Jeukendrup, Craig, & Hawley, 2000). The assessment of aerobic metabolism is readily available, since the rate of pulmonary oxygen uptake ( $\dot{V}O_2$ ) closely reflects aerobic energy production during whole-body exercise. Unfortunately, the assessment of anaerobic energy production is difficult and there is currently no gold-standard method to quantify AnC.

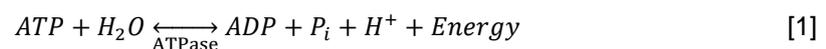
This literature review aims to first analyse how energy is produced during exercise, with particular focus on anaerobic energy production. Subsequently, factors determining AnC are discussed, and finally different approaches to estimate AnC are critically reviewed. Particular attention is given to the accumulated oxygen deficit (AOD) and the curvature constant of the work rate-duration relationship ( $W'$ ).

## 1.2. Energy production during exercise

### 1.2.1. Anaerobic energy production

#### ***ATP / ATPase***

At the onset of exercise, muscle contraction relies on ATP stores as the immediate source of energy for muscle contraction. The hydrolysis of ATP is catalysed by the enzyme adenosine triphosphatase (ATPase) as described in equation 1.

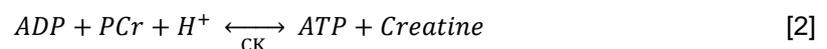


As a result of ATP hydrolysis, adenosine diphosphate (ADP), inorganic phosphate ( $P_i$ ) and hydrogen ions ( $H^+$ ) are formed, and energy is released. Under physiological conditions ( $pH = 7.0$ , magnesium ( $Mg^{2+}$ ) = 10 mmol, temperature = 25°C), 7.3 kCal are released from each molecule of ATP. At rest, the

high molar mass of ATP (507 g·mol<sup>-1</sup>) limits [ATP] in skeletal muscle to ~25 mmol·kg dry muscle (dm)<sup>-1</sup> (Gaitanos, Williams, Boobis, & Brooks, 1993; Green, Dawson, Goodman, & Carey, 1996; Spriet, 2006). Nonetheless, although ATP can be depleted at turnover rates of ~15 mmol·s<sup>-1</sup>·kg dm<sup>-1</sup> during a short, maximal effort (Greenhaff & Timmons, 1998), [ATP] remains tightly controlled. During submaximal exercise [ATP] remains close to resting values. After high-intensity exercise, however, [ATP] decreases from its resting values, but typically remains ≥ 50% of its resting value (Bogdanis, Nevill, Boobis, Lakomy, & Nevill, 1995; Cheatham, Boobis, Brooks, & Williams, 1986; Karatzaferi, de Haan, Ferguson, van Mechelen, & Sargeant, 2001; Karlsson & Saltin, 1970; Mendez-Villanueva, Edge, Suriano, Hamer, & Bishop, 2012). From the onset of exercise, a series of metabolic signals associated with muscle contraction prevent [ATP] from decreasing precipitously (Haseler, Kindig, Richardson, & Hogan, 2004).

### ***Phosphocreatine / creatine phosphate***

Increases in [ADP] (equation [1]) affect the equilibrium of the creatine kinase (CK) reaction (Chance et al., 2006; Sahlin & Harris, 2011), facilitating the donation of a phosphate from phosphocreatine (PCr) to ADP to resynthesise ATP, as shown in equation [2] :



The simplicity of this single-reaction metabolic pathway allows high rates of ATP resynthesis. During the initial 1.3 s of all-out exercise ATP can be resynthesised from PCr at ~9 mmol·s<sup>-1</sup>·kg dm<sup>-1</sup> (Greenhaff & Timmons, 1998). The concentration of PCr ([PCr]) at rest is four- to six-fold greater than that of ATP, ranging from 75 to 90 mmol·kg dm<sup>-1</sup> (Bangsbo, 1998; Bangsbo, Krstrup, González-Álonso, & Saltin, 2001; Cheatham et al., 1986; Gaitanos et al., 1993; Méndez-Villanueva et al., 2012), which allows ATP-PCr to be the primary energy system during the first ~10 seconds of maximal exercise (Gastin, 2001; Spriet, 2006, 1992). At the onset of exercise, the rate of [PCr] depletion is determined by the intensity of the exercise (Rossiter, Ward, Doyle, Howe, Griffiths, & Whipp, 1999; Rossiter, 2011; Whipp, Ward, & Rossiter, 2005). During moderate intensity exercise below the gas exchange threshold (GET), the decrease in [PCr] reaches a steady state within 2-3 minutes (Francescato, Cettolo, & Di Prampero, 2003). Above GET, but below critical power (CP), often referred to as heavy exercise domain, the fall in [PCr] does not stabilise after the initial decrease (2-3 min), but continues to slowly decrease until, eventually (after 5-10 min), a steady state is reached. Above CP (defined for the purposes of the current

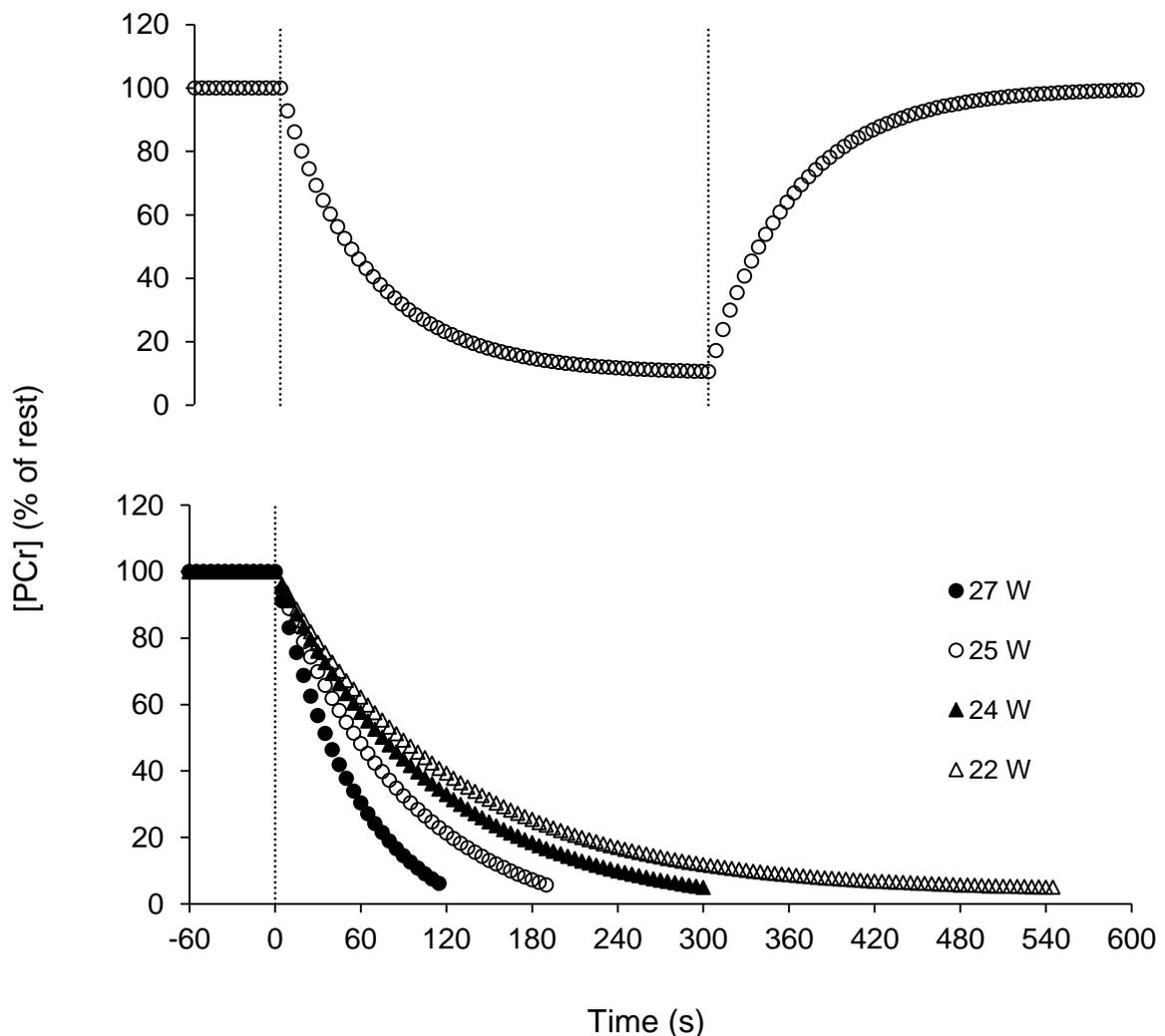
PhD thesis as either 'high-intensity' or within the 'severe exercise domain'), [PCr] does not attain a steady state, but continues to decrease until the point of exhaustion, when [PCr] reaches values < 30% of those at rest (Jones, Wilkerson, DiMenna, Fulford, & Poole, 2008c; Karlsson & Saltin, 1970; Sahlin & Harris, 2011; Vanhatalo, Fulford, DiMenna, & Jones, 2010). Moreover, the kinetics of [PCr] and  $\dot{V}O_2$  follow an inverse linear relationship, irrespective of the domain of the exercise (Cannon et al., 2013; Rossiter et al., 1999), so the characterisation of  $\dot{V}O_2$  kinetics has been used to as a proxy measure of [PCr] kinetics (Koga et al., 2005; Rossiter, Howe, Ward, 2005; Whipp & Rossiter, 2005).

At exhaustion, there is a large within-individual variability in [PCr] which might be consequence of variability in fibre type (Cannon et al., 2013; Chance et al., 2006; Karatzaferi et al., 2001; Sargeant, 2007). Human skeletal muscle is composed of different muscle fibres, with distinctive biochemical and structural characteristics, and consequently different bioenergetics (Greising, Gransee, Mantilla, & Sieck, 2013). Despite the continuum in the characteristics of muscle fibres, they are, for convenience, grouped and classified in humans as type I, type II and type IIx. In brief, type I fibres, have a high oxidative capacity and are more fatigue resistant than type II fibres, which have lower oxidative capacity and are less resistant to fatigue (Greising et al., 2013). Since type II fibres are more powerful but less fatigue-resistant than type I fibres, it has been suggested that PCr decreases to a larger extent in type II fibres (Greenhaff & Timmons, 1998; Karatzaferi, de Haan, Ferguson, van Mechelen, & Sargeant, 2001; Sargeant, 2007).

Not all studies report a large within-individual variability in [PCr] at exhaustion. Vanhatalo et al. (2010), for example, reported similar values for PCr at exhaustion in exercise at different work rates within the severe exercise domain (Figure 1.1). Similarly, Chidnok et al. (2013b, 2013c), observed similar [PCr] at the point of exhaustion in continuous and intermittent exercises with different work:rest ratios. In the above studies, [PCr] was determined using phosphorus-31 magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS) (Chidnok et al., 2013b, 2013c; Vanhatalo et al., 2010). The authors of the above studies interpreted these data as being suggestive of a threshold in the accumulation/depletion of metabolites at exhaustion, regardless of the intensity that prevented exercise from continuing.

The quantification of [PCr] offers an insight into muscle energetics, either on its own or combined with other phosphates and pH. The  $P_i/PCr$  ratio, an index of oxidative phosphorylation, has been used, for example, to investigate the metabolic stability of the exercising muscle (Barker, Welsman, Fulford,

Welford, & Armstrong, 2010; Chance et al., 2006; Raymer, Forbes, Kowalchuk, Thompson, & Marsh, 2007). Moreover, together with other metabolites such as ATP and ADP, the quantification of [PCr] is required to for the direct evaluation of AnC. The quantification of [PCr], however, is technically difficult. In the above studies, [PCr] has been measured using either phosphorus-31 magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS) (e.g. Chidnok et al., 2013b, 2013c; Vanhatalo et al., 2010) or muscle biopsies (e.g. Bogadinis et al., 1995, 1996). The assumptions needed to calculate AnC from measurements of muscle metabolite concentration, including [PCr], are discussed in Section 1.5.1. Often, due to the difficulties in determining [PCr], it is sometimes estimated (e.g. Binzoni, Ferretti, Schenker, & Cerretelli, 1992; Riberiro et al., 2015; Sousa et al., 2013).



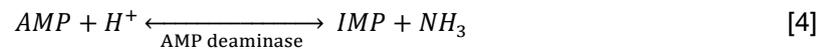
**Figure 1.1.** Top panel. PCr on- and off-kinetics during high intensity exercise to exhaustion. Exercise starts at time = 0 and exhaustion occurs at 300 s (denoted by discontinuous horizontal lines); followed by 5 min of passive rest. At exhaustion, [PCr] has dropped to ~10% of resting [PCr], however it is largely replenished after 5 minutes of rest. Adapted from Rossiter et al., 2002. Bottom panel. PCr depletion during four severe-intensity leg-extension exercise bouts to exhaustion. Note there is a similar end-exercise concentration of PCr at exhaustion (~6%), irrespective of the rate at which [PCr] is being depleted. Adapted from Vanhatalo et al., 2010.

### ***ADP / Adenylate kinase reaction***

During intense exercise, the phosphagen (ATP-PCr) system may not be sufficient to meet ATP demands. In those instances, two molecules of ADP combine to produce ATP and adenosine monophosphate (AMP):



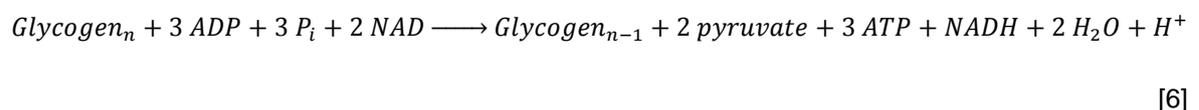
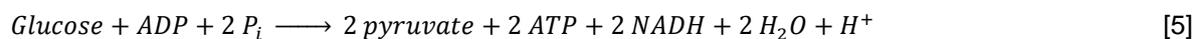
AMP is further deaminated to ammonia (NH<sub>3</sub>) and inosine monophosphate (IMP) in a reaction catalysed by AMP deaminase:



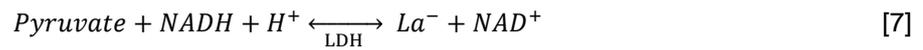
The emergence of ammonia in skeletal muscle can be related to fatigue processes (Ament & Verkerke, 2009), and an increase in the blood concentration of ammonia has been interpreted to represent the extent of the involvement of anaerobic metabolism in high-intensity exercise (Naughton et al., 1997; Ravier, Dugué, Grappe, & Rouillon, 2006).

### ***Glycolysis***

Glycolysis is a series of catabolic reactions that breaks down glucose-6-phosphate derived from glycogen or blood-borne glucose to pyruvate (Figure 1.2). The summary reactions for glycolysis, beginning with glucose and glycogen, are summarised in equations 5 and 6 (Robergs, Ghiasvand, & Parker, 2004):

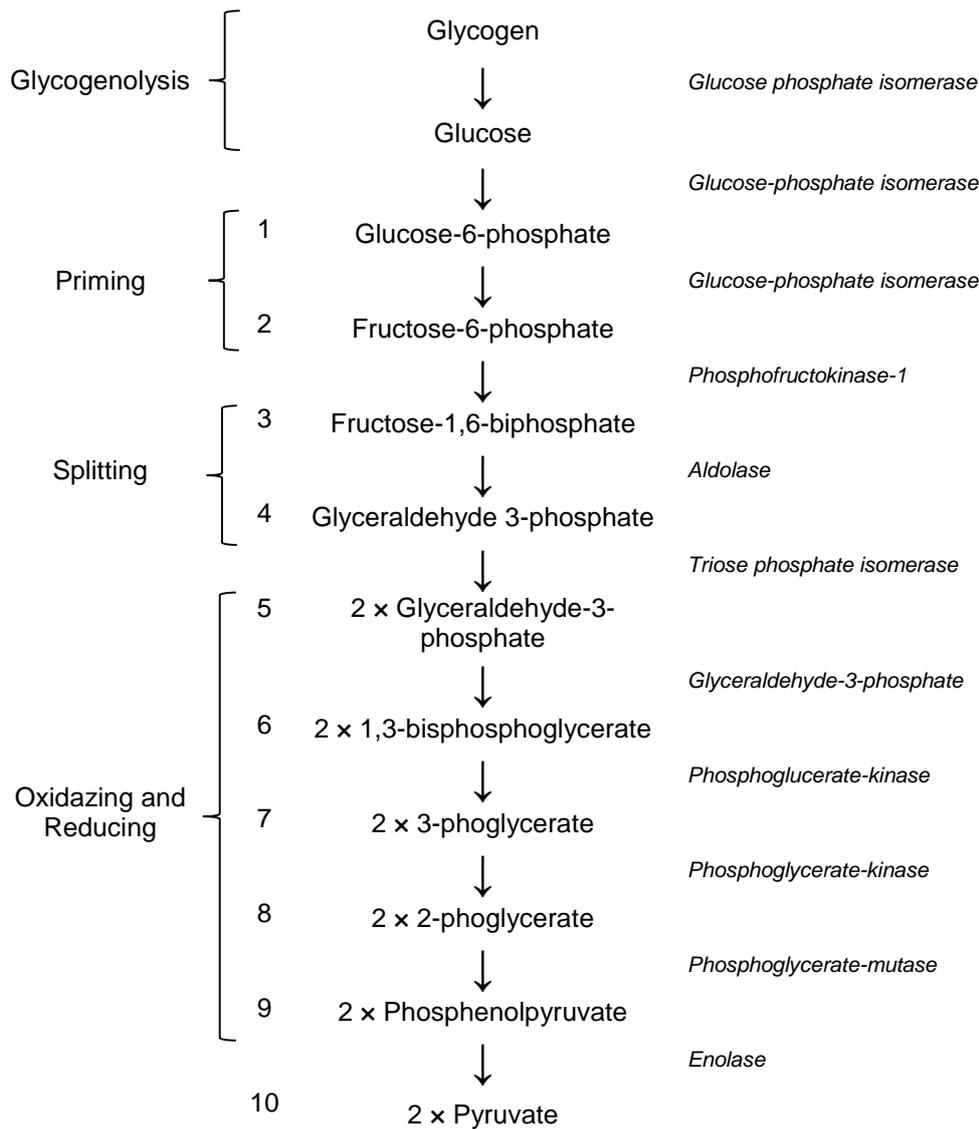


The above equations occur in the cytoplasm, outside the mitochondria and, therefore, do not rely on oxygen and are considered anaerobic. If the degradation of glycogen to pyruvate occurs at slow rates, pyruvate enters the mitochondria and is completely oxidized to water and carbon dioxide via the Krebs cycle (known as aerobic glycolysis, see oxidative phosphorylation). At higher metabolic rates, pyruvate is converted to lactate (La<sup>-</sup>) in a reaction catalysed by the enzyme lactate dehydrogenase (LDH):



As a result, at high metabolic rates, more pyruvate is converted to  $\text{La}^-$ , which results in the accumulation of this metabolite in the blood (i.e. the lactate threshold, see Messinier et al., 2013). Contrary to PCr, where its concentration determines capacity, the capacity of anaerobic glycolysis to supply ATP during high intensity exercise is not determined by the overall muscle concentration of glycogen (Ørtenblad, Westerblad, & Nielsen, 2013; Spriet, 2006), but by the ionic changes associated with glycolytic flux. Note that muscle acidosis (i.e.  $\text{H}^+$  accumulation) does not occur exclusively as a result of anaerobic glycolysis; other metabolic reactions such as ADP hydrolysis (Equation [1]) also produce  $\text{H}^+$  (for a review, see Robergs et al., 2004). It has been estimated that the contribution of anaerobic glycolysis ( $\sim 300 \text{ mmol} \cdot \text{kg} \cdot \text{dm}^{-1}$ ) to the total AnC is, approximately, four-fold greater than that of PCr (Bangsbo et al., 1990; Spriet, 2006).

Traditionally,  $\text{La}^-$  has been considered an end-product of anaerobic glycolysis (Hartree & Hill, 1923; Hill & Lupton, 1923). However, the role of  $\text{La}^-$  during exercise metabolism is much more complex. For example,  $\text{La}^-$  serves in the complete oxidation of pyruvate through acetyl co-enzyme A and the tricarboxylic acid cycle (Figure 1.2.). In addition,  $\text{La}^-$  can be converted to glucose in the liver, in a process known as gluconeogenesis (Brooks, 2007; Gladden, 2004; Van Hall, 2000).



**Figure 1.2.** Schematic outline of glycolysis.

### 1.2.2. Oxidative phosphorylation

Oxidative phosphorylation, or aerobic metabolism, produces ATP, H<sub>2</sub>O and CO<sub>2</sub> from substrates obtained from food in a process triggered by increases in ADP and P<sub>i</sub>. Substrates and the electrons from the two pairs of H<sup>+</sup> formed during anaerobic glycolysis enter the mitochondria. These electrons enter the electron transport chain reducing NAD<sup>+</sup> to NADH and H<sup>+</sup>, in a process resulting in ATP, H<sub>2</sub>O and CO<sub>2</sub> production ( $\dot{V}CO_2$ ). Carbohydrate and fat metabolism produce ATP in different metabolic processes. However, the aerobic contribution is easily assessed as  $\dot{V}O_2$ , which is largely representative of muscle  $\dot{V}O_2$  during major forms of physical activity such as walking, running, rowing, or cycling (Whipp, Ward, & Rossiter, 2005).

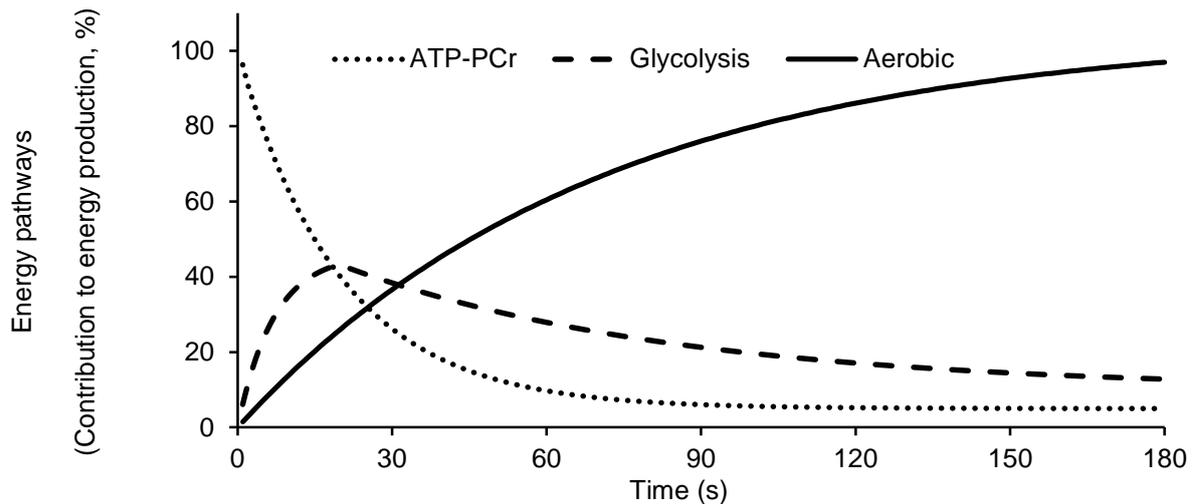
A major difference between the aerobic and anaerobic energy systems is their ability to contribute to energy release. On the one hand, anaerobic ATP production (via ATP-PCr and anaerobic glycolysis) has a finite capacity to release energy; whereas oxidative phosphorylation is virtually unlimited in its capacity. On the other hand, the rate of ATP turnover is much higher in anaerobic than aerobic metabolic pathways, which is limited by the maximal  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ).

### **1.3. Aerobic and anaerobic interaction and their relative contribution to total energy production during a single bout to exhaustion**

The relative contribution of each energy system varies substantially during exercise, but follows a hierarchical order. Initially, at the onset of the exercise, the anaerobic energy system provides high rates of energy readily available for muscle contraction. As exercise progresses, energy yield from aerobic metabolism increases, and anaerobic energy production relative to total ATP resynthesis decreases. First, at the onset of exercise, the ATP-PCr system provides energy for ATP resynthesis at high rates. However, the relative contribution of ATP-PCr to total energy release decreases from the beginning of exercise. In cycle-ergometer exercise it has been estimated that during a single 6 s sprint 6.3% and 49.6% of the energy released from anaerobic pathways correspond to ATP and PCr degradation, respectively, with the remaining anaerobic energy yielded from glycolysis (Gaitanos, Williams, Boobis, & Brooks, 1993). During a 30 s all-out sprint, anaerobic energy production derived from ATP stores and PCr degradation decreases to 5.5% and 26.8%, respectively (Bogdanis, Nevill, Boobis, & Lakomy, 1996). Similar values (5.5% and 31.7% for ATP and PCr, respectively) have been reported during a 30 s running sprint on a non-motorised treadmill (Cheetham, Boobis, Brooks, & Williams, 1986). Secondly, the partial contribution of anaerobic glycolysis to total energy release increases from the beginning of exercise attaining a peak within the initial ~30 s of exercise, usually after ~5 s. Then the energy contribution from anaerobic glycolysis progressively decreases (Figure 1.3). Consequently, the contribution of glycolysis to anaerobic energy production increases from 44.1% in a 6 s sprint (Gaitanos et al., 1993) to 67.7% for a 30 s sprint (Bogdanis et al., 1996). Thirdly, as the exercise continues, aerobic metabolism progressively increases its contribution to total energy released (Figure 1.3; Grassi, 2006; Jones & Burnley, 2009; Poole & Jones, 2012; Xu & Rhodes, 1999).

This hierarchical interaction between aerobic and anaerobic metabolism holds true during repeated exercises at high intensity. Thus, during repeated sprint exercise, as the number of sprints increases, the relative contribution of anaerobic energy supply for each sprint decreases, whereas aerobic metabolism increases its contribution (Gaitanos et al., 1993). Moreover, during two bouts consecutive bouts of 5 repeated sprints, the aerobic contribution is greater in the second bout compared to the first bout (McGawley & Bishop, 2015). Importantly, relatively small changes in the duration of the recovery period between sprints within a single repeated sprint exercise have been shown to affect mean and peak power, perhaps because longer rest durations between sprints allows larger [PCr] resynthesis, therefore reducing fatigue in subsequent sprints (Brown & Glaister, 2014; Glaister, Stone, Stewart, Hughes, & Moir, 2005). The effect that alterations in the duration of the recovery period during a repeated spring exercise have on the aerobic-anaerobic interaction has not been studied. Nonetheless, it appears that the hierarchical nature of aerobic and anaerobic metabolism is preserved even if the duration of the rest period between sprints is increased. McGawley and Bishop (2015) determined the mean power output during two consecutive bouts of 5 × 6 s sprints, interspersed with ~10.9 min of passive rest that allowed full (i.e. > 98%) recovery between first and second repeated sprint exercise. The authors noted an increase in  $\dot{V}O_2$  in the second repeated sprint bout, suggesting that anaerobic metabolism was decreased in the latter repeated sprint bout.

Given the progressive decrease in anaerobically-derived energy production, concomitant with the increase in ATP resynthesised aerobically, a crossover point at which both anaerobic and aerobic metabolic pathways equally has been suggested. Most likely, the crossover point is suggested to occurs after ~70 s of high-intensity exercise, but may be affected by the mode of exercise, the intensity, factors affecting  $\dot{V}O_2$  kinetics and/or the method used to determine anaerobic energy production (Gastin, 2001; Li, Niessen, Chen, & Hartmann, 2015; Losnegard & Halle, 2012; Losnegard, Myklebust, & Hallen, 2012; Medbø & Tabata, 1993). The interaction and relative contribution of aerobic and anaerobic energy systems during an exhaustive 3-min exercise bout is represented in Figure 1.3.



**Figure 1.3.** Aerobic (solid line) and anaerobic (broken lines) relative contribution to total energy yield during a bout of exercise to exhaustion at any given intensity. ATP-PCr and anaerobic glycolysis contribute to the majority of energy released at the onset of exercise. After ~1 min of exercise, there is a crossover in metabolic pathways, and the aerobic energy system supplies the majority of the energy. Adapted from Gastin, 2001.

## 1.4. Determinants of anaerobic capacity

The descriptive physiology of variables determined by oxidative metabolism, such as  $\dot{V}O_{2max}$ , have been well reported (e.g. Ferretti, 2014). However, since measuring anaerobic energy production is difficult, as previously discussed, a comprehensive review of factors affecting AnC is lacking. Therefore, the following section reviews the effects of a number of variables (mode of exercise, gender, age, body composition, and genetics) on AnC. In addition, the potential effect of environmental factors (hypoxia, heat and cold) and training interventions on AnC are also discussed.

### 1.4.1. Mode of exercise

Anaerobic capacity has been determined using different modes of exercise, with the majority of the research conducted using either cycling or running. It is generally accepted that exercise that engages a large muscle mass results in a greater anaerobic energy production. For example, running activates more muscle mass than cycling, and indeed AnC determined during running is greater than that determined during cycling (Hill & Vingren, 2011; Hill, Davey, & Stevens, 2002). Furthermore, compared to running on the flat, uphill running results in an increased muscle mass activation and, also, increased AnC (Medbø & Burgers, 1990; Olesen, 1992; Olesen, Raabo, Bangsbo, & Secher, 1996; Sloniger et al., 2011). Similarly, Weyand, Cureton, Conley, and Higbie (1993), determined AnC during one-legged

and two-legged cycling exercise. The latter resulted in ~50% higher anaerobic energy production than the former.

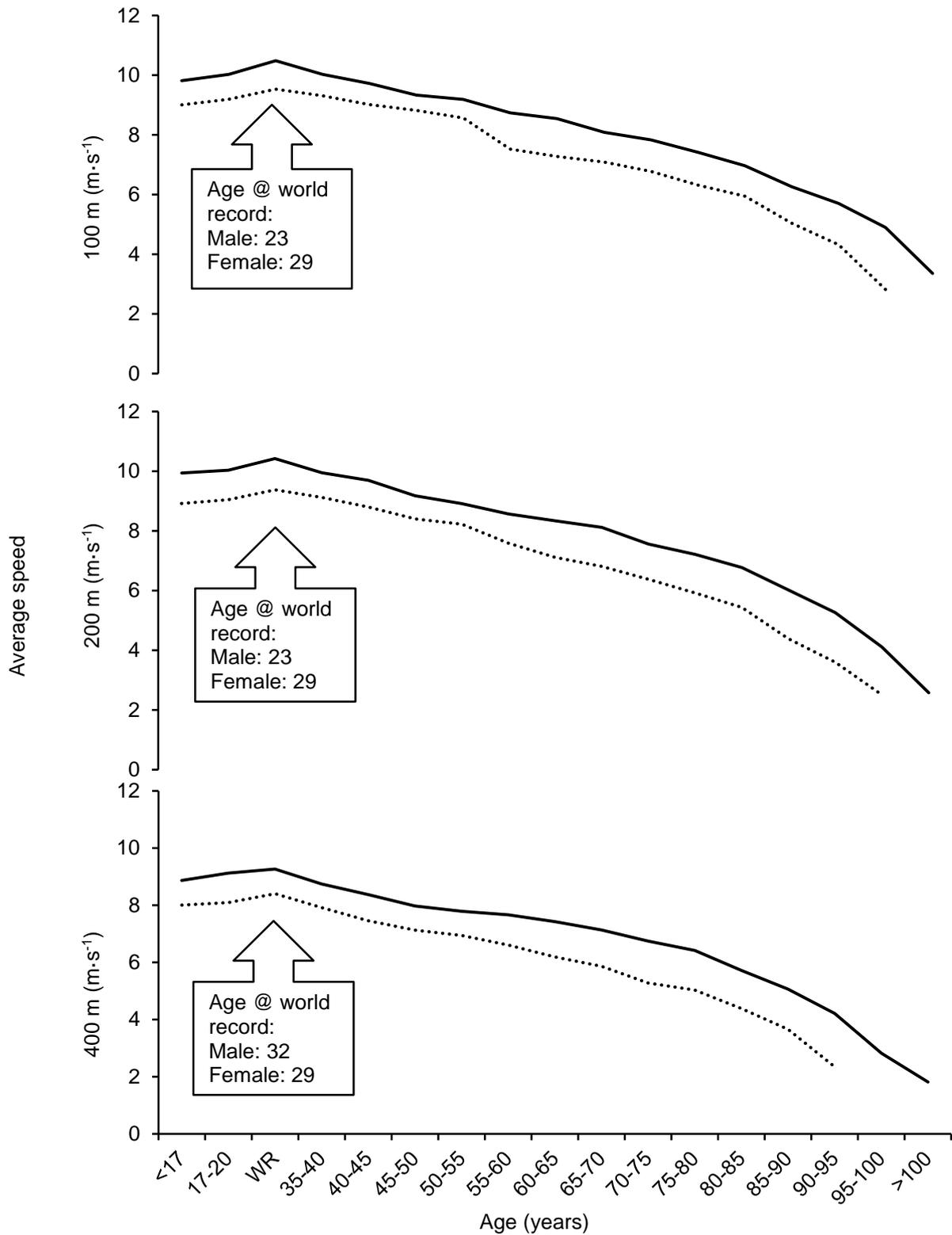
#### **1.4.2. Sex**

Men consistently exhibit a greater AnC than women, though the magnitude changes from ~20 to 100%, depending on the method used to determine AnC (Hill & Vingren, 2014; Pérez-Gómez et al., 2008; Ramsbottom et al., 1997; & Weber & Schneider, 2000, 2002). The chief mechanism to explain the higher AnC in men is largely attributed to a greater muscle mass (Reaburn & Dascombe, 2008). Nevertheless, Pérez-Gómez et al. (2008) reported that men completed 22% more work than women in a 30 s all-out test, even when work was expressed relative to lean-mass. Similarly, Weber and Schneider (2000) observed that the gender differences in AnC, estimated as the accumulated oxygen deficit (AOD), did not disappear when AOD was expressed relative to active muscle mass. Further physiological differences other than higher muscle mass, therefore, need to be considered in order to explain sex differences in AnC. Specifically, it has been suggested that the larger percentage of type II fibres and enzymatic activity observed in men, together with the increased concentration of certain hormones (e.g. testosterone) might also contribute to explain sex differences in AnC (Pérez-Gómez et al., 2008; Reaburn & Dascombe, 2008). In summary, men consistently have a larger AnC compared to women, though the precise mechanism(s) to explain those differences remain to be elucidated.

#### **1.4.3. Age**

Across the life-span, the human body experiences important changes in its physiology. Unfortunately, and in contrast to research on aerobic metabolism, the effect of age on anaerobic metabolism has received very little attention (Praagh & Doré, 2002; Reaburn & Dascombe, 2008). Indeed, no study has directly measured the changes in AnC with age across the life-span. There are, however, a number of cross-sectional studies that compare AnC in different age groups. Anaerobic capacity is lower in children and adolescents compared to adults. Indeed, AnC appears to peak around the third decade of life and, thereafter, steadily decreases until death (Praagh & Doré, 2002; Reaburn & Dascombe, 2010). Given the absence of longitudinal studies, the effect of age on AnC can be studied using performance in athletic events with a significant contribution from the anaerobic energy system as a proxy measure of AnC (e.g. Reaburn & Dascombe, 2010). For instance, the average speed of world records for three athletic events that are considered to be largely anaerobic: 100 m, 200, and 400 m (Gastin, 2001; IAAF,

2015). For clarity, the corresponding world record for hurdle events have not been included in Figure 1.4. Moreover, the anaerobic contribution has been shown to be greater in a 400-m flat race, compared to a 400-m hurdle race (Zouhal et al., 2010). Mean speed increases consistently in both sexes and for both sporting events until adulthood is reached (20-35 years). However, once athletes enter master categories (i.e. > 35 years), performance consistently declines.



**Figure 1.4.** World-records in athletic events considered to be largely determined by anaerobic performance: 100-, 200-, and 400-run. Solid lines represent men's records; broken lines represent women's records.

The increase in AnC observed during growth and maturation may be mediated by the increases in muscle mass and/or enzymatic activity. Indeed, from birth to young adulthood, there is a 20-fold

increase in muscle cross sectional area for both type I and type II muscle fibres (Armstrong, Barker, & Mcmanus, 2015). The effect of muscle mass on AnC is discussed below (Section 1.4.4). Peak power output attained during high-intensity exercise increases during maturation at a faster rate than body mass (Armstrong et al., 2015), suggesting that factors other than muscle mass regulate the increase in AnC during growth. Anaerobic enzymes such as CK, adenylate kinase or LDH (see equations [2, 3]) were 28%, 20% and ~400% lower, respectively, in healthy, relatively sedentary children (3–11 years) compared to healthy sedentary adults (29–54 years) (Kaczor, Ziolkowski, Popinigis, & Tarnopolsky, 2005). When expressed relative to protein content, however, only LDH remained different (~359%) between children and adults, being greater in the adult group (Kaczor et al., 2005). Although a higher activity of anaerobic enzymes in adulthood is not consistent in the literature (Ratel, Tonson, Cozzone, & Bendahan, 2010), children do consistently exhibit a reduced lactate accumulation (Leclai, Borel, Theveret, Baquet, Mucci, & Berthon, 2010; Machado, Guglielmo, Greco, & Denadai, 2009; Ratel et al., 2010) and overall reduced metabolic disturbance (Barker et al., 2010; Tonson et al., 2010), suggesting that anaerobic energy production is indeed compromised. Tonson et al. (2010), using <sup>31</sup>P-MRS, studied metabolic responses in children vs. adults during a 3-min submaximal exercise task. The authors reported that maturation had no effect on the energy released from anaerobic glycolysis (Tonson et al., 2010). Barker et al. (2010) studied boys and girls aged 9 to 12 years and adults (~24 years) during incremental exercise. Whilst at moderate intensities adults and children exhibited similar metabolic profiles (i.e. [Pi]/[PCr] and pH); at heavy and severe intensities adults exhibited an greater [Pi]/[PCr] and reduced in pH compared to children (Barker, Welsman, Fulford, Welford, & Armstrong, 2010), implying a larger disturbance of the cellular milieu. Discrepancies in the results from the studies described above might be explained by methodological considerations and different intensities used in both studies. Indeed, Ratel et al. (2010) highlighted a myriad of confounding variables when studying whether anaerobic energy production changes with maturation. Alongside the effect of the intensity of the exercise (Barker et al., 2010), these confounding variables included differences in allometric scaling (to account for differences in body composition), difficulties in differentiating between chronological and biological age (Armstrong et al., 2015), and musculoskeletal differences between children and adults. Moreover, children typically exhibit lower reductions in pH during high-intensity exercise, which may result in different oxidative ATP flux and/or different muscle recruitment patterns (Ratel et al., 2010).

Though the mechanism(s) remain to be completely understood, anaerobic energy production is reduced in children compared to adults.

After the age of 35, there is a steady decrease in anaerobic performance which appears to accelerate from the age of 70 (Figure 1.4). Although the precise mechanisms underpinning the decrease in anaerobic performance remain elusive, they most likely involve genetic factors (including sex), muscle quantity (i.e. decrease in muscle mass) and quality (changes in fibre type, fibre size and muscle architecture), reduced substrate availability (mainly PCr), decreased enzymatic activity, increased accumulation of reactive oxygen species, and sociological factors (reduced training volume) (Reaburn & Dascombe, 2008). Nonetheless, there is growing evidence suggesting that training at high-intensities, as opposed to the traditional model based on submaximal continuous endurance exercise, provides long-term health benefits and increases the likelihood of successful ageing (Ramos, Dalleck, Tjonna, Beetham, & Coombes, 2015; Kusy & Zielin, 2015). In summary, AnC increases from birth until, approximately, the age of 30, when it peaks. After the age of 35, AnC seems to decrease at a moderate rate until the age of 65-70, and more rapidly in the last decades of life. Although the precise mechanisms underpinning this decrease in AnC are not completely understood, high intensity exercise might prevent (or, at least, decelerate), the rate of decline.

#### **1.4.4. Body composition**

The effects that mode of exercise, gender and age have on anaerobic performance are, to a certain extent, mediated by body mass. Indeed, a large body mass has been suggested to be beneficial for anaerobic energy production (Chikani, Cuneo, Hickman, & Ho, 2015). Importantly, body mass includes fat mass, a passive tissue that tends to be minimised in athletes, particularly in those competing in weight-bearing activities such as running (Huovinen et al., 2015). Therefore, increases in muscle mass have been shown to induce larger increases in cycling anaerobic power than increases in body mass alone (Maciejczyk, Wiecek, Szymura, Szygula, & Brown, 2015). Moreover, lean mass, is positively correlated with AnC, irrespective of the test used to estimate AnC (Miura, Endo, Sato, Barstow, & Fukuba, 2002). Pizza et al. (1996) studied the AnC, estimated as AOD, of resistance-trained, endurance-trained and untrained men. The authors observed that AOD was greatest in resistance-trained, intermediate in endurance-trained and lowest in the untrained group. Moreover, Pizza et al.

(1996) reported a strong correlation between AnC and leg muscle mass in the resistance-trained group ( $r = 0.85$ ) but not in the endurance-trained and untrained groups ( $r = 0.55$  and  $r = 0.20$ , respectively).

#### **1.4.5. Genetics**

It is widely acknowledged that inter-individual differences in the physiological response to short (i.e. acute response) and long term (i.e. training adaptation) exercise are determined by the interaction between environmental and genetic factors. How genetics and environmental factors interact is a complex issue (e.g. Eynon et al., 2011; Tucker & Collins, 2012) beyond the scope of the current literature review. Nonetheless, research has been conducted to investigate the heritability of AnC and to determine which gene(s) may be responsible. Regarding heritability, Fagard, Bielen, and Amery, (1991) concluded that the variation in anaerobic energy production was not determined by genetic factors. However, the approach that these authors used to assess anaerobic energy production, the  $\dot{V}O_2$  at a respiratory exchange ratio of 0.95, is not a common method of estimating AnC (see Section 1.5). Calvo et al. (2002) determined the heritability index, which ranges from 0 (no variability due to genetic factors) to 1 (all variability due to genetic factors) in 16 pairs of male Caucasian twins for a range of anaerobic tests. The results revealed a significant heritability index for anaerobic power (determined as the peak power output during a 5 s sprint) and AnC (determined as the mean power output and post-exercise BLA in a 30 s all-out cycling test) (Calvo et al., 2002). However, the heritability index (0.222) was not significant if AnC was determined using AOD (Calvo et al., 2002). The authors acknowledged the difficulty in quantifying AnC, which might explain dissimilar results from different approaches used to estimate whether AnC is heritable (Calvo et al., 2002).

Further to heritability studies on AnC, the identification of genetic variants (i.e. polymorphisms) that contribute to sporting success has received considerable attention in recent years. It is now acknowledged that a single polymorphism has little effect on athletic performance (Eynon et al., 2011; Tucker & Collins, 2012). Instead, there are certain combinations of polymorphisms that have been associated with performance in either sprint/power or endurance sports. With regards to sprint/power performance, where AnC plays a significant role, the polymorphism that has received the most attention regarding AnC is, arguably, the genetic R477 variation of the  $\alpha$ -actinin-3 gene (Eynon et al., 2013; Macarthur & North, 2007). There are three possible genotypes of R577: XX, RX, and RR. Individuals with a XX genotype have a complete deficiency of the  $\alpha$ -actinin-3 protein, which forms part of the

contractile machinery in type II fibres, and as such, these individuals typically possess a high aerobic capacity (Silva et al., 2015). In contrast, RR has been proposed to favour anaerobic energy production, and perhaps increased AnC (Eynon et al., 2013; Macarthur & North, 2007). A higher peak power during a 30 s all-out cycling test has been observed in subjects with the RR genotype of the  $\alpha$ -actinin-3 R477X polymorphism (Kikuchi, Nakazato, Min, Ueda, & Igawa, 2014). However, the authors reported that there were no differences between XX, RX and RR for mean power output during the 30 s all-out spring, which might provide a better estimate of AnC than peak power (Kikuchi et al., 2014).

In summary, the difficulties in obtaining a valid and reliable measure of AnC seem to undermine the ability to determine the extent to which AnC is determined genetically. Nonetheless, AnC appears to be heritable, with the RR genotype of the  $\alpha$ -actinin-3 R477X polymorphism suggested to favour anaerobic performance.

#### **1.4.6. Environmental factors**

##### ***Altitude***

At sea level, the atmosphere exerts a pressure of ~760 mm Hg (normobaria). The fraction of inspired oxygen ( $F_{iO_2}$ ) is 0.2093 (i.e. 20.93% oxygen), the partial pressure of inspired oxygen ( $P_{iO_2}$ ) is ~149 mm Hg. As altitude increases, the pressure of the atmosphere decreases, resulting in a decrease in  $P_{iO_2}$ , known as hypobaric hypoxia. Alternatively, the effects of altitude can be simulated at sea level by reducing  $F_{iO_2}$ , which is known as normobaric hypoxia. Whether normobaric and hypobaric hypoxia induce the same physiological responses remains debated and is beyond the scope of this literature review (Beidleman, Fulco, Staab, Andrew, & Muza, 2014; Coppel, Hennis, Gilbert-Kawai, & Grocott, 2015; Millet, 2012). In the current PhD thesis, both methods will be used interchangeably as “hypoxia”. This section, therefore, aims to summarise the effects of hypoxia on anaerobic energy production.

Hypoxia has a profound, dose-dependent, detrimental effect on aerobic metabolism, as indicated by the decrease in  $\dot{V}O_{2max}$  with increasing altitude (Wehrlin & Hallén, 2005). In contrast, it has been hypothesised that anaerobic power and AnC should not be affected under hypoxic conditions (Álvarez-Herms, Julià-Sánchez, Gatterer, Viscor, & Burtscher, 2015; Coudert, 1992; Peronnet, Thibault, & Cousineau, 1991). Indeed, the assumption that hypoxia does not affect anaerobic metabolism has been used to test the external validity of tests aiming to quantify AnC (Broxterman, Ade, Craig, Wilcox,

Schulp, & Barstow, 2015a; Dekerle, Mucci, & Carter, 2012; Feriche et al., 2007; Friedmann, Frese, Menold, & Bärtsch, 2007; Medbø et al., 1988; Morales-Álamo et al., 2012; Ogura, Katamoto, Uchimar, Takahashi, & Naito, 2006; Simpson, Jones, Skiba, Vanhatalo, & Wilcerson, 2015; Valli et al., 2011). In the above studies, it is assumed that hypoxia has no effect on AnC, but the results are not consistent. Unfortunately, the effect of hypoxia on AnC has been assessed using different approaches to estimate AnC, and hypoxia has been generated by employing different techniques (hypobaric hypoxia, normobaric hypoxia, and blood flow occlusion), making comparisons problematic. Moreover, recent evidence suggests that chronic hypoxic exposure might stimulate anaerobic glycolysis (Horscroft & Murray, 2014). Indeed, the up-regulation of key glycolytic enzymes observed after high intensity training (MacDougall et al., 1998) is increased if training is undertaken in hypoxia compared to normoxia (Puype, Van Proeyen, Raymackers, Deldicque, & Hespel, 2013). Nevertheless, hypoxic exposure is still considered a powerful tool to determine the validity of tests intended to estimate AnC. The specific effects of hypoxia on  $W'$  and AOD are presented in Section 1.6 and Section 1.7, respectively.

The detrimental effect of altitude on aerobic metabolism does not necessarily translate to an impaired performance in all athletic events. Whilst the negative effect of altitude on endurance performance is well known, mathematical models have predicted an enhanced running performance at moderate altitudes compared to sea level in events up to 800 m (Arsac, 2002; Peronnet et al., 1991; Quinn, 2011), and historical data appears to support these predictions (Hamlin, Hopkins, & Hollings, 2015). This is because the reduced air resistance at altitude and the relatively low contribution of the aerobic metabolism compensate for the impaired aerobic metabolism in events with a high reliance on anaerobic energy production (Arsac, 2002).

### ***Ambient temperature***

The effect of exercising in hot and cold environments has been widely studied, with the majority of the research focusing on aerobic metabolism, cardiovascular responses and endurance performance (Doubt, 1991; Febbraio, 2001; Martola & Maskrey, 2011; Nybo, Rasmussen, & Sawka, 2014). Heat stress is determined by environmental factors (temperature, humidity, clothing), individual characteristics (acclimatization, fitness, body size/surface area, hydration), and the characteristics of the exercise task (duration, intensity). Recently, Nybo et al. (2014) proposed an integrative model incorporating the systems suggested to underpin the increase in fatigue and decrease in performance

typically observed in the heat. This model included psychological factors, changes in the central nervous system, alteration of substrate utilization, cardiovascular changes, peripheral and muscular factors, and respiration. The authors acknowledged the complexity of fatigue, and therefore recommended the use of the model in a task-specific manner (Nybo et al., 2014). Regardless of the exercise task, aerobic performance appears to be negatively affected in the heat, most likely due to a cascade of cardiovascular responses, as blood flow is redistributed to the skin in an attempt to dissipate heat. In contrast, the effect on AnC of exercising in the heat has received little attention. It has been hypothesised that the rate of anaerobic energy production increases in the heat in order to compensate for a reduced cardiovascular function, as suggested by the observed reduction in  $\dot{V}O_2$  and increase in blood lactate concentration (BLa) (Dimri, Malhotra, Sen Gupta, Kumar, & Arora, 1980; González-Alonso, Calbet, & Nielsen, 1999; Gonzalez-Alonso & Calbet, 2003; Logan-sprenger, Heigenhauser, Jones, & Spriet, 2015; Nybo, Jensen, Nielsen, Jensen, & Nielsen, 2001). However, most evidence contradicts the hypothesised increase in AnC in the heat (Finn, Wood, & Marsden, 2003; Maxwell, Aitchison, & Nimmo, 1996; Maxwell, Gardner, & Nimmo, 1999; Nybo et al., 2001), with only one study supporting it (Dotan & Bar-Or, 1980). Whilst heat stress might increase the rate of anaerobic energy production (i.e. anaerobic glycolysis, as denoted by BLa); performance (e.g. TTE) is negatively affected by heat stress during high intensity exercise (Nybo et al., 2001). Therefore, whilst it is plausible that heat stress increases the rate of anaerobic energy release, the impaired performance and TTE observed might result in an overall unaffected AnC under heat stress. In summary, whilst the negative effect of heat stress on aerobic metabolism is well established, the effect of heat stress on AnC is less clear. It is possible that heat stress stimulates the rate anaerobic ATP resynthesis, although whether AnC is affected is uncertain.

The effect of cold temperatures on AnC has also received little attention. Indeed, only one study has appeared to have investigated the topic (Hackney, Shaw, Hodgdon, Coyne, & Kelleher, 1991). The authors assessed, by means of a 30 s all-out cycling test, the AnC in military personnel before and after 5 days of military field operations in either cold (range -2 to -20 °C) or control temperatures (range 10 to 30 °C). There were no differences before the field operation between the two groups in the mean or peak power of the 30 s all-out cycling test. Mean power output decreased in both groups after the field operations, though its decrease in the cold-exposure group (9.8%) was greater than that of the control group (3.1%). Similarly, peak power was lower in the cold-exposure group compared to the control

group (8.4% vs. 1.6%, respectively) (Hackney et al., 1991). This study suggests that 5 days of cold exposure impairs subsequent anaerobic performance.

#### **1.4.7. Training and training status**

Anaerobic capacity is rarely included in the physiological profiling of athletes, partly because it is difficult to assess, and because indirect tests are time and effort demanding (Bishop & Spencer, 2011). Despite this, AnC has been compared in cross-sectional studies using participants with different training backgrounds. The overall conclusion from these studies is that athletes who regularly engage in high-intensity exercise, where there is a large contribution of the anaerobic energy pathways to overall ATP resynthesis (Figure 1.3), show a greater AnC when compared with endurance-trained individuals or their untrained peers (Craig & Norton, 2001; Craig et al., 1995; Greco, Caritá, Dekerle, & Denadai, 2012; Pizza et al., 1996; Ramsbottom et al., 1997; Scott, Ruby, Lohman, Bunt, 1991; Sloniger, Cureton, Prior, & Evans, 1997; Weber & Schneider, 2000). Similar effects have also been reported in studies that have monitored the effects of high-intensity training on AnC. Typically, these studies have used interval training as the stimulus to produce the adaptive response. However, nine variables have been identified to characterise high-intensity interval training (Buchheit & Laursen, 2013), making comparisons between studies difficult. The majority of studies have shown an increase in AnC after high-intensity interval training (Jenkins & Quigley, 1993; Medbø & Burgers, 1990; Mueller et al., 2015; Ravier, Dugué, Grappe, & Rouillon, 2009; Sawyer et al., 2014; Stevens, Olver, & Lemon, 2015; Weber & Schneider, 2002). Nevertheless, there are two studies that have reported no changes in AnC, estimated by means of  $W'$ , after a high intensity training program (Mueller et al., 2015; Vanhatalo, Doust, & Burnley, 2008a). Unlike those studies showing a positive effect on AnC, both Mueller et al. (2015) and Vanhatalo et al. (2008a) used training sessions where the high-intensity component was performed at relatively low intensities (i.e. 105% CP, 75% peak power output attained in a ramp incremental test), and for a relatively long duration (two to five minutes), suggesting that the training stimulus might not have challenged anaerobic energy production sufficiently to induce an increase in AnC (Mueller et al., 2015; Vanhatalo et al., 2008a). Indeed, endurance training has been found to have no effect on AnC (Glaister, Stone, Stewart, Hughes, & Moir, 2007). It appears, therefore, that a training program with a high intensity component, consisting of repeated maximal or near maximal efforts, is necessary in order to elicit an increase in AnC. Moreover, there is growing evidence that repeated bouts of high-intensity

exercise have a beneficial effect for both performance and health (for reviews, see Gibala, Little, Macdonald, & Hawley, 2012; Laursen, 2010; Ramos et al., 2015).

#### **1.4.8. Summary**

Anaerobic capacity is determined by a number of factors, largely influenced by muscle mass. Hence, AnC tends to be greater in exercise which engages a large muscle mass. Similarly, the larger muscle mass typically observed in men compared to women, and in adulthood compared to childhood and old age, explains why peak values of AnC are reported during the third decade of life, and why men typically exhibit greater AnC than women, although other mechanisms might also contribute. Whilst some of the variability in AnC seems to be determined by genetic factors, it can be trained through high-intensity training interventions. Unfortunately, there is a myriad of tests used to quantify AnC and direct comparison between studies is rather difficult. Moreover, the validity of some of these tests to quantify AnC has been challenged, so conclusions drawn from those data might be compromised. It follows, therefore, to critically review methods to quantify AnC.

### **1.5. Quantification of anaerobic capacity**

The quantification of AnC has been a difficult challenge for exercise physiologists for many decades. On the one hand, attempts to directly quantify AnC require the measurement of muscle metabolites during and/or immediately after exercise, invasively via muscle biopsies or confined in a MRS scanner. These techniques are expensive, technically challenging and not readily available in most exercise physiology laboratories (Bishop & Spencer, 2011; Brooks, 2012). On the other hand, a number of indirect tests have been purposed to estimate AnC. These assess a number of physiological or mechanical variables such as  $\dot{V}O_2$ , BLA, power output or speed, and work completed during or after high intensity exercise. Two indirect tests embed the majority of research on the assessment of AnC. First, the curvature constant of the hyperbolic relationship between intensity and time to fatigue, represented as  $W'$ , has been proposed to represent a measure of the finite amount of work that can be accomplished from anaerobic energy sources (Hill, 1993; Morton, 2006). Secondly, the oxygen deficit incurred at the onset of a single bout of exercise to exhaustion has been proposed, and is currently believed to best represent AnC (Medbø et al., 1988; Noordhof, De Koning, & Foster, 2010). Unfortunately, both direct and indirect approaches to quantify AnC suffer from a number of assumptions

and limitations, and currently there is no gold standard test to quantify AnC (Bishop & Spencer, 2011). The following sections review a number of approaches to quantify AnC, though  $W'$  and AOD are discussed in detail in Section 1.6 and Section 1.7, respectively.

### 1.5.1. Quantification of muscle metabolites

The determination of changes in muscle metabolites due to an exercise bout to exhaustion has been used to determine AnC (Bangsbo, 1998; Bangsbo et al., 1990; Bangsbo et al., 2001; Green et al., 1996; Medbø & Tabata, 1993). Changes in metabolite concentrations are quantified *in vitro* from the difference in biopsied muscle tissue pre- vs. post-exercise ( $\Delta$ ) to calculate muscle anaerobic ATP yield ( $ATP_{an}$ ), using the equation first proposed by Chasiotis, Bergström, and Hultman (1987):

$$ATP_{an} = 1.5 \Delta[La^-] + \Delta[PCr] + (2 \Delta[ATP] - \Delta[ADP]) \quad [7]$$

It should be noted that energy derived from  $La^-$  released into blood is not taken into account, and therefore anaerobic energy production is underestimated (Green & Dawson, 1993). This can account for approximately one third of the total anaerobic energy release (range 5-38%, Bangsbo, 1998). In addition, this approach requires an estimation of active muscle mass for each particular mode of exercise (Bangsbo et al., 1990; Hill et al., 2002; Medbø et al., 1988; Weyand et al., 1993). This is unfortunate because there is no direct technique to establish active muscle mass during exercise. Furthermore, the site of muscle biopsy needs to be the same pre- and post-exercise, and tissue analysed is assumed to be representative of the entire active muscle mass (Bangsbo et al., 2001). Nonetheless, there are a number of studies showing close agreement between estimations of AnC derived from direct quantification of changes in muscle metabolites before and after exercise and other tests, namely AOD, in some (Bangsbo et al., 1990; Medbø et al., 1988; Medbø & Tabata, 1993; Whitters et al., 1991), though not all studies (Bangsbo, 1998; Green et al., 1996). Nonetheless, metabolite determination is considered the best technique for AnC estimation for small muscle groups (Gastin, 2001).

The use of  $^{31}P$ -MRS allows an *in vivo* determination of muscle metabolites during exercise (Chance et al., 2006; Kemp, Meyerspeer, & Moser, 2007). Combined with measures of intracellular pH, measures of energy metabolites have been used to estimate AnC (Volianitis, Secher, & Quistorff, 2010). Nonetheless, though there are several ways of reporting  $^{31}P$ -MRS spectra, this approach also requires

an estimation of active muscle mass during exercise, and it is common to express metabolic changes relative to resting values rather than by using absolute quantification (see Figure 1.1) (Chance et al., 2006; Jones et al., 2008c; Vanhatalo et al., 2010b). Unfortunately, there are two main limitations with using  $^{31}\text{P}$ -MRS as a means of estimating AnC. First, as mentioned above this is an expensive technique which is not readily available for athletes and coaches, and even most exercise physiology laboratories. In addition, exercise within the MRS bore is limited to relatively small muscle groups, and muscle bioenergetics cannot be determined for whole body exercises. Secondly, from a physiological standpoint, it is necessary to accept a number of assumptions.  $^{31}\text{P}$ -MRS does not allow interrogation of a specific muscle (e.g. vastus lateralis), but a region (e.g. quadriceps). As a result, surrounding muscles might contaminate the  $^{31}\text{P}$ -MRS spectra. For example, Cannon et al. (2013), demonstrated that the limit of tolerance during an incremental test to exhaustion was attained with wide heterogeneity in substrate depletion and fatigue-related metabolite accumulation. By using localized  $^{31}\text{P}$ -MRS however, this limitation has partly been resolved. Localized  $^{31}\text{P}$ -MRS separates the region of interest, and allows an enhanced temporal and spatial resolution (Chance et al., 2006). The localised  $^{31}\text{P}$ -MRS technique allows, for example, an evaluation of the differences between sprint and endurance athletes and untrained subjects in four regions within a muscle group (Pesta et al., 2013).

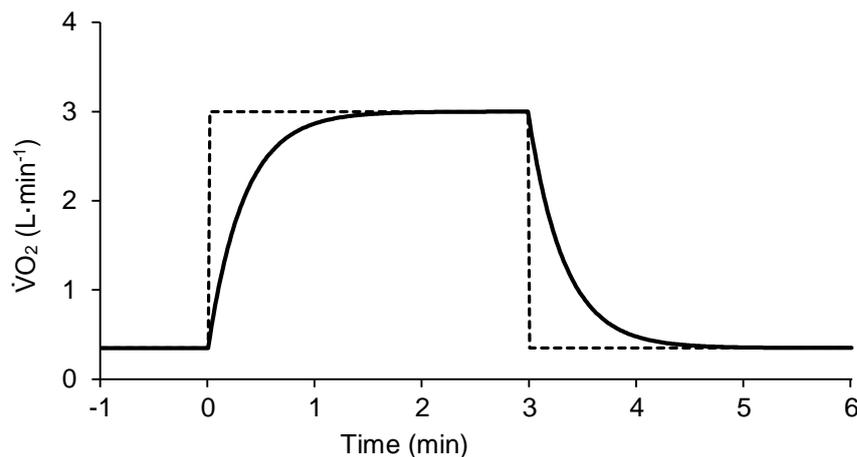
In summary, anaerobic energy production can be estimated from muscle biopsies and  $^{31}\text{P}$ -MRS via interrogation of the skeletal muscle during or immediately after strenuous exercise. However, whilst these techniques have largely contributed to enhancing the understanding of muscle metabolism, they offer limited value to the assessment of AnC on a regular basis.

### **1.5.2. Excess post-exercise oxygen consumption**

The excess post-exercise oxygen consumption (EPOC) was first described by Hill and Lupton (1923) as the  $\dot{V}\text{O}_2$  above resting  $\dot{V}\text{O}_2$  after exercise. Originally, what is now known as EPOC was termed oxygen debt, and explained by the “oxygen deficit repayment” hypothesis (Hill, 1924). Based on this theory, metabolic rate (i.e.  $\dot{V}\text{O}_2$ ) remains elevated after exercise to “pay back” the oxygen deficit incurred at the onset of exercise, making the magnitude of the oxygen deficit and debt equal (Figure 1.5). As the oxygen deficit can be considered to provide an estimation of AnC (Linnarsson et al., 1974; Medbø et al., 1988; Noordhof et al., 2010; see Section 1.7), it was hypothesised that EPOC could estimate AnC. However, evidence does not support symmetrical on- and off-transients in the kinetics of  $\dot{V}\text{O}_2$  within

the moderate or heavy exercise domains (Børsheim & Bahr, 2003; Kilding, Challis, Winter, & Fysh, 2005; Rossiter et al., 2002). Moreover, whilst pulmonary  $\dot{V}O_2$  on-kinetics seem to represent muscle  $\dot{V}O_2$  on-kinetics with an acceptable degree of fidelity (see Section 1.7 for further details), this is not the case for the pulmonary and muscle  $\dot{V}O_2$  off-kinetics (Krustrup, Jones, Wilkerson, Calbet, & Bangsbo, 2009). The hypothesis that EPOC represents the oxygen deficit, and therefore provides an estimate of AnC, is therefore not accepted.

The quantification of EPOC, nonetheless, can still be useful to evaluate anaerobic metabolism. The EPOC can be divided into the rapid phase and the prolonged phase. The rapid phase of EPOC represents the sudden, decrease in  $\dot{V}O_2$  immediately after exercise, and is mainly associated with the replenishment of oxygen stores, [ATP] and [PCr] resynthesis. In contrast, the prolonged phase of EPOC is more complex, and its precise mechanisms are not fully understood. In particular, after exercise at high intensities, increased ventilation, temperature, blood flow, circulating hormones, and  $La^-$  removal appear to contribute to the prolonged phase of EPOC (Børsheim & Bahr, 2003; Di Prampero, Peeters, & Margaria, 1973; Green & Dawson, 1993; Krogh & Lindhard, 1919; LaForgia, Withers, & Gore, 2006; Özyener, Rossiter, Ward, & Whipp, 2001; Vanderwalle, Pérès, & Monod, 1987). Di Prampero, Peeters, and Margaria (1973) proposed a method to estimate AnC that differentiates between the alactic and lactic components of AnC. More recently, Bertuzzi et al. (2010) used a combination of the fast component of EPOC and BLa accumulation, in an attempt to account for energy derived from PCr and anaerobic glycolysis, respectively (Bertuzzi et al., 2010). Although estimations of AnC were strongly correlated with the AOD ( $r = 0.78$ ), this method has failed to gain popularity, perhaps because the use of BLa, itself, has a number limitations (see below, Section 1.5.3).



**Figure 1.5.** Time-course of  $\dot{V}O_2$  during rest-to-work and work-to-rest transitions. The oxygen deficit is determined from the difference between energy demand (broken line) and  $\dot{V}O_2$  (solid line), whilst EPOC is determined from the extra  $\dot{V}O_2$  above resting values after exercise.

### 1.5.3. Peak blood lactate concentration

Lactate is a by-product of anaerobic glycolysis, which may account for up to 80% of the total anaerobic energy released during supramaximal exercise to exhaustion (Medbø & Tabata, 1989; Medbø et al., 1988). At intensities above the lactate threshold, in the heavy and severe exercise domains, lactate production exceeds the capacity for lactate removal (Messonnier et al., 2013), and as a result there is an accumulation of BLA and elevated post exercise BLA levels. Measures of BLA after a bout of high intensity exercise, therefore, were proposed as a way of estimating energy production from anaerobic glycolysis (Di Prampero, Peeters, & Margaria, 1973; Jacobs, 1986; Margaria, Cerretelli, di Prampero, Massari, & Torelli, 1963). Di Prampero et al. (1999) proposed an energy equivalent for each mmol of BLA accumulated after exhaustive exercise of 3 mL  $O_2 \cdot kg^{-1}$ .

Unfortunately, the use of BLA to estimate AnC has a number of limitations. First, energy yield from the ATP-PCr system is not taken into account if peak BLA is used solely to estimate AnC. Secondly, despite a strong correlation between muscle  $[La^-]$  and BLA ( $r > 0.80$ ; Cheetham et al., 1986), muscle  $[La^-]$  starts to decrease straight after the end of exercise, but BLA continues to increase for a few minutes (Bogdanis et al., 1995, 1996). As a consequence, the timing of sample collection is likely to affect peak BLA (Bassett et al., 1991; Howley, Bassett, & Welch, 1995). This issue can be somewhat accounted by taking several BLA samples post-exercise. Thirdly,  $La^-$  can be oxidised and used as a fuel during exercise (Brooks, 2007; Gladden, 2004; Van Hall, 2000). If  $La^-$  is used as a fuel, it does not escape the cell into the blood stream and it cannot be measured as BLA, thereby underestimating AnC from BLA. BLA reflects increases in muscle  $[La^-]$ , but it also represents other processes regulating transport and

elimination of La out of the blood (Beneke, Hu, Jung, Leitha, & Renate, 2005). Therefore, the use of post-exercise BLa seems to be unrepresentative of AnC. In summary, the solely quantification of BLa might offer a rough estimation of anaerobic glycolysis, but limited information on the total anaerobic energy production or the interaction between aerobic and anaerobic metabolism during exercise (Heck et al., 2003).

#### **1.5.4. The Wingate anaerobic test**

The Wingate anaerobic test (WAnT) was developed by the Wingate Institute for Physical Education as an inexpensive, simple to administer, indirect test to assess anaerobic performance (Bar-Or, 1987). In the WAnT, participants perform all-out cycling for approximately 30 s against a constant resistance that aims to elicit intensities of 200-400%  $\dot{V}O_{2max}$ . There are three main outcomes from the WAnT: peak power, fatigue index, and mean power/total work. Work done in a WAnT has been considered, with assumptions, to provide an indication of AnC (Bar-Or, 1987), and together with peak power and fatigue index, provides an evaluation of anaerobic performance (Bar-Or, 1987; Green & Dawson, 1993; Vandewalle et al., 1987).

There are two main methodological considerations in the WAnT: the resistance of the flywheel during the test and the duration of the test. The former is typically determined relative to body mass as  $0.085 \text{ N}\cdot\text{m}\cdot\text{kg}^{-1}$ . Bar-Or (1987) and MacIntosh, Rishaug, and Svedahl (2003) suggested that higher resistances might be optimal ( $0.090$  and  $0.100 \text{ N}\cdot\text{m}\cdot\text{kg}^{-1}$  for untrained and trained adults, respectively). Moreover, Glaister et al. (2014) observed that the resistance that individually elicits peak power was attained at  $\sim 0.113 \text{ N}\cdot\text{m}\cdot\text{kg}^{-1}$ . The authors, however, used 6 s sprints so it is not known if such a high resistance elicits optimal performance in the WAnT. Vargas and Klopp (2015) compared the parameters of the WAnT determined using an optimal load and the traditional  $0.085 \text{ N}\cdot\text{m}\cdot\text{kg}^{-1}$ . The authors reported that neither peak power output nor mean power output were different during the WAnT using the optimal or traditional resistance. The duration of the WAnT is the second main methodological consideration, and has been suggested to affect peak and mean power. Specifically, peak power output appears to be higher and mean power lower during short (15-20 s) compared to longer WAnT (40 s) (Bar-Or, 1987). The duration of longer all-out test protocols have increased up to 3 minutes (Burnley, Doust, & Vanhatalo, 2006; Calbet et al., 2003; Gastin et al., 1995; Vandewalle et al., 1987). All-out efforts longer

than 30 s seconds have been used to quantify AnC, using AOD and  $W'$  (see Section 1.7.2 and Section 1.6.2, respectively).

It bears repeating the absence of a gold-standard test to quantify AnC. As a consequence, it is difficult to determine the validity of the WAnT to assess AnC. Nonetheless, the WAnT has been compared with other tests intended to quantify AnC. Two studies have reported strong ( $r \geq 0.64$ ), positive correlations between both the peak and mean power output attained during a WAnT and estimations of AnC derived from the AOD test (Minahan et al., 2007; Scott et al., 1991). Moreover, estimations of AnC derived from the WAnT (i.e. peak and mean power output) correlate with the percentage of type II muscle fibres, remain unaffected by hypoxia, differentiate between power and endurance athletes and correlate with performance in 'anaerobic' events (Inbar & Bar-Or, 1986). With regards to the reliability of the WAnT, there is a general agreement that the peak power and mean power have good reproducibility with test-retest  $r$  values typically  $> 0.90$ . The fatigue index seems to be the least reliable index ( $r = 0.43$ ) of the WAnT (Bar-Or, 1987; Driss & Vandewalle, 2013; Vandewalle et al., 1987).

There are, however, a number of limitations to consider before using the WAnT as a measure of AnC. First, 30 s might not be long enough to fully deplete the anaerobic energy stores (Beneke, Pollmann, Bleif, Leithäuser, & Hütler, 2002; Calbet et al., 2003; Medbø & Tabata, 1993). Secondly, during the WAnT it is not possible to determine the relative contribution of the aerobic energy system (Bar-Or, 1987; Gastin, 2001; Scott, Roby, Lohman, & Bunt, 1991). Specifically, it has been estimated that aerobic metabolism represents approximately 20-30% to the total work completed during a 30 s WAnT (Bar-Or, 1987; Beneke et al., 2002; Nummela, Albert, Rijntjes, & Ruskol, 1996a).

In summary, the WAnT is a popular, simple to administer, inexpensive test that seems to provide a good overview of anaerobic performance. However, there are limitations that compromise the use of WAnT as a measure of AnC.

### **1.5.5. Maximal anaerobic running test**

The maximal anaerobic running test (MART) was developed and validated by Nummela and colleagues (Nummela & Rusko, 1995; Nummela et al., 1996a; Nummela, Andersson, Hakkinin, & Ruskob, 1996b; Nummela, Mero, Stray-Gundersen, & Rusko, 1996c; Nummela, Mero, & Rusko, 1996) as a test to estimate AnC during running exercise. Participants are required to complete 20 s sprints at increasing speeds interspersed with 100 s rest intervals on a treadmill at a 10.5% incline. Alternatively, in an

attempt to better simulate game sports, the original MART protocol has been modified, so that, instead of running on a treadmill, participants run between two parallel lines 20 m apart (Dardouri et al., 2014). Irrespective of the protocol, exercise is continued until exhaustion, so that AnC is assumed to be fully depleted. It has been estimated that the AnC contributes to ~70% of the total energy demand in the MART test (Nummela et al. 1996a). The MART test relies on the same principles as the AOD in that at the onset of high intensity exercise, the majority of the energy is released from anaerobic energy sources. Indeed, the MART estimates AOD during intermittent running, though the oxygen demand is estimated following the guidelines of the American College of Sports Medicine (ACSM, 1986). Specifically, the oxygen demand is determined as a function of speed, grade and a constant, to account for resting  $\dot{V}O_2$ . The correlation between AOD and MART, however, is not consistent, with reports showing a strong relationship between AOD and MART ( $r = 0.83$ ; Maxwell & Nimmo, 1996) and no significant correlation (Zagatto et al., 2011). A potential limitation of the MART comes from the fact that the test requires running at very high speeds on an uphill gradient. There is evidence that the ability to run at very high speeds might be limited by biomechanical factors, instead of metabolic factors (Bundle & Weyand, 2012), so factors other than AnC may determine performance during the MART (Maxwell & Nimmo, 1996). Despite these limitations, the MART benefits from a relatively simple approach that requires inexpensive and readily available equipment.

#### **1.5.6. Alternative approaches**

A further approach that has been suggested for assessing AnC is derived from the levelling off in  $\dot{V}O_2$  observed towards the end of an incremental test to exhaustion (i.e. the so called plateau). Gordon et al. (2011) reported a strong negative correlation ( $r = -0.77$ ) between the increase in  $\dot{V}O_2$  in the last 60 s of a ramp test to exhaustion and AOD values. However, the plateau phenomenon in  $\dot{V}O_2$  during an incremental test to exhaustion is the subject of debate because it is not always observed (Howley et al., 1995; Howley, 2007; Midgley et al, 2007; Poole, Wilkerson, & Jones, 2008). Furthermore, the criteria used to determine whether  $\dot{V}O_{2max}$  has reached a plateau are critical to this approach, and yet they have not been standardised (Poole, Wilkerson, & Jones, 2008). It is typically assumed, somewhat arbitrarily, that an increase  $< 2.1 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  despite an increase in power output (Taylor, Buskirk, & Henschel, 1955) represents a plateau in  $\dot{V}O_2$ . However, the existence of a plateau in  $\dot{V}O_2$  during incremental exercise to exhaustion is still debated, and not always observed (Poole et al., 2008). As

such, this approach to estimate AnC has been criticized and not been further employed (Shephard, 2011).

### **1.5.7. Summary**

A number of approaches have been suggested for estimating AnC, either through direct measures of anaerobic-related metabolites ([ATP], [ADP], [PCr], [La<sup>-</sup>]), before and after exercise, or by indirect estimation from mechanical (e.g. power output, speed) or metabolic (e.g. BLa,  $\dot{V}O_2$ ) variables. Direct quantification of muscle metabolites during exhaustive exercise or indirect tests, whereby AnC is derived from measurements of EPOC, peak BLa, the WAnT or MART may all be representative of the AnC. Unfortunately, these approaches have limitations that constrain their validity and are currently disregarded as tests to determine AnC.

## **1.6. The curvature constant of the power output-duration relationship**

### **1.6.1. Definition**

As early as 1925, A.V. Hill observed that the decrease in world-record speeds for running, walking, swimming, and rowing was non-linear as the distance of the athletic event increased. Currently, it is well known that the relationship between power output and the duration that exercise can be sustained before exhaustion (*P-d* relationship) is hyperbolic, as initially demonstrated in a synergic muscle group (Scherrer and Monod, 1960), and subsequently for whole body exercises such as cycling (Moritani, Nagata, DeVries, & Muro, 1981) running (Hughson, Orok, & Staudt, 1984), swimming (Wakayoshi et al., 1993) or rowing (Kendall, Smith, Fukuda, Dwyer, & Stout, 2011).

The *P-d* relationship can be characterised by two parameters. First, the asymptote of the hyperbola, which is referred to as critical power (CP) (Scherrer & Monod, 1960). Secondly, the curvature constant, which represents a finite amount of work that can be performed above CP (*W'*). The physiological mechanisms underpinning CP and *W'* are discussed below, but it is anticipated that CP is an aerobic parameter and demarcates the boundary between heavy and severe exercise domains. Thus, CP can be defined as the highest sustainable work rate, where physiological variables attain a steady state. With regards to *W'*, it has been traditionally linked to anaerobic energy production and it is sometimes

referred as anaerobic work capacity. The precise aetiology of  $W'$ , however, is still debated. Note that, where appropriate (e.g. running exercise), CP and  $W'$  are replaced by critical speed (CS) and  $D'$ , respectively. This section aims to summarise the  $P$ - $d$  relationship, with particular focus on  $W'$  as a potential measure of AnC.

### 1.6.2. Determination of critical power and $W'$

#### ***Several constant work-rate bouts to exhaustion***

The determination of CP and  $W'$  has typically required each athlete to complete a series of exercise bouts to exhaustion. In this traditional approach, time to exhaustion (TTE) is determined from 3 to 5 exercise bouts to exhaustion at constant work rates (CWR), lasting between 2 and 15 minutes. From these data, CP and  $W'$  can be estimated from linear and non-linear models (Figure 1.6, Gaesser, Carnevale, Garfinkel, Walter, & Womack, 1995; Jones, Vanhatalo, Burnley, Morton, & Poole, 2010; Hill, 1993; Jones, Vanhatalo, Burnley, Morton, & Poole, 2010).

The parameters of the  $P$ - $d$  relationship were originally determined using non-linear models (Scherrer & Monod, 1960). The power output and TTE from each CWR test to exhaustion is plotted in a scatter graph. The asymptote of the hyperbolic function represents CP (Panel A, Figure 1.6), and the area above CP stands for  $W'$ . Thus, at any given power output above CP, the duration that exercise can be tolerated (i.e. TTE) is determined as:

$$TTE = \frac{W'}{\text{Power output} - CP} \quad [9]$$

In equation [9], as TTE approaches zero, power output becomes infinite. In order to overcome this limitation, a third parameter,  $k$ , has been included (Bosquet, Duchene, Lecot, Dupont, & Leger, 2006; Chatagnon, Pouilly, Thomas, & Busso, 2005; Gaesser, Carnevale, Garfinkel, Walter, & Womack, 1995; Morton, 1996):

$$TTE = \left( \frac{W'}{\text{Power output} - CP} \right) + k \quad [10]$$

Where  $k$  accounts for peak power. Hence, with the inclusion of  $k$ , as TTE approaches zero, power output approaches peak power.  $k$  is substituted as:

$$TTE = \left( \frac{W'}{\text{Power output} - CP} \right) - \left( \frac{W'}{\text{Peak power output} - CP} \right) \quad [11]$$

The non-linear equation [9] can be mathematically transformed to a linear function by plotting power output from each CWR trial against the inverse of exercise duration ( $1 \times TTE^{-1}$ ). Here, the slope represents the  $W'$  and the y-intercept represents CP (Panel B, Figure 1.6).

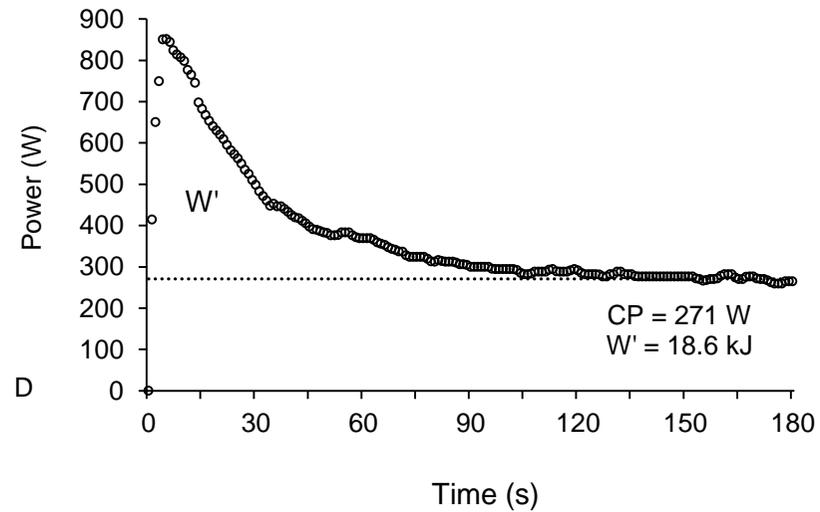
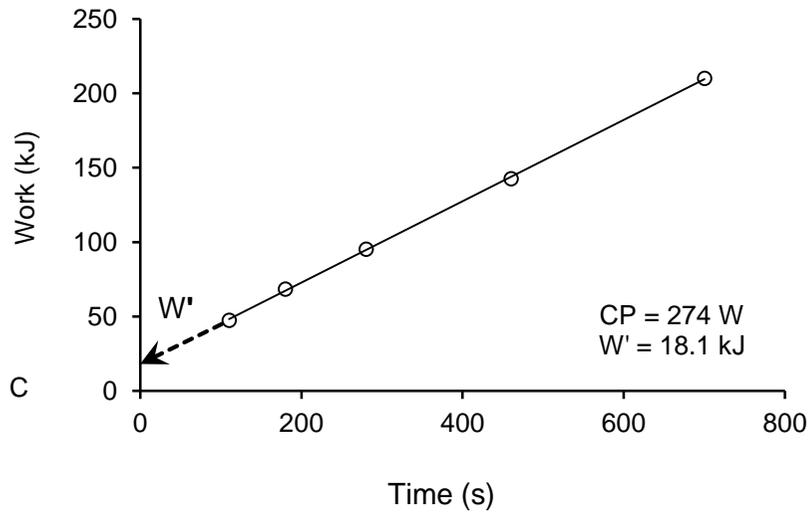
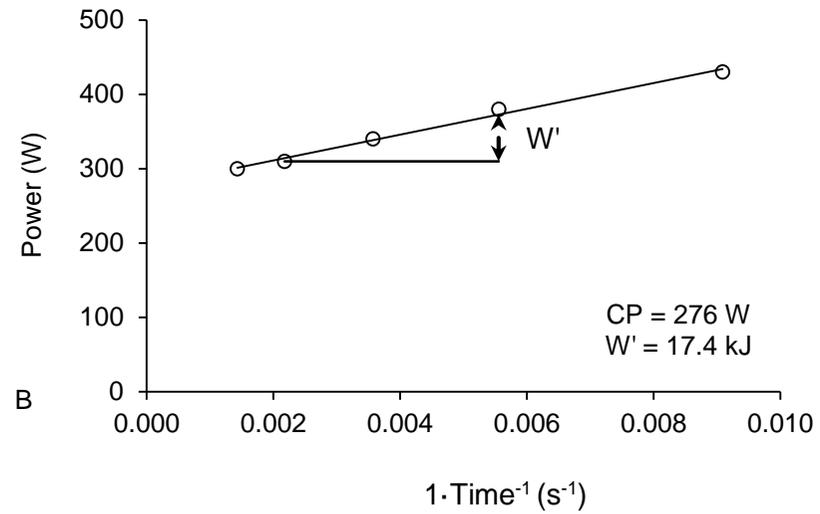
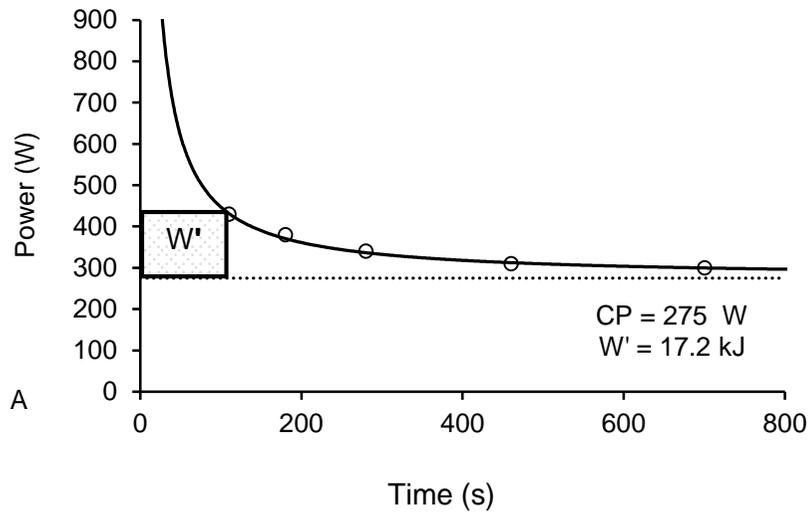
$$\text{Power Output} = CP + W' \times TTE^{-1} \quad [12]$$

Another linear function of the  $P-d$  relationship is obtained by plotting the work accomplished in each bout ( $Work = \text{power output} \times TTE$ ) against exercise duration (Panel C, Figure 1.6). The y-intercept of the linear relationship represents  $W'$ ; and the slope represents CP:

$$Work = W' + CP \times TTE \quad [13]$$

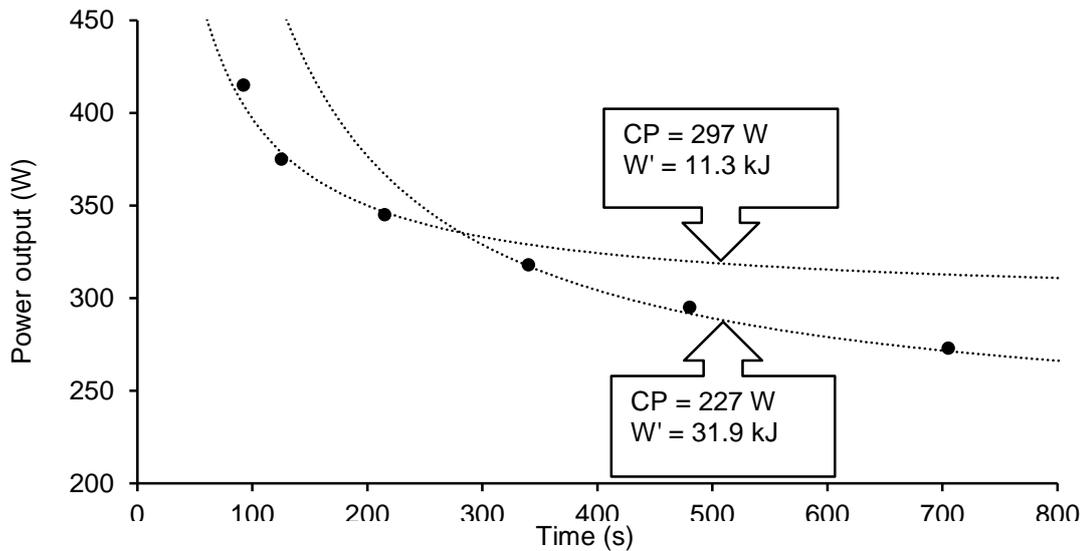
It should be noted that CP can also be determined using an exponential model (Bergstrom et al., 2014; Bosquet et al., 2006; Gaesser et al., 1995). However, the exponential model does not allow  $W'$  to be calculated, and it is therefore not discussed within this literature review.

Using any of the above models (equations [9-13]), CP and  $W'$  can be estimated with an excellent individual goodness of fit for all individuals ( $R^2 = 0.818-1.000$ ) (Bergstrom et al., 2014; Bull, Housh, Johnson, & Rana, 2008; Gaesser et al., 1995). Importantly, although equations [9, 12, 13] discussed above are mathematically equivalent, they typically result in relatively similar, albeit statistically different, estimations of CP and  $W'$  (Bergstrom et al., 2014; Bull, Housh, Johnson, & Perry, 2000; Bull, Housh, Johnson, & Rana, 2008; Gaesser et al., 1995). There have been two approaches to decide which mathematical model should be used to determine CP and  $W'$ . On the one hand, Hill (1993) proposed to use the model with the highest goodness-of-fit and/or lowest standard error. On the other hand, some authors have favoured the 3-parameter nonlinear model since it accounts for peak power when time approaches zero (equation [11]), as this can be seen as a more ecologically valid approach from a physiological standpoint (Bergstrom et al., 2014; Bosquet et al., 2006; Chatagnon et al., 2005; Gaesser et al., 1995). Then again, peak power is typically determined during sprint exercise, which can be affected by several factors (Sargeant, 2007; see Section 1.5.4). Unfortunately, there is no consensus on which mathematical modelling approach derived from several CWRs to exhaustion should be used to estimate CP and  $W'$ .



**Figure 1.6.** Individual determination of CP and  $W'$  using exhaustive exercise bouts at constant intensity (Panels A, B, C) and a 3-min all-out test (Panel D). Note that linear and non-linear models from several CWRs to exhaustion and the 3AO test produce similar estimates of CP and  $W'$ . See test for further details.

Further to the effects that mathematical modelling might have on CP and  $W'$ , the characteristics of the predictive trials have also been shown to affect the parameters of the  $P-d$  relationship. Specifically, predictive trials with longer duration (i.e. lower power output) result in lower CP and increased  $W'$  compared to predictive trials with relatively short duration (i.e. higher power output; Figure 1.7) (Bishop, Jenkins, & Howard, 1998; Busso, Gimenez, & Chatagnon, 2010; Jenkins, Krettek, & Bishop, 1998). Alternatively, CP and  $W'$  have been determined using closed-loop tests (i.e. time-trials), where participants were instructed to complete a target amount of work as quickly as possible (Black, Jones, Bailey, & Vanhatalo, 2015). The target work for the time trials was matched with that of a corresponding CWR to exhaustion trial. Despite being matched by work, mean power output was higher, and therefore TTE shorter, during time-trials. As a result, CP was 7% higher using time trials, whereas  $W'$  was not affected by the type of predictive trials. However, the percentage change in CP and  $W'$  determined from CWR compared to time trials were strongly correlated ( $r = -0.74$ ), suggesting that  $W'$  might have also been affected (Black et al., 2015). There have also been attempts to determine CP and  $W'$  in the field. Karsten et al. (2014b) determined CP and  $W'$  using three CWRs to exhaustion in a laboratory setting (90, 100 and 105% maximal aerobic power, which induced exhaustion in ~12, 4 and 2.5 min) and three time trials in a field, where the target was to ride as fast as possible for 12, 7 and 3 min. The authors reported that estimations of CP from the laboratory and the field were very close (~0.2 W), but field estimations of  $W'$  were ~5 kJ higher than in laboratory tests. Since BLa was also higher in field tests, the authors speculated that the acceleration phase at the beginning of the field trials resulted in a higher utilization of type II muscle fibres, which is a proposed determinant of  $W'$  (see below). In a follow-up study, Karsten et al. (2015) reported that CP determined from field tests was a valid and reliable measure of CP, but the estimation of  $W'$  using field tests could not be considered valid or reliable. In summary, CP and  $W'$  can be determined from mathematical modelling of power and TTE determined from 3-5 exercise bouts to exhaustion at various CWRs. Unfortunately, both the mathematical model chosen to estimate CP and  $W'$  and the characteristics of the predictive trials seem to affect the parameters of the  $P-d$  relationship.



**Figure 1.7.** Effect of the intensity (and duration) of predictive constant-load bouts to exhaustion on estimations of CP and  $W'$ . Short trials (< 300 s) resulted in greater critical power and lower  $W'$  estimates than longer trials (> 300s), and vice-versa. Adapted from Busso et al. (2010).

### ***The 3-min all-out test***

Beyond the effect that the mathematical modelling and predictive trials have on CP and  $W'$ , the traditional approach to determine CP and  $W'$  requires multiple visits to the laboratory to perform several CWR tests to exhaustion, therefore being a time- and effort-consuming protocol, which represents a practical drawback in the determination of the parameters of the  $P$ - $d$  relationship. As a consequence CP and  $W'$  are not standard measures in exercise physiology laboratories despite their relevance and applicability (Rossiter, 2011). In contrast to the multiple day test to determine the parameters of the  $P$ - $d$  relationship, all-out exercises have been used as a means of assessing AnC within a single laboratory visit (see WAnT, Section 1.5.4). However, Calbet et al. (1997, 2003) and Beneke et al., (2002) suggested that the traditional duration of 30 s may not be enough to deplete anaerobic energy stores. Indeed, power-output is still decreasing at the end of a WAnT. Dekerle et al. (2006) extended the duration of all-out efforts up to 90 s, but power output was still decreasing at the end of the test, suggesting that the end-test power output was greater than CP and, therefore, AnC was not fully depleted. Burnley et al. (2006) further extended the duration of all-out exercise to 180 s, and observed that the decrease in power output stabilised in the last 30 s of the test (Panel D, Figure 1.7). In this 3-min all-out (3AO) test, the end-test power output represented the upper limit at which physiological variables (i.e.  $\dot{V}O_2$  and BLa) reached a steady state (Burnley et al., 2006). In a follow-up study, Vanhatalo, Doust, and Burnley (2007) reported close agreement between CP and the end-power of the

3AO test ( $r = 0.99$ ; standard error of estimate = 6.4 W). Moreover, the work performed above the end-power during a 3AO test was similar to  $W'$  ( $r = 0.84$ ; standard error of estimate = 2.6 kJ). The 3AO test has since become a popular alternative, more practical approach to determine CP and  $W'$  (Dekerle, Vanhatalo, & Burnley, 2008).

The original protocol (Burnley et al., 2006; Vanhatalo et al., 2007) consists of a ramp incremental test to determine preferred cadence, gas exchange threshold (GET), and  $\dot{V}O_{2max}$ . In a subsequent trial, the cycle ergometer is set in the isotonic mode, where power output is a function of cadence. The alpha factor (i.e. the power output) is set so that cycling at the participant's preferred cadence results in a power output of 50% of the difference between the GET and maximal power output (i.e. 50% $\Delta$ ).

The original protocol of the 3AO test developed by Burnley et al. (2006) and Vanhatalo et al. (2007) has been modified, chiefly because the two separate trials required make it a less attractive test from a practical perspective (e.g. Bergstrom et al. 2012; Clark, Murray, & Pettitt, 2013; Constantini, Sabapathy, & Cross, 2014; Karsten et al., 2013). Clark et al. (2013), for instance, determined the alpha factor of the 3AO test as a percentage of body mass. Specifically, the authors used 3, 4, or 5% body mass in recreationally active, aerobic/anaerobic athletes (i.e., teamsports), and endurance athletes, respectively. There were no differences in either CP or  $W'$  determined from the 3AO test with the traditional approach to determine the alpha factor, and an alpha factor dependent on body mass. Mechanically braked ergometers (e.g. Monark, Varberg, Sweden) may provide an accessible alternative to estimate CP and  $W'$  from a single exercise bout. Bergstrom et al. (2012) determined CP and  $W'$  using the modelling of several exercise bouts to exhaustion at CWR, the traditional 3AO test, and a modified 3AO test. The modified 3AO test consisted of a 3AO effort on a mechanically braked ergometer with resistances equivalent to 3.5% and 4.5% of body weight. Using the latter resistance,  $W'$  was not different to the other procedures; however, a resistance equivalent to 3.5% of body weight resulted in a smaller  $W'$  compared to that determined from the conventional 3AO test (Bergstrom et al., 2012). These data suggest that the 3AO test might be determined within a single laboratory visit. Lastly, it has been hypothesised that the mode of contraction should not affect CP and  $W'$  estimates. Karsten et al. (2013) investigated whether a 3-min all-out isokinetic test performed on an electronically-braked cycle ergometer (Schober Rad Messtechnik, Jülich, Germany) at participants preferred cadence, resulted in valid estimates of CP and  $W'$ . Both parameters of the  $P-d$  relationship derived from the

isokinetic 3AO test were different from estimates derived from CWR tests to exhaustion. Specifically, the isokinetic mode appeared to overestimate CP and underestimate  $W'$ . Alternatively, Constantini, Sabapathy, and Cross (2014) hypothesised that the ramp incremental test to exhaustion and the 3AO test could be determined in a single trial. The authors reported that a 3AO test performed 20 min after the conclusion of the exhaustive ramp resulted in a CP and  $W'$  similar to that obtained in a separate trial (standard error of estimate of 5 W and 1.81 kJ for CP and  $W'$ , respectively). Overall, although these alternative approaches to estimate CP and  $W'$  from a 3AO are promising from a practical perspective, the preferred protocol is still that originally outlined by Burnley et al. (2006).

There are a number of studies looking at the validity of the 3AO test to assess CP and  $W'$ . On the one hand, close agreement between the 3AO test and the traditional protocol (modelling several CWRs to exhaustion) has been reported (Bergstrom et al., 2013b; Vanhatalo et al., 2007, 2008a). Moreover, the 3AO test has been repeatedly shown to be a robust test (Simpson et al., 2015; Vanhatalo, Doust, & Burnley, 2008b). However, all the above studies were conducted using adults. Barker, Bond, Toman, Williams, and Armstrong (2012) observed no differences in either CP or  $W'$  estimates derived from the traditional CWR approach and the 3AO test in a group of 14-15 year old adolescents. However, both parameters of the  $P-d$  relationship derived from the 3AO test had large typical errors compared to the traditional CWR approach, and the authors deemed that the 3AO test was not a valid test for this population. It is possible to account for this result based on the reliability of CP and  $W'$  derived from the 3AO test. Using the traditional approach, repeated estimates of CP and  $W'$  differ by ~5% and ~15%, respectively (Hill, 1993). In contrast, the test-retest variability of the parameters of the  $P-d$  relationship determined using the 3AO test are higher than those from the conventional approach for both CP (7%) and, in particular, for  $W'$  (28%) (Johnson, Sexton, Placek, Murray, & Pettitt, 2011).

The parameters of the  $P-d$  relationship derived from the 3AO test have been correlated with endurance performance. Black et al. (2014) observed that performance in a 16.1 km cycling time-trial was strongly correlated with CP ( $r = 0.83$ ) and the total work done during the 3AO test ( $r = 0.86$ ); which is consistent with previous research on CP as a predictor of performance (see Section 1.6.2). Further evidence to support the validity of the 3AO test can be found in the sensitivity of the test to detect changes induced by training (Vanhatalo et al., 2008a). These authors reported an increase in CP after a 4 week period of high-intensity training, irrespective of the approach used to determine CP. Moreover, the increased

in CP using the 3AO test and the traditional approach was strongly correlated ( $r = 0.77$ ) (Vanhatalo et al., 2008a).

Whilst the original 3AO test was designed to assess CP and  $W'$  during cycle-ergometer exercise (Burnley et al., 2006; Vanhatalo et al., 2007, 2008b), similar all-out protocols have been developed for other whole-body exercises, including running (Broxterman, Ade, Poole, Harms, & Barstow, 2013; Pettitt, Jamnick, & Clark, 2012), rowing (Cheng, Yang, Lin, Lee, & Wang, 2012) and swimming (Kalva-Filho et al., 2015). The running 3AO test requires the participants to run as fast as possible for 3 minutes. Peak speed is attained at the beginning of the test, and progressively decreases until a plateau (i.e. CS) occurs in the last 20 s (Broxterman et al., 2013) or 30 s (Pettitt et al., 2012) of the test. The distance covered above CS is defined as  $D'$ . Whereas estimates of CS derived from the running 3AO test and the traditional approach seem to be similar,  $D'$  appears to be underestimated if determined from the 3AO test (Broxterman et al., 2013; Pettitt et al., 2012). The extra energy required to accelerate to maximal speeds and overcome air resistance have been suggested a plausible cause of the underestimation of  $D'$  using the 3AO test (Broxterman et al., 2013). Indeed,  $D'$  determined in field tests using the traditional approach is also underestimated compared to that determined in the laboratory (Galbraith, Hopker, Lelliott, Diddams, & Passfield, 2014). With regards to the rowing and swimming 3AO test, they both follow the same principles described above for cycling and running tests (i.e. maximal effort, avoiding pacing, during 3-min). It is important to note that the swimming 3AO test proposed by Kalva-Filho et al. (2015) is performed in tethered swimming to avoid influences caused by turns. Thus, instead of power output, velocity, or stroke rate, the authors used an elastic cord with a dynamometer cell to connect the swimmer and the starting block to continuously measure force during the test. The rowing and swimming 3AO tests produce valid and reliable measures of CP (Cheng et al., 2012; Kalva-Filho et al., 2015), although  $W'$  derived from the rowing 3AO test overestimated the traditionally determined  $W'$  (Cheng et al., 2012), and it did not correlate with the work performed above the maximal lactate steady state (Kalva-Filho et al., 2015).

Similar all-out approaches have been used to determine critical torque during maximal isometric contractions (Burnley, Vanhatalo, Fulford, & Jones, 2010; Burnley, 2009; Kellawan & Tschakovsky, 2014). Burnley (2009) observed that force of maximal isometric contractions (3 s contraction, 2 s rest) progressively decreased during a 5-min all-out test from an initial value of 97% of maximal voluntary

contraction at the start of the test until it stabilised at ~29% of maximal voluntary contraction in the last 30 s. Similarly, using a 600 s all-out test, critical force was determined as the average force during the last 30 s of the test (Kellawan & Tschakovsky, 2014).

In summary, the above studies demonstrate that the parameters of the  $P$ - $d$  relationship can now be determined from all-out tests in a variety of exercises. In these approaches, the work-rate (power output, speed, force, or torque) reaches its peak at the beginning of the test, and progressively decreases until it stabilises in the last portion of the test. Critical power is determined as the average power output (or speed) once stabilised, and  $W'$  (or  $D'$ ) is determined as the work completed above CP (or CS).

### **1.6.3. The components of the power output-duration relationship**

#### ***Critical power***

The asymptote of the  $P$ - $d$  relationship, CP, represents the boundary between the heavy and severe exercise domains. Physiological variables (i.e.  $BLa$ ,  $\dot{V}O_2$ ,  $pH$ ,  $[H^+]$ ,  $[PCr]$ ,  $[Pi]$ ) attain a steady state below CP, but at intensities above CP these variables continue to increase or decrease until a critical value is attained, and exhaustion occurs (Jones et al., 2008; Poole et al., 1988). Although mechanistically different, CP has been suggested to occur at a similar metabolic rate to other physiological thresholds, such as the maximal lactate steady state, the respiratory compensation point, the electromyographic threshold or the deoxyhemoglobin ( $[HHb]$ ) breakpoint or the ventilatory threshold (Bergstrom et al., 2013; Dekerle, Baron, Dupont, Vanvelcenaher, & Pelayo, 2003; Greco et al., 2012; Keir et al., 2015; Pringle & Jones, 2002; Pringle et al., 2003; Smith & Jones, 2001; Vautier, Vandewalle, Arabi, & Monod, 1995; Wakayoshi et al., 1993). Although frequently reported as a mechanical intensity (i.e. power output or speed), CP is best described as a metabolic rate (Barker, Poole, Noble, & Barstow, 2006; Keir et al., 2015). Given that CP is an index of aerobic energy production, any alteration in oxygen delivery has been hypothesised to have a profound effect on CP. Indeed, CP is increased by hyperoxia (Vanhatalo, Fulford, DiMenna, & Jones, 2010), decreased by hypoxia (Dekerle, Mucci, & Carter, 2012; Simpson et al., 2015; Valli et al., 2011), and further decreased to a theoretically, albeit physiologically impossible, negative value by blood flow occlusion (Broxterman et al., 2015). Lastly, the practical utility of CP has long been recognised. Critical power correlates with traditional markers of aerobic fitness, such as the gas exchange threshold (GET), the lactate threshold (LT), the maximal lactate steady state, and the

time constant ( $\tau$ ) of the primary phase and mean response time of  $\dot{V}O_2$  kinetics (Black et al., 2015; Brickley et al., 2007; Brickley, Doust, & Williams, 2002; Dekerle et al., 2003; Murgatroyd, Ferguson, Ward, Whipp, & Rossiter, 2011; Poole et al., 1988). Moreover, evidence suggests that CP is a strong predictor of performance in endurance events (Black et al., 2014; Bosquet et al., 2006; Greco et al., 2012; Kendall et al., 2011; Smith et al., 1999). Another important practical application of CP is to evaluate training interventions, since CP seems to increase after a period of endurance training (Greco, Caritá, Dekerle, & Denadai, 2012; Jenkins & Quigley, 1993; Vanhatalo et al., 2008a). Finally, CP has been suggested to be a useful tool to prescribe the intensity of exercise. Traditionally, exercise has been prescribed as a percentage of  $\dot{V}O_{2max}$ . Instead, CP has been favoured as a tool to prescribe or define the intensity of exercise (Clark et al. 2013; Rossiter, 2011; Whipp et al., 2005). Given the aerobic nature of CP, the reader is referred to previous review articles on the underpinning physiology and practical applications of CP (Dekerle et al., 2008; Hill, 1993; Jones, Vanhatalo, Burnley, Morton, & Poole, 2010; Morton, 2006; Vanhatalo, Jones, & Burnley, 2011a, Walsh, 2000).

### ***Curvature constant ( $W'$ )***

Since CP is representative the highest intensity sustainable from the aerobic energy system,  $W'$  has been traditionally linked with non-oxidative energy production. Exercising at intensities above CP, where  $W'$  is progressively depleted, requires a constant supply from anaerobic energy pathways (Jones et al., 2008c). Indeed, some authors refer to  $W'$  as anaerobic work capacity (e.g. Heubert et al., 2005; Hill, 1993; Moritani et al., 1981; Morton, 2006; Scherrer & Monod, 1960; Vandewalle et al., 1987). Here, however, the nomenclature of  $W'$  is preferred for consistency with current literature (Dekerle et al., 2008).

Supporting the view that  $W'$  represents AnC, Miura et al. (2002) and Chatagnon et al. (2005) reported strong correlations ( $0.63 \leq r \leq 0.76$ ), between  $W'$  and AOD in cycling. Leclair et al. (2010) observed a significant correlation between AOD and  $W'$  in children ( $r = 0.56$ ), but not adults ( $r = 0.04$ ). Moreover, the relationship between AOD and  $D'$  has shown conflicting results. Bosquet et al. (2007) observed moderate correlations ( $0.48 \leq r \leq 0.57$ ) between AOD and  $D'$  determined from the two- and three-parameter models (equations [8, 10, 11]); Zagatto et al. (2013) reported weak correlations ( $r \leq 0.25$ ) between AOD and  $D'$  irrespective of the modelling approach. It is difficult to explain these differences. It is anticipated that factors other than AnC might underpin  $W'$ . For example, Murgatroyd et al. (2011)

suggested a link between  $W'$  and the amplitude of the slow component of  $\dot{V}O_2$  kinetics (further discussed below). However, the amplitude of the slow component of  $\dot{V}O_2$  kinetics is larger in cycling than in running (Carter et al., 2000; Hill, Halcomb, & Stevens, 2003). Thus, differences in  $\dot{V}O_2$  kinetics between running and cycling might, at least in part, explain the conflicting results in regards to the correlation between AOD and  $W'$ .

Critical power demarcates the heavy and severe domains of exercise, which in turn have markedly different  $\dot{V}O_2$  kinetics. Indeed,  $\dot{V}O_2$  in the severe exercise domain does not attain a steady state, but increases inexorably until  $\dot{V}O_{2max}$  is reached, and soon afterwards exercise is terminated. Murgatroyd et al. (2011) investigated whether the parameters of  $\dot{V}O_2$  kinetics might determine CP and  $W'$ . The key findings of the study were that CP was strongly correlated with the primary phase  $\tau$  of  $\dot{V}O_2$  kinetics and  $\dot{V}O_{2max}$  ( $r = -0.95$  and  $r = 0.89$ , respectively), whereas  $W'$  was strongly correlated with the amplitude of the slow component of  $\dot{V}O_2$  ( $r = 0.84$ ). The  $\tau$  of the primary phase of  $\dot{V}O_2$  and  $\dot{V}O_{2max}$  are, like CP, measures of the aerobic energy system. However, the aetiology of the slow component of  $\dot{V}O_2$  is not yet completely understood. In brief, the slow component of  $\dot{V}O_2$  represents a decrease in efficiency, and has been linked to fatigue of type I muscle fibres and/or additional recruitment of type II (see Section 1.7.5 for a more thorough discussion on the slow component of  $\dot{V}O_2$ ). Whether exercise above or below CP results in different recruitment patterns in humans, however, is difficult to measure. Pringle and Jones (2002) failed to detect differences in the integrated electromyographic signal during exercise below and above CP. Moreover, Bergstrom et al. (2013c) did not observe an increase in the amplitude of electromyography signal during exercise at CP, as would be expected with an additional recruitment of motor units during (Bergstrom et al., 2013c). Whilst these data suggest no differences in muscle activation during exercise below vs. above CP, or at CP, the high intersubject variability observed in electromyography measurements (Pringle & Jones, 2002) and the different approaches to determine CP might have confounded the results.

The hyperbolic  $P-d$  relationship seems to be an intrinsic characteristic of skeletal muscle, at least in mammalian species (Billat, Mouisel, Roblot, & Melki, 2005; Lauderdale & Hinchcliff, 1999; Poole & Erickson, 2011). Using animal models, the recruitment patterns during exercise below vs. above CP (or CS) have been investigated. Copp, Hirai, Musch, and Poole (2010) observed that blood flow in rats exercising at a work-rate 15% above CP was consistently higher than at intensities 15% below CS.

Moreover, the authors noted that the increase in blood flow below vs. above CS was disproportionately high in muscles with a high percent of type IIb/d/x fibres. The authors interpreted these data as an additional recruitment of the less-efficient types II above CS, which ultimately led to fatigue. Interestingly, it has recently been suggested that CP/CS might represent a threshold of the recruitment of type II fibres (James & Green, 2012; Skiba, Fulford, Clarke, Vanhatalo, & Jones, 2015).

The advancement in techniques to assess the exercising muscle has provided further insight into the mechanisms underpinning CP and  $W'$ . Jones et al. (2008c) interrogated the quadriceps muscle during single-leg knee-extension exercise at intensities ~10% below and above CP using  $^{31}\text{P}$ -MRS. At intensities above CP, metabolites derived from anaerobic energy production such as  $\text{P}_i$  (equation [1]), PCr (equation [2]), and  $\text{H}^+$  (as denoted by pH; equations [1] and [6]) did not reach a steady-state but continued to decrease ([PCr] and pH) or increase (and [ $\text{P}_i$ ]) until a critical threshold was reached, concomitant to  $W'$  depletion, and exercise was subsequently terminated. Subsequent studies have demonstrated that the rate of accumulation/depletion of these metabolites is proportional to the rate of  $W'$  depletion (Chidnok et al., 2013b; Vanhatalo et al., 2010). During exercise, however, exercise is not performed at a constant intensity, but typically alternates between intensities above CP and below CP (e.g. Skiba, Clarke, Vanhatalo, & Jones, 2014; Figure 1.9). In these instances, it appears that the average intensity dictates metabolic responses, as measured by  $\dot{V}\text{O}_2$  (Brickley et al., 2007). Nonetheless, it has also been shown that for a duty cycle of 1:2, when average power was held constant, increasing the duration of the exercise from 10 s to 90 s resulted in different metabolic responses, and increased exercise tolerance (Turner et al., 2006). Metabolic responses during intermittent exercise above and below CP need to be further investigated, though there is some evidence that at the point of exhaustion, metabolic disturbances within the exercising muscle reach a consistent critical threshold, preventing exercise from continuing irrespective of the intermittent protocol (Chidnok et al., 2013b).

Throughout this literature review, a consistent approach to determine the validity of tests intending to measure AnC has been derived from the assumption that aerobic metabolism is sensitive to changes in oxygen delivery, but AnC remains constant. Within the  $P$ - $d$  model, if CP and  $W'$  are measures of aerobic and anaerobic energy production, respectively, the magnitude of CP has been hypothesised to be affected by changes in oxygen delivery, but  $W'$  should remain unaffected. However, whereas CP

has responded as hypothesised in studies where oxygen delivery has been modified experimentally, growing evidence suggests that  $W'$  is also sensitive to changes in oxygen delivery. Specifically,  $W'$  decreases in hyperoxia (Vanhatalo, et al., 2010), increases (Simpson et al., 2015) or remains unchanged (Dekerle et al., 2012) in mild hypoxia, and was estimated to have a mathematically, albeit physiologically impossible, negative value during blood flow occlusion (Broxterman et al., 2015a). Furthermore, these studies report a strong negative correlation ( $-0.88 \leq r \leq -0.72$ ) between percentage change in CP under experimental (hypoxia or hyperoxia) conditions compared to control, and the percentage change in  $W'$  under experimental compared to control (Dekerle et al., 2012; Simpson et al., 2015; Vanhatalo, et al., 2010). These studies, therefore, suggest a possible interaction between CP and  $W'$ , such that  $W'$  trends to increase in participants whose CP is most severely reduced by hypoxia exposure, and to decrease in subjects whose CP is most augmented in hyperoxia.

Studies looking at the effects of prior high-intensity exercise on the parameters of the  $P-d$  relationship might also offer an insight of the underpinning mechanisms of  $W'$ . Consistent with the traditional view of  $W'$  as a measure of AnC, prior high-intensity exercise decreases  $W'$  in a subsequent exercise (Burnley, Davison, & Baker, 2011; Coats, Rossiter, Day, Miura, Fukuba, & Whipp, 2003; Ferguson et al., 2010; Heubert et al., 2005; Johnson, Mills, Brown, & Sharpe, 2014; Skiba, Chidnok, Vanhatalo, & Jones, 2012; Vanhatalo & Jones, 2009). Overall, these studies suggest that prior severe or all-out exercise, compromises the tolerance to subsequent exercise at intensities within the severe exercise domain. Furthermore, this decrease in exercise tolerance after high-intensity exercise seems to be mediated exclusively by a decrease in  $W'$ , since CP has been consistently shown to remain unaffected (Burnley et al., 2011; Coats et al., 2003; Ferguson et al., 2010; Heubert et al., 2005; Johnson et al., 2014; Skiba et al., 2012; Vanhatalo & Jones, 2009). However, although  $W'$  is rapidly recovered and ~15 min has been shown to allow an almost complete recovery of  $W'$  (Ferguson et al., 2010), the recovery of  $W'$  does not mimic that of markers of anaerobic energy production such as [PCr] (Skiba et al., 2015) or BLa (Ferguson et al., 2010). A recent study investigated whether prior severe intensity exercise reduces exercise tolerance due to a reduced  $W'$  or due to the accumulation of fatigue-inducing metabolites. Johnson et al. (2014) determined cycling CP and  $W'$  four minutes after  $8 \times 1$ -min arm cranking exercise bouts ( $1.5$ – $2.0$   $W \cdot kg^{-1}$ ). Compared to a control condition (i.e. no prior exercise), CP was not affected, but  $W'$  was reduced by ~31% (17.3 vs. 11.8 kJ). Given that, at the point of exhaustion (i.e.  $W'$  depletion), [PCr], [ADP] or [Pi] have reached a critical, consistent value irrespective pacing

strategies and exercise conditions (Chidnok et al., 2013b, 2013c; Vanhatalo et al., 2010), the authors suggested that prior upper-body exercise increased the concentration of fatigue-inducing metabolites, so a critical threshold in the accumulation of metabolites was reached sooner (Johnson et al., 2014).

The hypothesis that  $W'$  might be underpinned by fatigue, instead to represent exclusively a measure of AnC, has been further strengthened by two recent studies (Broxterman et al., 2015b; Nicolò, Bazzucchi, Felici, Patrizio, & Sacchetti, 2015). Nicolò et al. (2015) observed a correlation between power output and the muscle fibre conduction velocity ( $r = 0.87$ ), which is affected by changes on muscle membrane properties due to changes in potassium concentration, and therefore is considered a marker of peripheral fatigue. Broxterman et al. (2015b) also reported that the magnitude of  $W'$  was significantly related to the magnitude of global ( $r = 0.91$ ) and peripheral ( $r = 0.83$ ) fatigue. Broxterman et al. (2015b) suggested that  $W'$  might be related to the magnitude of fatigue accrued during exercise. This hypothesis is compatible with the large interindividual, but relatively small intraindividual variability in the muscle metabolites concentration at the point of exhaustion (see discussion above).

To summarise,  $W'$  has traditionally been considered to be a measure of AnC, and indeed several authors refer to it as anaerobic work capacity. However, recent evidence suggests that the precise aetiology of  $W'$  is complex and multifactorial. Recently, in addition to anaerobic energy production, the magnitude of  $W'$  has been suggested to be determined by the attainment of a critical threshold of fatigue-inducing metabolites and/or the magnitude of fatigue accrued during severe exercise.

#### **1.6.4. Assumptions of the power output-duration relationship model**

The parameters of the  $P-d$  relationship, CP and  $W'$ , have been suggested to broadly represent aerobic and anaerobic energy production, respectively. In turn, this bioenergetics model relies on a number of assumptions (Heck et al., 2003; Jones et al., 2010; Morton, 2006; Walsh, 2000), that are discussed below.

#### ***Exercise tolerance is limited by energy supply***

Bioenergetics for working muscles cannot be explained as simply as two separate, independent compartments, represented by CP and  $W'$  respectively. In turn, alternative approaches have been proposed that incorporate other physiological parameters into the  $P-d$  relationship (James & Green, 2012; Morton, 2006). Wilkie's correction, for instance, takes into consideration the effect of  $\dot{V}O_2$  kinetics

during a CWR exercise bout on  $W'$  (Wilkie, 1980). Unfortunately, alternative approaches have not gained popularity, likely because they still rely on additional assumptions (Morton, 2006). Despite an oversimplified view of bioenergetics, the CP- $W'$  model successfully predicts times to exhaustion and is still extensively used (Chidnok et al., 2013b, 2013c; Vanhatalo et al., 2010).

### ***Aerobic energy supply is instantaneous and limited by rate***

Mathematically, the  $P$ - $d$  relationship assumes that there is no exhaustion during exercise at any intensity below CP. Fatigue does indeed develop during prolonged exercise at intensities below CP, but for different reasons (glycogen depletion, hyperthermia, dehydration, psychological factors, etc.) to those observed at intensities above CP (Abbiss & Laursen, 2005; Rossiter, 2011, Walsh, 2010). Pinot and Grappe (2011) reported field power output data from elite cyclists over a competitive season. The authors developed the record power profile test, which determines the relationship between mean power output and time over 13 timespans (between 1 s to 4 hours). Unsurprisingly, mean power decreases as the duration increases (akin to the  $P$ - $d$  relationship). However, power output did not stabilize as the duration increased, but continued to decrease. The authors identified three phases in the decrease of power output over time. Initially, between 1 s to 5 min, mean power output decreases very rapidly, from ~1250 W to ~430 W, respectively. Between 10 min to 60 min, the decrease continues but reduces its rate, from ~400 W to 335 W, respectively. Consistent with the  $P$ - $d$  relationship, for times in excess of 60 min up to 4 hours, power output approaches the asymptote of the hyperbola, and therefore the decrease is lower: from ~295 W in 2 hours to ~270 in 4 hours. The data reported by Pinot and Grappe (2011) suggests that the  $P$ - $d$  might not be purely hyperbolic (i.e. attain a hyperbola where exercise is sustained without fatigue). Moreover, when required to exercise to exhaustion at the intensity corresponding to CP, there seems to be a large interindividual differences in TTEs, ranging from ~20 min to ~60 min (Bergstrom et al., 2014; Brickley, Doust, & Williams, 2002; McMellan & Cheung 1992; Housh et al., 1989; Hill et al. 1991, Hill 1993). More recently, using the 3AO test, exercise was only maintained for 12 – 15 min at CP when calculated from the 3AO, suggesting that this approach perhaps overestimates CP (Bergstrom et al., 2013a, 2013b; McClave, LeBlanc, & Hawkins, 2011). Irrespective of the protocol used to determine CP, sustained exercise fractionally below or at CP cannot be sustained for a long period of time without exhaustion (> 60 min).

The CP- $W'$  model assumes that aerobic metabolism supplies instantaneously the energy required to exercise below CP, which in turn means that  $\dot{V}O_2$  kinetics are infinitely fast (i.e.  $\tau = 0$  s). However, as discussed below, at the onset of exercise aerobic metabolism exhibits a slowed response relying on anaerobic energy sources. In practical terms, assuming a  $\tau = 0$  may result in earlier exhaustion than that predicted from the  $P$ - $d$  relationship, in particular during CWR exercise of short duration (< 3 min). Another implication of this assumption is that  $W'$  would only be used when work-rate exceeds CP. However, since  $\dot{V}O_2$  rises exponentially in response to changes in metabolic demand, exhaustion might occur earlier than what would be predicted. This is, likely, because some  $W'$  is tagged at the onset of exercise to account for the anaerobic energy production at the onset of the (i.e. the oxygen deficit). There have been some attempts to account for this limitation by introducing a  $\tau$  that accounts for the  $\dot{V}O_2$  kinetics (Busso & Chatagnon, 2006; Chatagnon & Busso, 2006; Wilkie, 1980). Moreover, although this underestimation of  $W'$  is sometimes deliberately ignored because of its relatively small magnitude (Heubert et al., 2005), it may be important, particularly in the in shorter exercise bouts (Busso et al. 2010; Morton, 2006).

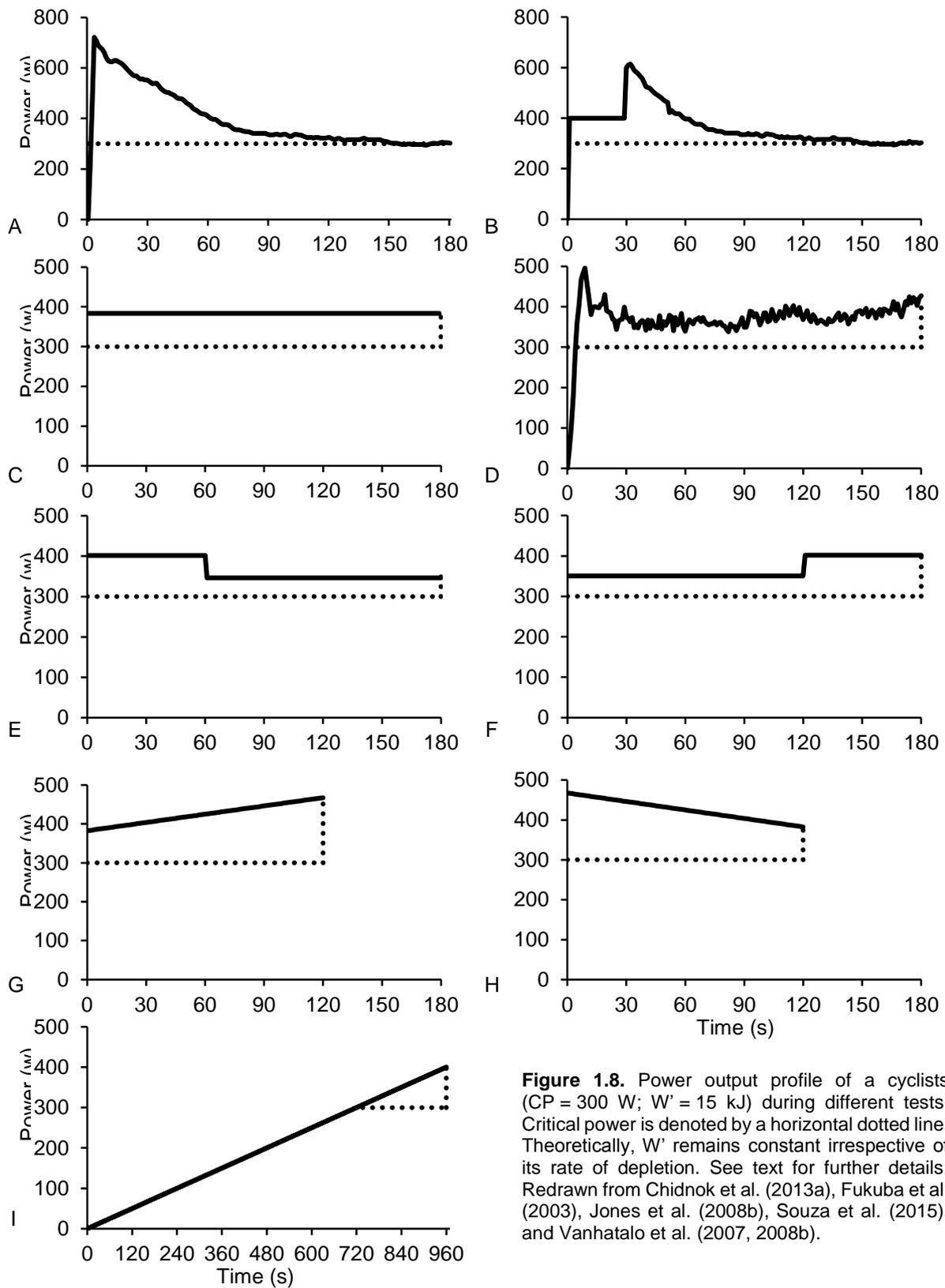
### ***$W'$ remains constant, irrespective of the rate at which it is used***

The curvature constant  $W'$  represents a fixed amount of work which becomes depleted during exercise above CP (Hill, 1993; Morton, 2006). Exhaustion during supra-CP exercise intensities is predicted to occur at  $W'$  depletion, regardless of the rate at which  $W'$  has been depleted (Figure 1.8). It has been investigated whether fatigue and  $W'$  were indeed concomitant events (Chidnok et al., 2013; Fukuba et al., 2003; Jones et al., 2008; Vanhatalo et al., 2008). Vanhatalo et al. (2008) determined  $W'$  from the 3-min all-out test (Panel A, Figure 1.8). Subsequently, they required their subjects to exercise for 30 s at a CWR at 100% and 130% of the power output attained during a maximal ramp test immediately followed by 2.5 min of all-out isotonic effort (Panel B, Figure 1.8). The work performed above CP in a modified 3AO tests (0.5 min at CWR + 2.5 min all-out effort) was not different from  $W'$  (Vanhatalo et al., 2008). Chidnok et al. (2013) recently reported that the magnitude of  $W'$ , was again consistent with the work performed above CP during a 3AO test, a CWR exercise to exhaustion (predicted TTE of ~3 min; Panel C, Figure 1.8), a ramp test to exhaustion (Panel I, Figure 1.8), and a 3-min time-trial (Panel D, Figure 1.8). Moreover, Souza et al. (2015) recently reported that, in a group of subjects with similar aerobic fitness ( $\dot{V}O_{2max}$ , LT and CP), those with larger  $W'$  were able to continue exercising longer in a

ramp test to exhaustion (i.e. higher peak power output) than those with a low  $W'$ . In ramp incremental tests, TTE cannot be calculated using the standard formulae of equations [9-13]. Morton (2011) derived the two parameter model (equation [9]), so TTE can be estimated during ramp incremental tests from the CP- $W'$  model:

$$TTE = \frac{CP}{s} + \sqrt{\frac{2 W'}{s}} \quad [14]$$

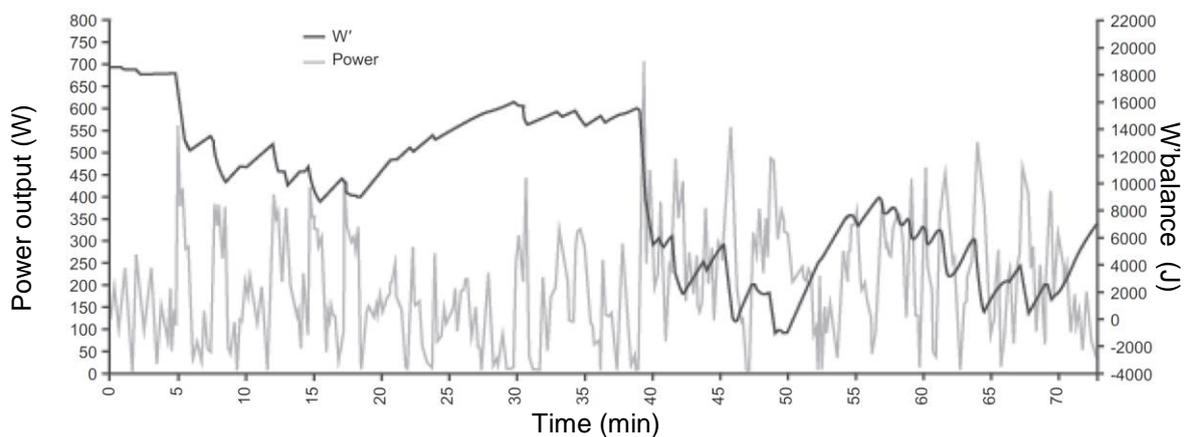
where  $s$  is the slope of the ramp test (e.g.  $0.5 \text{ W} \cdot \text{s}^{-1}$ ). Fukuba, Miura, Endo, Kan, Yanagawa, and Whipp (2003) compared the work completed above CP from two approaches. The “DOWN” protocol required participants to exercise at a CWR corresponding to 134% CP for a period of time predicted to deplete 50%  $W'$ , and then the resistance was abruptly reduced to 117% CP and maintained to exhaustion (Panel E, Figure 1.8). In the “UP” protocol, the initial work-rate was 117% CP until  $W'$  was reduced to 50% of its magnitude, and then increased to 134% CP and sustained to exhaustion (Panel F, Figure 1.8). Both protocols resulted in a similar  $W'$  (Fukuba et al., 2003). Using a similar approach, however, Jones, Wilkerson, Vanhatalo, & Burnley (2008b) observed that work completed above CP was affected by pacing strategies. These authors reported no differences in the  $W'$  during either an ‘even’ pacing strategy test, which consisted of an exercise bout to exhaustion at a CWR predicted to fatigue participants after 2 min, or a ‘slow-start’ pacing strategy test, whereby the power output was initially 10% lower than that of the even strategy, and then progressively increased with time such that it was 10% above the power output of the even strategy after 2 min (Panel G, Figure 1.8). However, during the ‘fast’ pacing strategy test, which was the inverse of the ‘slow’ pacing strategy test (Panel H, Figure 1.8), both TTE and  $W'$  were increased by ~45% and ~15%, respectively. The authors suggested that large energy demands at the start of the fast-start protocol accelerated the kinetics of  $\dot{V}O_2$ , leading to an increased TTE.



**Figure 1.8.** Power output profile of a cyclist ( $CP = 300$  W;  $W' = 15$  kJ) during different tests. Critical power is denoted by a horizontal dotted line. Theoretically,  $W'$  remains constant irrespective of its rate of depletion. See text for further details. Redrawn from Chidnok et al. (2013a), Fukuba et al. (2003), Jones et al. (2008b), Souza et al. (2015), and Vanhatalo et al. (2007, 2008b).

### **Practical applications of power output-duration relationship**

The parameters of the  $P$ - $d$  relationship,  $CP$  and  $W'$ , have received renewed attention in the last few years. This is, in part, based on the assumption that the depletion of  $W'$  is concomitant with exhaustion within the severe exercise domain. Fukuba and Whipp (1999) were the first to theoretically explore the interaction between the parameters of the  $P$ - $d$  relationship, pacing during the first and second halves of a running race, and running performance. This study only considered the first and second half of running events. However, pacing is constantly adjusted, and exercise is rarely performed at a CWR (Atkinson, Peacock, St Clair Gibson, & Tucker, 2007). Morton and Billat (2004) first attempted to model TTE during intermittent exercise using the  $P$ - $d$  relationship. Notably, whilst the model produced good estimations of TTE, the authors assumed that the recovery of  $W'$  was linear. Subsequent research, however, has shown that  $W'$  recovery follows a non-linear, exponential-like kinetics (Ferguson, Rossiter, Whipp, Cathcart, Murgatroyd, & Ward, 2010; Skiba, Clarke, Vanhatalo, & Jones, 2014; Skiba, Chidnok, Vanhatalo, & Jones, 2012; Skiba, Fulford, Clarke, Vanhatalo, & Jones, 2015). Using non-linear models, Skiba and co-workers (2012, 2014, 2015) have recently developed a model that estimates the remaining  $W'$  at any given time during exercise ( $W'$  balance). This is a promising tool for athletes and coaches, as live information of the energetic status of the athlete might now be available (Figure 1.9).



**Figure 1.9.** Critical power and  $W'$  during cycling exercise. Reproduced from Clarke and Skiba (2013).

#### **1.6.5. Summary**

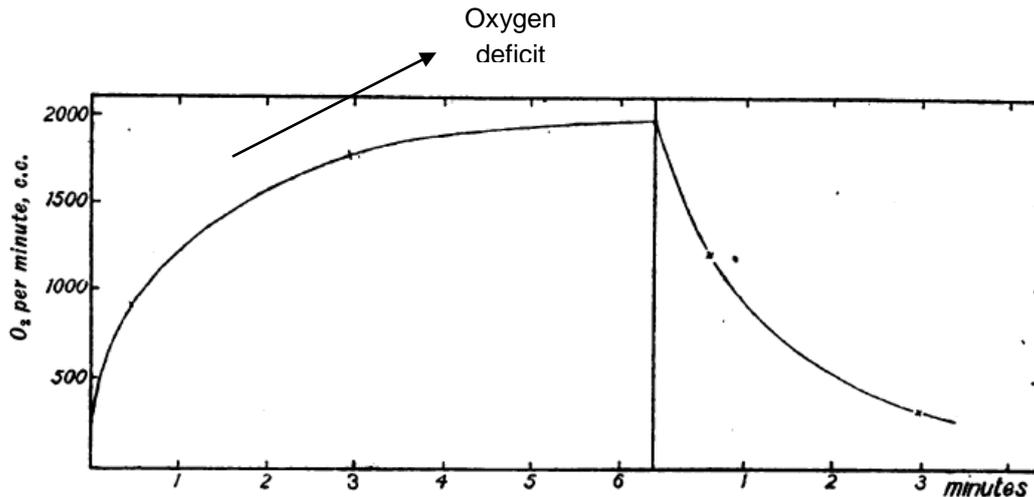
The  $P$ - $d$  relationship is hyperbolic, and distinguishes two compartments. The asymptote of this relationship is the  $CP$  which represents the highest intensity at which metabolic and muscular variables

(i.e.  $\dot{V}O_2$ ,  $[P_i]$ ,  $[PCr]$ ,  $pH$ ) can stabilise in a submaximal steady state. Above CP, there is a finite amount of work that can be completed before exercise is terminated (denoted by  $W'$ ). Despite a number of physiological limitations compromising the CP- $W'$  model, it is of great interest and has been extensively used given its potential to predict exhaustion and monitor fatigue status. Currently, it is suggested that CP is a parameter of the aerobic metabolism, such as  $\dot{V}O_{2max}$ , the  $\tau$  of the primary phase of kinetics of  $\dot{V}O_2$ , the concentration of type I fibres, or the maximal lactate steady state. Regarding  $W'$ , it is generally accepted to represent a finite source of energy, associated with AnC, but its putative mechanisms remain to be completely understood. Recently, the aetiology of  $W'$  has been also linked to the capacity to acquire and tolerate fatigue. Instead of the traditional time-consuming protocol, CP and  $W'$  can be determined from a single 3AO tests, which in turn represents a simple approach to evaluate whether  $W'$  actually represents AnC, and gain further insight into its putative physiological mechanisms.

## **1.7. The maximal accumulated oxygen deficit**

### **1.7.1. Definition**

From the  $P$ - $d$  relationship, a limitation in the consideration of  $W'$  as a measure of AnC was to assume that  $\dot{V}O_2$  responds with infinitely fast kinetics at the onset of exercise, so that aerobic energy production is instantaneously and completely meeting energy demands during exercise at intensities below CP. Instead, the respond in  $\dot{V}O_2$  to any change in metabolic rate is slow (Grassi, 2006; Poole & Jones, 2012). As a consequence, at the onset of exercise there is a mismatch between the oxygen demand and the oxygen uptake, known as the oxygen deficit (Figure 2.10). The oxygen deficit at the onset of exercise was first defined by Krogh and Lindhard (1919) as the difference between predicted oxygen demand and actual oxygen uptake at the onset of exercise. Assuming  $\dot{V}O_2$  provides a measurement of aerobic energy release (Bangsbo et al. 2000; Barstow, Lamarra & Whipp, 1990; Krusturp et al., 2009; Poole et al., 1991), it follows that the oxygen deficit provides an estimate of anaerobic energy production (Bangsbo, 1996; Graham, 1996; Green & Dawson, 1993; Karlsson & Saltin, 1971; Krogh & Lindhard, 1913; Medbø et al., 1988; Noordhof et al., 2010, Saltin, 1988).



**Figure 1.10.** Oxygen deficit, determined as the difference between oxygen demand and  $\dot{V}O_2$ , at the onset of exercise at a constant intensity. Reproduced from Krogh and Lindhard (1919; p. 434).

### 1.7.2. Determination of the maximal accumulated oxygen deficit

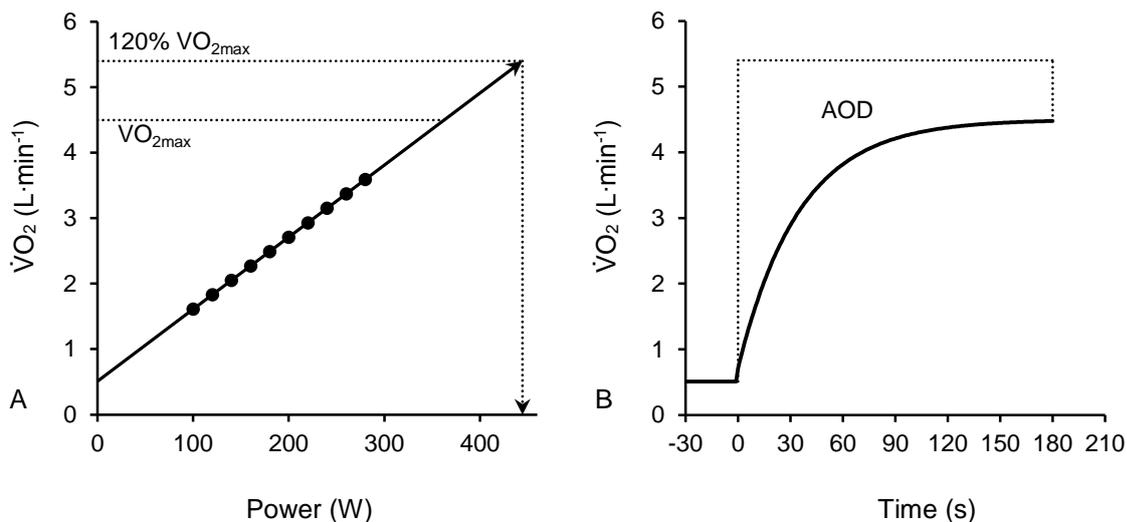
After Krogh and Lindhard (1919) first proposed the concept of oxygen deficit, early studies determined the oxygen deficit as the difference between accumulated oxygen uptake and oxygen demand. The latter, however, was determined by dividing the work done by an assumed efficiency of 23% (Åstrand, Åstrand, Christensen, & Hedman 1960). Instead, Medbø and colleagues (1988) developed the accumulated oxygen deficit, or AOD, to estimate AnC from a single bout of exercise at supramaximal intensities. In this approach, the oxygen demand is not derived from an assumed efficiency individually determined, but individually determined from the extrapolation of the  $\dot{V}O_2$ -work rate relationship (Medbø, 1996; Medbø & Burgers, 1990; Medbø & Tabata, 1989, 1993; Medbø et al., 1988).

The determination of AOD consists of two phases (Medbø et al., 1988). First, participants perform a series of bouts of exercise at submaximal intensities to establish the  $\dot{V}O_2$ -work rate relationship, typically assuming a linear regression. Secondly, participants are asked to perform, at least, one CWR test to exhaustion. The AOD is then determined as the difference between the accumulated oxygen demand (estimated from the linear projection of the  $\dot{V}O_2$ -work rate relationship) and the accumulated oxygen uptake. In the initial protocol proposed by Medbø et al. (1988), the  $\dot{V}O_2$ -running speed relationship was determined using 20 × 10 min bouts at 30-95%  $\dot{V}O_{2max}$  intensities. The average  $\dot{V}O_2$  in the last two minutes of each submaximal bout was plotted against running speed to establish the  $\dot{V}O_2$ -speed linear relationship using. Subsequently, the linear  $\dot{V}O_2$ -speed relationship was rejected to estimate supramaximal speeds so that exhaustion would occur after 15 s to 5 min. Medbø et al. (1988)

observed that the AOD was not different in exercise bouts to exhaustion lasting 2 to 5 minutes, and assumed that this corresponded with the maximal AOD (MAOD), which in turn represents an estimation of AnC.

To reduce such a time consuming protocol, Medbø et al. (1988) proposed a method of determining the  $\dot{V}O_2$ -speed relationship from  $2 \times 10$  min bouts at intensities near  $\dot{V}O_{2max}$  combined with a y-intercept value corresponding to resting  $\dot{V}O_2$  ( $\sim 5.1 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). However, the procedures to construct the  $\dot{V}O_2$ -work rate (speed or power output), and the subsequent supramaximal test to determine AOD, are far from consistent. Tables 1.1 and 1.2 provide an overview of the protocol used to determine AOD in performing running and cycling exercise, respectively. Green and Dawson (1996) were amongst the first to address the effect that the protocol used to construct the  $\dot{V}O_2$ -work rate relationship has on the prediction of supramaximal oxygen demands. They determined the  $\dot{V}O_2$ -work rate relationship using continuous (4 min) and discontinuous (15 min) exercise bouts. In the discontinuous protocol, mean  $\dot{V}O_2$  was determined in the 4<sup>th</sup>, 4<sup>th</sup> to 6<sup>th</sup>, 8<sup>th</sup> to 10<sup>th</sup>, and 13<sup>th</sup> to 15<sup>th</sup> min, and the characteristics of the  $\dot{V}O_2$ -work rate linear relationship (y-intercept and slope) were determined. The y-intercept was not different between protocols. The slope, however, was similar between the continuous and discontinuous protocols of the same duration (i.e. 4 min). However, increasing the duration in the discontinuous protocols (4<sup>th</sup> to 6<sup>th</sup>, 8<sup>th</sup> to 10<sup>th</sup>, and 13<sup>th</sup> to 15<sup>th</sup> min), also increased the slope of the  $\dot{V}O_2$ -work rate relationship, and so increased the oxygen demand (Green & Dawson, 1996). Green and Dawson (1996) recommended using relatively short exercise bouts ( $\sim 4$  min) in a continuous test. Nonetheless, some authors have recommended using 10 min bouts (Buck & McNaughton, 1999a). However, using longer durations may exacerbate the effect of slow component of  $\dot{V}O_2$  on predicted supramaximal work rates (see limitations below for further discussion). Indeed, in a recent review on the AOD, Noordhof et al. (2010) favoured the use of relatively short bouts to prevent (or minimize) the emergence of the  $\dot{V}O_2$  slow component. Similar to the duration of the submaximal bouts, the number of submaximal bouts of exercise required to construct the  $\dot{V}O_2$ -work rate relationship has not been consistent (Table 2.1, Table 2.2). Bickham, Le Rossignol, Gibbons, and Russel (2002) observed similar AODs using 10, 4 or, 2 submaximal bouts and a forced y-intercept of  $\sim 5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ . However, Buck and McNaughton (1999b) reported an increase in the precision of AOD (Buck & McNaughton, 1999b). It seems, therefore, that either 10 submaximal bouts or 2-5 submaximal bouts combined with a forced y-intercept of  $\sim 5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  should be used to construct the  $\dot{V}O_2$ -work rate relationship (Noordhof et al., 2010).

Finally, the AOD is calculated during a CWR test to exhaustion. In the example below (Figure 1.11), a projection of the the  $\dot{V}O_2$ -work rate relationship enables was determined from ten exercise bouts (100-280 W), and AOD was subsequently determined from supramaximal CWR test to exhaustion at 120%  $\dot{V}O_{2max}$ . Using a supramaximal CWR exercise bout to exhaustion is indeed the most common approach to estimate AOD (Table 1.1. and Table 1.2.), although, all-out efforts have been used to determine AOD (Gastin et al., 1995). During 30- and 45-s WAnT, the AOD represents ~80 and ~90% of the AOD incurred during a CWR exercise to exhaustion, respectively (see Section 1.5.4, Calbet et al., 1997; Whithers et al., 1991, 1993). Instead, the AOD during longer all-out cycling tests (60- to 90-s) seems to plateau (Whithers et al., 1991, 1993), so that AOD during these longer all-out tests is not different from the value obtained in an CWR exercise bout to exhaustion (Gastin et al., 1995). It seems, therefore, that all-out tests need a duration > 60 s in order to fully deplete AnC. Whether the AOD can be determined from from the 3AO test and a CWR test to exhaustion remains unknown.



**Figure 1.11.** Outline of the traditional protocol for AOD determination. Panel A represents the construction of the  $\dot{V}O_2$ -power output relationship to predict supramaximal energy demand, assuming a linear relationship. Once  $\dot{V}O_{2max}$  is known, the  $\dot{V}O_2$ -power output relationship can be projected to predict an exercise intensity that causes exhaustion in 2-5 min (in the example 120%  $\dot{V}O_{2max}$  - or 445 W). Panel B represents  $\dot{V}O_2$  (solid line) and oxygen demand (broken line) during an exercise bout to exhaustion at a supramaximal intensity (120%  $\dot{V}O_{2max}$ ; 445 W). Because the oxygen demand is known, AOD is calculated as the area between accumulated oxygen demand and  $\dot{V}O_2$ . Adapted from Medbø et al. (1988).

Further to methodological variation in the protocols discussed above, there have been two alternative approaches to determine AOD. First, Hill and colleagues (Hill, 1996; Hill et al., 1998, 2002, 2011) modified the original protocol proposed by Medbø et al. (1988) and developed an alternative approach to determine AOD, assuming AnC (and therefore AOD) remains constant during a short sporting event

to exhaustion. This conceptually similar approach allows AOD to be determined without completing a series of submaximal bouts to construct the  $\dot{V}O_2$ -work rate relationship. Instead, participants are required to perform 3-5 supramaximal exercise bouts to exhaustion at CWR, so exhaustion occurs between 2 and 7 min. Using a forced y-intercept (either 0 or 5 mL·kg<sup>-1</sup>·min<sup>-1</sup>, depending on the study), the slope (and therefore oxygen demand) is determined iteratively, so that the variation in the AOD for all supramaximal tests to exhaustion is minimised.

With the popularisation of online gas analysers, the temporal resolution of  $\dot{V}O_2$  improved considerably, so that breath-by-breath measurements became the standard. Moreover, from breath-by-breath measurements,  $\dot{V}O_2$  can be interpolated to produce second to second data, and thus characterise the kinetics of  $\dot{V}O_2$  (Poole & Jones, 2012). Whipp and Ward (1993) determined the AOD from analysis of  $\dot{V}O_2$  kinetics. Specifically, the mean response time was determined during a CWR test to exhaustion:

$$\dot{V}O_{2(t)} = \dot{V}O_{2(b)} + A \times \left(1 - e^{\left(-\frac{t}{\tau}\right)}\right) \quad [15]$$

where  $\dot{V}O_{2(t)}$  is the  $\dot{V}O_2$  at a time  $t$ ,  $\dot{V}O_{2(b)}$  is the baseline  $\dot{V}O_2$ ,  $A$  is the amplitude of the  $\dot{V}O_2$  response, and  $\tau$  is the constant of the  $\dot{V}O_2$  response. It is important to differentiate between the mean response time of the overall response, and the time constant of the primary phase of the  $\dot{V}O_2$  kinetics. The former includes the overall  $\dot{V}O_2$  response with no time delay, whilst the latter only includes data from the primary phase (phase II) of  $\dot{V}O_2$ . The AOD, therefore, is determined using the overall response, and does not distinguish between phases. Finally, the AOD is calculated as:

$$AOD = \tau \times A \quad [16]$$

Where  $A$  is the amplitude of  $\dot{V}O_2$  at the point of exhaustion. During high intensity exercise to exhaustion, provided the duration of the exercise exceeds  $\tau$  (Whipp & Rossiter, 2007), the amplitude of  $\dot{V}O_2$  will reach its maximum value (i.e.  $\dot{V}O_{2max}$ ):

$$AOD = \tau \times \dot{V}O_{2max} \quad [17]$$

Hill et al. (2002) compared these three approaches to determine AOD: the traditional protocol, as proposed by Medbø et al. (1988), the alternative protocol proposed by Hill et al. (Hill, 1996; Hill et al., 1998), and the AOD derived from the parameters of  $\dot{V}O_2$  kinetics, as proposed by Whipp and Ward

(1993). The authors reported that the highest AOD was derived using Medbø et al. (1988) approach, intermediate using Hill's approach, and lowest if derived from equation [17].

**Table 1.1.** Outline protocols using AOD, as proposed by Medbø et al. (1988), in running.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -speed relationship	AOD determination
Medbø et al. (1988)	11 men (24 y, 60.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) of varied training backgrounds.	Last 2 min of 20 × 10-min bouts at 35-100% $\dot{V}O_{2max}$ at 10.5% incline.	Constant speeds; TTE ranged 15 to 300 s. AOD levelled off at TTE > 120 s (~65 mL·kg <sup>-1</sup> ).
Medbø & Burgers (1990)	END (n = 6, 27 y, 51.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and SPR (n = 8, 24 y, 48.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) male athletes; and a CON group (20 y, 38.2 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ). PA men (n = 5, 35 y, 38 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and women (n = 7, 29 y, 36.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	4+ × 10-min bouts at 70-95% $\dot{V}O_{2max}$ at 10.5% incline.	Cosntant speed, TTE ranged 2-3 min. AOD: 2.86 and 3.71 L·min <sup>-1</sup> for END and SPR, respectively.
		4+ × 10-min bouts at 70-95% $\dot{V}O_{2max}$ at 5.25% incline	Constant speed, TTE ranged 2-3 min. AOD ↑ in both groups by ~10% after training.
Scott et al. (1991)	Endurance (n = 4, 70.9 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ), middle distance (n = 5, 66.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and sprinters (n = 3, 59.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) athletes, and CON (n = 4, 51.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 3 min of 3 × 10-min bouts at 85-100% $\dot{V}O_{2max}$ . Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Constant speeds, TTE ranged 2-3 min. AOD: 59.9, 74.2, 78.3 and 56.1 mL·kg <sup>-1</sup> for END, middle-distance, SPR and CON group, respectively.
Olesen (1992)	6 men and women (range 17-27 y, 51-69 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 5-6 × 6 min bouts. 1, 15 and 20% incline.	Constant speeds, TTE: 125, 139 and 143 s for 1, 15 and 20% incline, respectively. AOD 39.5, 71.7 and 69.4 mL·kg <sup>-1</sup> for 1, 15 and 20% incline, respectively.
	5 trained male runners (range 17-27 y; 57-68 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 5-6 × 6 min bouts. 1, 10.5 and 15% incline.	Constant speeds, TTE: 155, 125 and 160 s for 1, 10.5 and 15% incline, respectively. AOD: 56.9, 78.3 and 99.8 mL·kg <sup>-1</sup> for 1, 10.5 and 15% incline, respectively.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -speed relationship	AOD determination
Weyand et al. (1994)	SPR (men: n = 9, 20.3 y and 60.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> , and women n = 7, 20.3 y and 49.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and END (men: n = 13, 28.2 y and 70.9 mL·kg <sup>-1</sup> ·min <sup>-1</sup> , and women: n = 12, 29.3 y and 59.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of ~7 × 5-min bouts. 0% incline.	Constant speed, TTE ranged 2-4 min. AOD for men: 55.1 vs. 46.8 mL·kg <sup>-1</sup> for SPR and END, respectively. For women: 45.3 vs. 37.8 mL·kg <sup>-1</sup> for SPR and END, respectively.
Ramsbottom et al. (1994)	12 male and female runners (25.8 y, 64.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).  14 male and female runners (27.5 y, 65 mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	Two runs (77.4 and 89.9% $\dot{V}O_{2max}$ ) at 10.5% incline. Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .  Two runs (81.0 and 90.1% $\dot{V}O_{2max}$ ) at 10.5% incline. Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Test-retest at 120% $\dot{V}O_{2max}$ . 172 and 182 s. AOD: 65.2 and 66.3 mL·kg <sup>-1</sup> .  ~117% $\dot{V}O_{2max}$ , TTE 186 s. AOD 66.1 mL·kg <sup>-1</sup> .
Nummela & Rusko (1995)	400-m (n = 8, 25 y, 64.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and END (n = 6, 72.2 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) men.	Last min of 4 × 3min bouts at 60-94% $\dot{V}O_{2max}$ at 1% incline. Y-int assumed 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	100% $\dot{V}O_{2max}$ . TTE: 49.5 and 49.4 s for 400-m and END, respectively. AOD: 53.9 and 43.1 mL·kg <sup>-1</sup> , respectively.
Maxwell & Nimmo (1996)	18 PA (24 y, 60.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 6 min of 2 × 10-min bouts at 80-95% $\dot{V}O_{2max}$ at 10.5 incline. Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	~139.1% $\dot{V}O_{2max}$ , TTE: 147 s. AOD: 74.6 mL·kg <sup>-1</sup> .
Olesen et al. (1996)	6 PA (28 y, 57.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 8 SPR and middle distance runners (23 y, 72.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 4-5 × 6 min bouts at 1% and 15% incline.	Speeds that exhausted in ~1 min, (15% incline) and 2-3 min (1% and 15% incline). AOD: 64.8 and 68.3 mL·kg <sup>-1</sup> for PA and SPR, respectively (15% incline, TTE 62 s), 86.8 and 82.9 mL·kg <sup>-1</sup> for PA and SPR, respectively (15% incline, TTE 2-3 min) and 53.0 and 52.2 mL·kg <sup>-1</sup> for PA and SPR, respectively (1% incline; TTE: 2-3 min).

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -speed relationship	AOD determination
Sloniger et al. (1997)	12 female college students (24 y, 49 mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	Last min of 6 × 6-min bouts. 0 and 10% incline.	~115% $\dot{V}O_{2max}$ , TTE: 177 s. AOD: 41 vs 49 mL·kg <sup>-1</sup> for 1 and 10% incline, respectively.
Naughton et al. (1997)	Young elite badminton players (male: n = 8, 14.8 y, 61.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ; females: n = 8, 14.2 y and 49.6 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Steady state during 4-5 × 6-8 min bouts at ~40-70% $\dot{V}O_{2max}$ at 6% incline.	120 and 130% $\dot{V}O_{2max}$ . 120% TTE ~195 s, AOD ~71.5 mL·kg <sup>-1</sup> . 130% TTE ~154 s, AOD ~67.6 mL·kg <sup>-1</sup> .
Ramsbttom et al. (1997)	37 men (n = 16, 24 y) and women (n = 11, 23 y) of various training backgrounds.	Last 2 min of 2-3 × 10-min bouts at 80-90% $\dot{V}O_{2max}$ at 10.5% incline. Y-int assumed 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	TTE: 177.6 and 173 s; AOD: 67.6 and 54.1 mL·kg <sup>-1</sup> for men and women, respectively.
Craig & Morgan (1998)	9 END (24.7 y, 67.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) men.	Last 2 min of 8 × 6-min bouts at 60-95% $\dot{V}O_{2max}$ . 1 and 10.5% incline.	Constant speed, TTE 147 vs. 116 s for 1 and 10.5% incline, respectively. AOD at 1% incline 45 and 59.3 mL·kg <sup>-1</sup> (linear and non-linear, respectively). AOD at 10.5%: 63.2 vs 93.6 (linear vs. non-linear).
Doherty (1998)	9 PA men (24 y, 60 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 3 × 6-min bouts at 80-90% $\dot{V}O_{2max}$ . 10.5% incline. Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	125% $\dot{V}O_{2max}$ . TTE: 181 s and 208 s for CON and caffeine, respectively. AOD: 68.6 vs. 76.5 mL·kg <sup>-1</sup> , respectively.
Renoux et al. (1999)	17 END men (17 y, 69 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	4-5 × 3-min bouts at 0% incline.	120% $\dot{V}O_{2max}$ , TTE 86 s. AOD 31.3 mL·kg <sup>-1</sup> .
Doherty et al. (2000)	15 PA men (22.4 y; 58 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 3 × 6-min bouts at a 10.5% incline. Y-int assumed 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	3 × 120% $\dot{V}O_{2max}$ (TTE: 194, 198 and 201 s). AOD: 69.0, 71.4 and 70.4 mL·kg <sup>-1</sup> .

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Spencer & Gastin (2001)	Male athletes, specialist of 200-m (n = 3, 56 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ); 400-m (n = 6, 59 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ); 800-m (n = 5, 67 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ), and 800-m (n = 6, 72 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 5-6 × 6-min bouts at 48-85% $\dot{V}O_{2max}$ . 1% incline.	Event-specific tests. Durations: 22.3, 41.3, 113 and 235 s for 100, 200, 400 and 800 m, respectively. AOD: 30.4, 41.3, 48.8 and 47.1 mL·kg <sup>-1</sup> for 100, 200, 400 and 800 m, respectively.
Ramsbotton et al. (2001)	16 men and women with various backgrounds (~28 y, 55 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last two min of 10 × 10-min bouts at ~75-90% $\dot{V}O_{2max}$ at 10.5% incline. Y-int assumed 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	120% $\dot{V}O_{2max}$ . TTE ranged 2-3 min. AOD ↑ in the training group (from ~65 to ~78). No change in control group (from 56 to 54 mL·kg <sup>-1</sup> ).
Hill et al. (2002)	9 PA men and women (~26 y; ~37 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of a 5-min bout that elicited a heart rate of 120-140 beats·min <sup>-1</sup> at 0% incline, and a Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Average AOD from constant-speed tests to exhaustion (TTE < 5 min) was 43.1 mL·kg <sup>-1</sup> .
Bickham et al. (2002)	7 middle-distance male runners (25 y, 64.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	10 × 10-min bouts (5 < LT, 1 @ LT and 4 > LT) at 1% incline. Y-int from resting $\dot{V}O_2$ .	Constant speeds, TTE range 2-4 min. AOD: 43.3, 44.2 and 42.6 mL·kg <sup>-1</sup> using all, 4 and 2 submaximal intensities, respectively.
Moore & Murphu (2003)	15 male rugby union players (22 y, 58.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 4 × 4-min bouts at 60-85% $\dot{V}O_{2max}$ at 10.5% incline.	125% $\dot{V}O_{2max}$ , TTE 214 s. AOD 99.4 mL·kg <sup>-1</sup> .
Duffield (2004)	100-m (men: n = 9, 25 y, 54 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ; women: n = 6, 24 y, 44 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 200-m (men: n = 8, 22 y, 57 mL·kg <sup>-1</sup> ·min <sup>-1</sup> and female: n = 5, 24, 45 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) specialists.	Last min of 5-6 × 4-6 min bouts at 40-95% $\dot{V}O_{2max}$ at 1% incline.	100-m run on the track: TTE 11.5 and 13.1 s; and AOD 17.4 and 12.4 mL·kg <sup>-1</sup> for men and women, respectively. 200-m run: TTE: 23.8 and 26.8 s, AOD: 28.4 and 22.5 mL·kg <sup>-1</sup> , for men and women respectively.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Ravier et al. (2005)	International (n = 10, 57.6 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and national (n = 8; 59.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) male karate athletes.	Last 2 min of 2 × 10-min bouts at intensities > 80% $\dot{V}O_{2max}$ . 10% slope.	140% $\dot{V}O_{2max}$ . TTE: 133 vs. 116 for attained 76 international vs national level, respectively. AOD: 67 vs. 64 mL·kg <sup>-1</sup> , respectively.
Duffield et al. (2005b)	400-m specialists (men: n = 11, 22 y, 60 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ; females: n = 5, 21 y, 52 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 800-m specialists (men: n = 9, 20 y, 62 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ; females: n = 2, 18 y, 55 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 6-9 × 4.5-7 min bouts at 30-90% $\dot{V}O_{2max}$ at 1% incline.	400-m run on the track: TTE 52.2 and 60.2 s; and AOD 48.0 and 41.8 mL·kg <sup>-1</sup> for men and women, respectively. 800-m run: TTE: 126 and 151 s, AOD: 65.9 and 43.8 mL·kg <sup>-1</sup> , for men and women respectively.
Duffield et al. (2005a)	1500-m (men: n = 10, 25 y, 66 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ; women: n = 4, 21 y, 50 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ), and 3000-m (men: n = 8, 26 y, 69 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ; women: n = 2, 26 y, 52 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) specialists.	Last min of 6-9 × 4.5-7 min bouts at 1% incline	1500-m run: TTE 263 vs. 216 s, AOD 71 vs. 61.5 mL·kg <sup>-1</sup> for men and women, respectively. 3000-m run: TTE 577 vs 695 s, AOD 88.1 vs. 47.1 mL·kg <sup>-1</sup> , respectively.
Friedmann et al. (2007)	18 male athletes (23.9 y; 67.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Between 3.50 and 4.50 min of 3 × 5 min bouts. 5% incline.	110 to 120% $\dot{V}O_{2max}$ . TTE: ~210 vs. ~150 s in normoxia vs. hypoxia, respectively. AOD ~90 vs. ~98 mL·kg <sup>-1</sup> in normoxia vs. hypoxia, respectively.
Feriche et al. (2007)	21 men, exercising in hypoxia (2320 m of altitude, n = 11, 23 y, 4.07 L·min <sup>-1</sup> ) or normoxia (690 m of altitude, n = 10, 23 y, 4.22 L·min <sup>-1</sup> ).	Last 5 min of 3 × 10-min bouts at 35-65% of $\dot{V}O_{2max}$ .	5 × 60 s runs at 90% of 400-m speed interspersed with 1, 3 and 5 min of rest. AOD was not different in hypoxia from normoxia, but ↑ alongside resting intervals from ~7.49 L with 1-min, to ~10.47 L with 3-min, and 12.76 L with 5-min.
Bosquet et al. (2008)	19 END (23 y; 66.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 30 s of several 2 min stages in an incremental test. Flat (0% incline).	110% $v\dot{V}O_{2max}$ . TTE: 193 s. AOD: 57 mL·kg <sup>-1</sup> (using sub-LT for $\dot{V}O_2$ -speed relationship) and 73.8 mL·kg <sup>-1</sup> (using all intensities for $\dot{V}O_2$ -speed relationship).

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Ravier et al. (2009)	14 elite male karate practitioners (~23 y, 58.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 2 × 10-min bouts at 10% incline. Y-int as resting $\dot{V}O_2$ .	TTE: 137 s. AOD ↑ from 63.9 to 70.5 mL·kg <sup>-1</sup> in the training group. Control did not change (65.5 vs. 62.0 mL·kg <sup>-1</sup> ).
Billat et al. (2009)	800-m (n = 8, 18 y, 64.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 1500-m (n = 7, 65.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) runners.	Last 6 min of a 10-min bout at 12 km/h in outdoor track. Y-int assumed at 5.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	800-m and 1500-m races. TTE: 129 and 270 s, respectively. AOD: 40 and 59 mL·kg <sup>-1</sup> , respectively.
Zouhal et al. (2010)	Six 400-m hurdle male runners (24 y, 64 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 4-6 × 6-min bouts.	400-m (52.04 s) and 400-m hurdles runs (55.69 s). AOD: 65.0 and 44.1 mL·kg <sup>-1</sup> , respectively.
Zagato et al. (2011)	11 armed forces personnel men (21 y, 56.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 5 × 7 min at 50-70% $\dot{V}O_{2max}$ .	120% $v\dot{V}O_{2max}$ . TTE: 208 s. AOD 70.8 mL·kg <sup>-1</sup> .
Loures et al. (2012)	9 male football players (16 y, 51.86 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 4 × 10-min bouts at 50, 60, 70 and 80% $\dot{V}O_{2max}$ .	100 and 110% $v\dot{V}O_{2max}$ (TTE: 355 and 220 s, respectively). AOD: 52.18 mL·kg <sup>-1</sup> .
Zagatto et al. (2013)	11 middle- and long-distance runners (20 y, 57.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 5 × 10-min bouts at 50-80% $v\dot{V}O_{2max}$ at 1% incline. Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	110% $\dot{V}O_{2max}$ . AOD: 61.6 mL·kg <sup>-1</sup> .
Dal Pupo et al. (2013)	14 men (21 y, 64 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	3 submaximal intensities, 11 and 12 km·h <sup>-1</sup> and $v\dot{V}O_{2max}$ . Y-int assumed 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	120% $v\dot{V}O_{2max}$ . TTE: 208 s. AOD 70.8 mL·kg <sup>-1</sup> .
Hill & Vingren (2014)	PA men (n = 104, 23 y) and women (n = 119, 23 y). Overall $\dot{V}O_{2max}$ 42 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Last 30 s of 4 × 4-min submaximal int at 0% incline. Linear at speeds <100 m·s <sup>-1</sup> , exponential (1.05) at speeds >100 m·s <sup>-1</sup> . Y-int 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	14.8 km·h <sup>-1</sup> , TTE: 302 s. AOD: 42 mL·kg <sup>-1</sup> .

y: years. END: endurance trained. SPR: sprint-trained. UT: untrained. PA: physically active (“healthy”, “not-specifically trained”, “active”, “recreationally active”, etc. also considered as PA). CON: control (or, where appropriate, placebo) group.  $v\dot{V}O_{2max}$ : velocity at  $\dot{V}O_{2max}$ . Y-int: Y axis of the  $\dot{V}O_2$ -speed relationship (not included if not reported).

**Table 1.2.** Outline protocols using AOD, as proposed by Medbø et al. (1988), in cycling.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Medbø & Tabata (1989)	17 PA men (25 y)	Last 2 min of 10-35 × 10 min bouts at 30-90% $\dot{V}O_{2max}$ .	Constant power output, TTE: 34, 75 and 156 s. AOD: 1.86, 2.25 and 2.46 L, respectively.
Withers et al. (1991)	6 END men (25 y, 4.63 mL·min <sup>-1</sup> ).	≥ 4 submaximal bouts	30-, 60- and 90-s all-out tests. AOD 3.18, 3.73 and 3.73 L, respectively.
Weyand (1993)	11 men (24.1 y, 63.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 9 women (23.9 y, 49.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 7 × 5-min bouts. From unloaded cycling to 85-100% $\dot{V}O_{2max}$ .	115 and 130% $\dot{V}O_{2max}$ (average if TTE > 70 s and < 4 min). One-legged AOD: 30.2 vs. 20.4 mL·kg <sup>-1</sup> for men and women, respectively. Two-legged AOD: 66.0 vs. 50.5 mL·kg <sup>-1</sup> , respectively.
78 Foster et al. (1993)	9 well-trained men and women, various training backgrounds.	~5 submaximal tests, using a non-linear model to account for the cycle-ergometer.	2-km time-trials with the first km completed at very slow, slow, even, fast or very fast pace. Duration changed with pacing (177, 170, 166, 170 and 171 s, respectively). Pacing had no effect on AOD (2.62, 2.84, 3.00, 2.57 and 2.82 L, respectively).
Craig et al. (1993)	18 elite END (n = 12) and SPR (n = 6) cyclists. 20 y, 68.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Last 2 min of 6-8 × 5-min bouts.	2- and 5-min time-trials. AOD 61.4 and 60.2 mL·kg <sup>-1</sup> , respectively.
Withers et al. (1993)	12 END men. 25 y, 70.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Last 2 min of 4 × 10-min bouts at 103-279 W.	45-, 60-, 75, and 90-s all-out tests. AOD: 48.0, 51.0, 51.3 and 51.1 mL·kg <sup>-1</sup> , respectively.
Gastin & Lawson (1994)	UT (n = 8, 20 y, 49 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ), END (n = 8, 26 y, 65 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and SPR (n = 6, 25 y, 58 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 6 × 5-min bouts at 46-83% $\dot{V}O_{2max}$ (excluded if slow component detected).	90-s all-out test. AOD: 44, 42 and 47 mL·kg <sup>-1</sup> for UT, END and SPR, respectively.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Gastin (1995)	9 active men (27 y, 57 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of several 7-min bouts at 29-84% $\dot{V}O_{2max}$ .	All-out isokinetic, 110% and 125% $\dot{V}O_{2max}$ to exhaustion. TTE 62, 186 and 94 s, respectively. AOD: 43.9, 44.1 and 42 mL·kg <sup>-1</sup> , respectively.
	12 END and UT men and women (23 y, 55 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Steady-state ( $\dot{V}O_2$ increase < 0.6 mL·kg <sup>-1</sup> from 4 <sup>th</sup> to 5 <sup>th</sup> min) of 5-6 × 5-min bouts at 46-81% $\dot{V}O_{2max}$ .	90-s all-out, and CWR at 110% $\dot{V}O_{2max}$ (TTE: 208 s). AOD 52.1 and 51.2 mL·kg <sup>-1</sup> , respectively.
Craig et al. (1995)	18 men (20 y). Sub-grouped in SPR (n = 6, 20 y, 62.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and END (n = 12, 20 y, 71.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 6-7 × 5-min bouts.	70 s, 120 s, 300 s time trials, and 115% $\dot{V}O_{2max}$ (TTE: 210 s). AOD was attained in the shortest test for SPR (~66.9 mL·kg <sup>-1</sup> ), and in the longest test for END (62.1 mL·kg <sup>-1</sup> ).
Green & Dawson (1996)	10 well-trained men (26 y, 69.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 8-12 × 4 min bout at 28.2 to 76.3% $\dot{V}O_{2max}$ (< LT).	~112% $\dot{V}O_{2max}$ , TTE 173 s. AOD 55.2 mL·kg <sup>-1</sup> .
Green et al. (1996)	Well-trained (n = 7, 26 y, 5.29 L·min <sup>-1</sup> ) and PA (n = 5, 33 y, 4.05 L·min <sup>-1</sup> ).	Last min of 12 × 4 min bouts < 85% $\dot{V}O_{2max}$ .	NA
		4 <sup>th</sup> and 10 <sup>th</sup> min of 8-10 × 10 min bouts in a step test (90 W + 44 W·4 min <sup>-1</sup> ).	NA
Tabata et al. (1996)	14 physically active men. 23 y, ~50 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Last 2 min of 6-9 × 10-min bouts at 39-87% $\dot{V}O_{2max}$ .	Constant power output, TTE range 2-3 min. AOD remains constant (~70 mL·kg <sup>-1</sup> ) after END, but ↑ from ~60 to 77 mL·kg <sup>-1</sup> with high-intensity training.
Pizza et al. (1996)	UT (n = 12, 22 y, 39.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ), RT (n = 11, 26 y, 42.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and END men (n = 10, 20 y, 61.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 4 min of 3 × 10-min bouts at 60, 70 and 90% $\dot{V}O_{2max}$ and last 2 min of a 6-min bout at 90% $\dot{V}O_{2max}$ .	Constant power output, TTE range 2-4 min. AOD 38.8, 52.5 and 53.7 mL·kg <sup>-1</sup> for UT, RT and END, respectively.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Calbet et al. (1997)	19 PA men (23 y, 56 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 3-5 × 6-min bout at 60-90% $\dot{V}O_{2max}$ .	120% $\dot{V}O_{2max}$ (TTE: 148 s), 30- and 45-s WanT. AOD 68.6, 53.7 and 60.9 mL·kg <sup>-1</sup> for CWR, 30- and 45-s WanT, respectively.
Faina et al. (1997)	8 END (24 y, 72 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 4-6 × 5-min bouts at 30-80% $\dot{V}O_{2max}$ .	~108% $\dot{V}O_{2max}$ , TTE 225 s. AOD 43.6 mL·kg <sup>-1</sup> .
Jacobs (1997)	26 men and women of various backgrounds (25.4 y).	Last min of 4 × 4-min bouts at 50, 65, 75 and 85% $\dot{V}O_{2max}$ .	125% $\dot{V}O_{2max}$ . Creatine supplementation ↑ TTE from 129.5 to 140.6 s and AOD from 4.038 to 4.405 L. Control group, no changes on TTE (131 vs. 134) or AOD (4.020 vs. 4.028 L).
Buck & McNughton (1999a)	8 men (25 y, 57.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	2-4, 4-6, 6-8 and 8-10 min of 10 × 10-min bouts at 30-90% $\dot{V}O_{2max}$ .	110% $\dot{V}O_{2max}$ . TTE 269 s. AOD 39.6, 47.8, 51.7 and 53.4 mL·kg <sup>-1</sup> using 2-4, 4-6, 6-8 and 8-10 min submaximal bouts.
Buck & McNughton (1999b)	8 men (25 y, 57.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	10 × 10-min bouts at 30-90% $\dot{V}O_{2max}$ . AOD determined using 2-10 exercise bouts, reducing highest, lowest and central bouts.	110% $\dot{V}O_{2max}$ . TTE 269 s. AOD 53.4 mL·kg <sup>-1</sup> . ↓ number of bouts resulted in different AODs.
Woolford (1999)	10 END. 17.4 y, ~75 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Step test. 100 W + 50 W/5 min, intensities 30-90% $\dot{V}O_{2max}$ , only < LT included.	120-s time-trial. AOD: 38.7, 56.4 and 56.8 mL·kg <sup>-1</sup> for cadences of 90-100, 120-130 rpm and 90-130 rpm, respectively.
Weber & Schneider (2000)	UT men (n = 10, 23.3 y, 43.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and women (n = 10, 24.7 y, 38.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 6 × 10-min bouts at 20-75% $\dot{V}O_{2max}$ .	120% $\dot{V}O_{2max}$ . TTE: 161 vs. 140 s for men and women, respectively. AOD: 46.3 vs. 38.2 mL·kg <sup>-1</sup> , respectively.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Bull (2001)	8 PA men (32 y, 43.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	4 × 4-min bouts at 50, 60, 70 and 80% $\dot{V}O_{2max}$ .	125% $\dot{V}O_{2max}$ . TTE 108 vs 117 (placebo and caffeine, respectively). AOD 3.214 vs 3.301 L, respectively.
Weber & Schneider (2001)	UT men (n = 7, 23.6 y, 44.6 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and women (n = 7, 25.3, 40.2 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 6 × 10 min bouts at 20-75% $\dot{V}O_{2max}$ .	Test-retest at 110 and 120% $\dot{V}O_{2max}$ . 110% $\dot{V}O_{2max}$ : TTE 226 and 223s, AOD: 2.62 and 2.54 L. 120% $\dot{V}O_{2max}$ TTE: 158 and 159 s, AOD: 2.64 and 2.63 L.
Hill et al. (2002)	9 PA men and women (~26 y; ~37 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of a 5-min bout that elicited a heart rate of 120-140 beats·min <sup>-1</sup> .	Average AOD from constant-speed tests to exhaustion (TTE < 5 min) was 70.8 mL·kg <sup>-1</sup> .
Miura et al. (2002)	17 men. 28 y, 42.9 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Ramp test: 4 min at 20W + 25W/min. $\dot{V}O_2$ determined < LT.	TTE < 2 min. AOD 2.29 L.
Weber & Schneider (2002)	7 UT men (23.7 y, 3.58 L/min) and women (22.7 y, 2.55 L·min <sup>-1</sup> ).	Last min of 6 × 10 min bouts at 20-75% $\dot{V}O_{2max}$ .	120% $\dot{V}O_{2max}$ , TTE ↑ from ~170 s to ~280 s after training. AOD increased from 3.93 and 2.75 L (men and women, respectively) to 4.82 and 3.28 L.
Russell et al. (2002)	8 men and 1 female triathletes (~27 y, 4.7 L·kg <sup>-1</sup> ·min <sup>-1</sup> ).	3 <sup>rd</sup> and 6 <sup>th</sup> min of 10 × 6-min bouts at 70% LT to 95% $\dot{V}O_{2max}$ .	110% $\dot{V}O_{2max}$ . AOD: 3.69 and 4.68 L using the 3 <sup>rd</sup> and 6 <sup>th</sup> min of the submaximal bouts, respectively.
Gardner et al. (2003)	10 men (25 y, 59 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Protocol 1. 1-3, 4-6 and 8-10 min of 5 × 10-min bout at 100-200 W. Protocol 2. Last 2 min of 5 × 3-min bouts at 100-200 W.	Protocol 1. 115% $\dot{V}O_{2max}$ (112.7 s) and 120-s time-trial. AOD: 52.33 mL·kg <sup>-1</sup> . Protocol 2. Two min after $\dot{V}O_2$ -power output determination, 120-s time-trial. AOD: 43.87 mL·kg <sup>-1</sup>
Calbet et al. (2003)	5 male SPR (19.0 y, 62 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 5 END (18.8 y, 72 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 12 × 6-min bouts at 60-90% $\dot{V}O_{2max}$ . Non-linear relationship to determine oxygen demand.	WAnT in normoxia and hypoxia (FiO <sub>2</sub> = 0.10). Normoxic AOD 67.7 and 51 mL·kg <sup>-1</sup> for SPR and END, respectively. Hypoxic AOD 64.4 vs. 54.4 mL·kg <sup>-1</sup> , respectively.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Roberts et al. (2003)	19 END men (n = 14) and women (n = 5). Overall: 27.7 y and 62.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	3 × 6-min bouts at 50, 65.5 and 75% $\dot{V}O_{2max}$ .	4-min time-trial. AOD no change CON group (~50 mL·kg <sup>-1</sup> ), but ↑ from 51.1 to 54.9 mL·kg <sup>-1</sup> after hypoxic training.
Finn et al. (2003)	6 male and female triathletes. 25 y, 56.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	6 × 5-min bouts at 75-225 W.	120% $\dot{V}O_{2max}$ . TTE 175 and 170 s in standard temperature and heat, respectively. AOD 3.3 and 3.5 L, respectively.
Aisbett et al. (2003)	6 PA men (25.2 y, 54 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	2.5 min at 2.5 W·kg <sup>-1</sup> + 50 W·2.5 min <sup>-1</sup> .	2 × 6-min time trials, first 2 min at ~108, ~100 and ~95% $\dot{V}O_{2max}$ . AOD 40.1, 37.5, 38.8 mL·kg <sup>-1</sup> , respectively.
Dorado et al. (2004)	10 students. 24 y, 58 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	6-7 × 6-min bouts at 60-90% $\dot{V}O_{2max}$ . Linear and curvilinear model.	4 × 110% peak power output (~120% $\dot{V}O_{2max}$ ), 5-min rest intervals. AOD ↓ from ~7 L to ~3.5 L in the first vs. last bout, irrespective of protocol during rest intervals.
Chatagnon et al. (2005)	12 physically active men (22.9 y, 49.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of a 10-min bout at 50% peak power output, slope from a ramp test (15 W·min <sup>-1</sup> ).	105% peak power output. TTE range 2-4 min. AOD 49.3 mL·kg <sup>-1</sup> .
Minahan and Woods (2007)	Recreationally active men (n = 7, 24 y, 44.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and women (n = 7, 23 y, 39.6 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 6 × 10 min bouts at 20-75% $\dot{V}O_{2max}$ .	120% $\dot{V}O_{2max}$ , TTE 171 s. AOD: 43.4 mL·kg <sup>-1</sup> .
Glaister et al. (2006)	25 men (20.6 y, 52 mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	Last 2 min of 7-min stages at 80, 120 and 180 W. Y-int assumed at 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	110% $\dot{V}O_{2max}$ . AOD 50.7 mL·kg <sup>-1</sup> .
Ogura et al. (2006)	7 male football players. 20 y, 3.69 L·min <sup>-1</sup> .	Last 2 min of 12 × 6-min bouts at 40, 50 and 80% $\dot{V}O_{2max}$ .	40-s WanT. AOD: 53.2, 61.1, 59.4 mL·kg <sup>-1</sup> for normoxia, 16.4% O <sub>2</sub> and 12.7% O <sub>2</sub> , respectively.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Mezzani et al. (2006)	10 chronic heart failure patients (n = 10, 66 y, 16.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ), 5 asymptomatic left ventricular dysfunction patients (n = 5, 65 y, 18 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and CON men (n = 5, 65 y, 28.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 3 × 10-min bouts at 30, 60 and 90% GET.	~135, 118 and 102% peak power output. TTE 86, 134 and 220 s, respectively. AOD not different between tests, mean 12.2, 23.7 and 24.8 mL·kg <sup>-1</sup> for chronic heart failure patients, asymptomatic left ventricular dysfunction patients and CON, respectively.
Glaister et al. (2007)	19 team sport male athletes (~20 y, ~3.88 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last 2 min of 3 × 7-min bouts at 80, 120 and 160 W.	110% $\dot{V}O_{2max}$ . No changes in AOD with END training (from 4.03 to 3.98 L, control group from 4.21 to 4.15 L).
Pouilly & Busso (2008)	12 PA men (22.7 y, 49.2 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Ramp test (15 and 30 W·min <sup>-1</sup> ).	105% peak power output. TTE: 174 s. AOD 2.33, 2.25 and 2.50 L for the CWR, 15 W·min <sup>-1</sup> and 30 W·min <sup>-1</sup> ramp tests.
Minahan & Wood (2008)	7 UT men (23 y, 46.9 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min of 5 × 4-min bouts at 20-75% $\dot{V}O_{2max}$ .	120% $\dot{V}O_{2max}$ . Resistance training ↑ TTE from 122 to 138 s. AOD ↑ from 2.65 to 2.81 L.
Mezzani et al. (2008)	19 men with left-ventricular dysfunction (66 years, 17.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 17 male CON (61 y, 28.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Steady-state of 3 × 10-min bouts at 30, 60 and 90% LT.	~145, 124 and 110% peak power output. TTE 93, 139 and 216 s, respectively. AOD at 145% 16.7 vs. 25.9 mL·kg <sup>-1</sup> (left-ventricular dysfunction vs CON group, respectively), at 124% 20.1 vs 28.3, and 145% 19.1 vs 28.2 mL·kg <sup>-1</sup> .
Palmer (2009)	8 male track cyclists (30 y, mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	63.7 8-10 × 5-min bouts at 40-90% $\dot{V}O_{2max}$ .	4000-time trial. TTE 345, 338 and 338 s for CON, prior heavy-intensity exercise and prior self-selected exercise, respectively. AOD: 63.2, 64.8 and 64.0 mL·kg <sup>-1</sup> , respectively.

Reference	Subjects		Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Aisbett et al. (2009a)	8 END men (30 y, 64 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).		Last 30 s of bouts at 150 W for initial 150 s, 200 W for subsequent 150 s, and increased by 25 W every 150 s.	~102.7 kJ time-trial time-trials with fast-start and all-out pacing strategy. TTE: 288 and 291 s, respectively. AOD: not different between (~4.6 L·min <sup>-1</sup> ).
Aisbett et al. (2009b)	26 END men (29 y, 62.8 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	62.8	Last 30 s of bouts at 150 W for initial 150 s, 200 W for subsequent 150 s, and increased by 25 W every 150 s.	~102.7 kJ time-trial time-trials with slow-, even-, and fast-start pacing strategy. TTE: 293, 304 and 309 s, respectively. AOD: 4.10, 4.00 and 3.80 L, respectively.
Bertuzzi (2010)	9 PA men (23 y, 41.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).		Last 30 s of 6 × 10 min bouts at 40-90% $\dot{V}O_{2max}$ .	110% peak power output. TTE: 154 s. AOD ~2.6 L.
Leclair et al. (2010)	Recreationally active men (n = 15, 23.5 y, 46.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and children (n = 16, 10.3 y, 43.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).		2 bouts at 70 and 90% LT. Resting $\dot{V}O_2$ included as Y-int.	110% peak power output. TTE: 147 and 98 s for men and children, respectively. AOD: 297 and 205 J·kJ <sup>-1</sup> , respectively.
Simmonds et al. (2010)	8 END men (26 y, 68 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).		Last min of 4 × 4-min bouts at 30, 60, 90 and 120% GET.	120% $\dot{V}O_{2max}$ (TTE: ~100 s).
Gordon et al. (2011)	9 well-trained cyclists (22.2 y, 59.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).		Last min of 5 × 8-min at 30-80% $\dot{V}O_{2max}$ .	125% $\dot{V}O_{2max}$ , TTE 153 s. AOD 69.4 mL·kg <sup>-1</sup> .
Noordhof et al. (2011)	15 well-trained men (26.5 y, 65.1 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).		4 <sup>th</sup> min and 8-10 min of 10 × 10-min bouts at 30-90% $\dot{V}O_{2max}$ (RER < 1.00). With and without assumed Y-int of 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Intensity of a 2.5 km time-trial, TTE 167 s. AOD 48.8, 41.4 and 43.0 mL·kg <sup>-1</sup> using 10-min, 4-min, and 4-min + Y-int, respectively.
Leclair et al. (2011)	15 male adolescents (n = 15, 10.3 y, 43.5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) and 15 men (n = 15, 23.5 y, 46.4 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).		Last min of 2 × 6-min bouts at 70-90% LT. Resting $\dot{V}O_2$ as Y-int.	110% $\dot{V}O_{2max}$ . TTE: 96 and 147 s for adolescents and men, respectively. AOD: 34.3 and 53.6 mL·kg <sup>-1</sup> , respectively.
Hill & Vingren (2011)	52 recreationally active men (n = 25) and women (n = 27). 23 y, 38 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .		4-min bout at submaximal bout (RER < 1). Y-int assumed 5 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Constant power output, TTE: ~3 min, 5 min and 7 min. AOD: ~59 mL·kg <sup>-1</sup> for all durations.

Reference	Subjects	Determination of $\dot{V}O_2$ to construct the $\dot{V}O_2$ -power output relationship	AOD determination
Hill & Vingren (2012)	18 men (n = 10) and women (n = 8). 23 y and 38 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	30 s steady-state of 2 × 4-min bouts at 25 and 75 W (< LT).	3 W·kg <sup>-1</sup> . TTE: 368, 299 and 220 s for cadence of 60, 80 and 100 rpm, respectively. AOD: 52, 52 and 52, mL·kg <sup>-1</sup> respectively.
Morales-Alamo et al. (2012)	10 physically active men. 25 y, 4.02 L·min <sup>-1</sup> .	Last 2 min of 8-11 × 10-min bouts at 50-90% $\dot{V}O_{2max}$ .	Isokenetic WanT. 2.874 vs 3.101 Lin normoxia and hypoxia (10.4%), respectively.
Lima-Silva et al. (2013)	6 physically active men (29.7 y, 46.7 mL·kg <sup>-1</sup> ·min <sup>-1</sup> ).	Last min during incremental stages (50 W + 20 W·3 min <sup>-1</sup> until exhaustion.	115% $\dot{V}O_{2max}$ . TTE 222, 180 and 264 s for control, low CHO and high CHO, respectively. AOD: 74.0, 73.9, 73.6 kJ for CON, low CHO and high CHO, respectively.
Adami & Capelli (2013)	14 physically active men. 26.5 y, 56.3 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	4 × 6-min bouts at 100, 125, 150 and 175 W.	120% $\dot{V}O_{2max}$ , TTE 160 s. AOD 77.6 mL·kg <sup>-1</sup> .
Hill (2014)	20 recreationally active men. 22 y, ~53 mL·kg <sup>-1</sup> ·min <sup>-1</sup> .	Last 30 s of 4 × 4-min bouts at 70, 80, 90 and 100% $\dot{V}O_{2max}$ .	CWR of ~5 min. TTE: 275 and 329 (am and pm test, respectively). AOD 70 and 75 mL·kg <sup>-1</sup> , respectively.
Hill & Vingren (2014)	216 recreationally active men (n = 110) and women (n = 106).	A 4-min bout at 78 W. Y-int of 8.2 mL·kg <sup>-1</sup> .	CWR of ~5 min. TTE 299 and 301 s for women and men, respectively. AOD 38 and 43 mL·kg <sup>-1</sup> , respectively.

y: years. END: endurance trained. SPR: sprint-trained. UT: untrained. PA: physically active (“healthy”, “not-specifically trained”, “active”, “recreationally active”, etc. also considered as PA). CON: control (or, where appropriate, placebo) group.  $v\dot{V}O_{2max}$ : velocity at  $\dot{V}O_{2max}$ . Y-int: Y axis of the  $\dot{V}O_2$ -speed relationship (not included if not reported). RER: respiratory exchange ratio. LT: lactate threshold. GET: gas exchange threshold. WanT: Wingate Anaerobic test.

### 1.7.3. Validity and reliability of maximal accumulated oxygen deficit

#### **Validity**

The validity of AOD as a test to estimate AnC has been studied from, at least, four different approaches. First, as a test to estimate AnC, it is assumed that AOD should not be affected by changes in  $\text{FiO}_2$ . Second, different values from the AOD test are expected in populations with, theoretically, different abilities to produce energy released anaerobically. Similarly, the changes in AOD after high-intensity training might offer insight into the validity of AOD to estimate AnC. Third, the AOD has been used to determine energy demands during various sporting events, with the assumption that the anaerobic energy production decreases as the duration of the sporting event increases. Moreover, AOD is assumed to correlate with performance in sporting events that heavily rely on anaerobic energy production. Fourth, it is assumed that the AOD remains constant provided the test has sufficient duration to fully deplete AnC. Exercise performance decreases in hypoxia due to an impaired aerobic function (Dekerle et al., 2012; Friedmann et al., 2007; Simpson et al., 2015; Wehrlin & Hallén, 2005); and increases in hyperoxia (Byrnes, Mihevic, Freedson, & Horvath, 1984; Macdonald et al., 1997; Vanhatalo et al., 2010). As a measure of AnC, however, the magnitude of AOD should remain constant in normoxia, hypoxia or hyperoxia. Similar AOD values have been reported during hypoxic and normoxic trials (e.g. Calbet et al., 2003; Friedmann et al., 2007; Weyand et al., 1999). Interestingly, two of these studies have reported that the reduction in  $\dot{V}\text{O}_{2\text{max}}$  in hypoxia was somewhat compensated by an increased oxygen deficit, so overall performance remains largely unaffected (Calbet et al., 2003; Weyand et al., 1999). As previously discussed for the parameters of the  $P$ - $d$  relationship (Section 1.6), it is indeed possible that acute hypoxia stimulates anaerobic glycolysis to compensate for the decrease in aerobic energy production. The effect of hyperoxia on AOD has received little attention. In fact, although no study has determined the AOD using the protocol proposed by Medbø et al. (1988) in hyperoxia, Macdonald et al. (1997) reported a reduced oxygen deficit submaximal cycling exercise in hyperoxia due to an accelerated mean response time of  $\dot{V}\text{O}_2$ . Subsequently, however, the parameters of the  $\dot{V}\text{O}_2$  kinetics in hyperoxia have been studied using more advanced modelling techniques (three-parameter models). Wilkerson, Berger, and Jones (2006) showed that the hyperoxia actually had no effect on the  $\tau$  of  $\dot{V}\text{O}_2$  kinetics, but rather caused a reduction in the amplitude of the slow component of

$\dot{V}O_2$ . Overall, AOD appears to remain unaffected by hypoxia, though there is some evidence of an increased AOD in normobaric hypoxia. The effect of hyperoxia on AOD remains to be determined.

The second approach used to study the validity of AOD focuses on whether the test is sensitive to different populations and energy demand in various sporting events. Greater AOD values have been reported in sprint-trained or resistance-trained athletes compared with endurance-trained athletes (Calbet et al., 2003; Gastin & Lawson, 1994; Nummela & Rusko, 1995; Pizza et al. 1996; Ramsbottom et al., 1997; Scott et al., 1991); and in healthy adults compared with patients suffering from various conditions (Mezzani et al., 2006, 2008). Moreover, AOD is lower in children, who are characterised by diminished AnC (see Section 1.4.3), compared with adults (Leclair et al., 2010, 2011). Alternatively, studies looking at the effect of training on AOD suggest that, whilst high intensity bouts of exercise interspersed with periods of light exercise or rest increase AOD (Medbø & Burgers, 1990; Ramsbottom et al., 2001; Ravier et al., 2009; Weber & Schneider, 2002), endurance training has no effect on AOD (Glaister et al., 2007). Overall, the evidence suggests that AOD is able to differentiate the AnC between populations, and is sensitive to changes in AnC after high-intensity training.

Given that AOD can detect energy demands in a single bout of exercise and differentiate subjects with different fitness levels, it has been hypothesised that it could be a predictor of performance (Craig & Morgan, 1998). On the one hand, significant correlations ( $0.40 \leq r \leq 0.88$ ) between AOD and athletic performance have been reported for various events (Billat et al., 2009; Del Pupo et al. 2013; Ramsbottom et al., 1994; Weyand, Cureton, Conley, Sloniger, & Lin Liu., 1994). Weyand et al. (1994), for instance, reported significant correlation between running performance and the AOD in athletes specialists in all Olympic distances from 100 m to 5,000 m ( $0.40 \leq r \leq 0.71$ ). Moreover, the correlation between AOD and performance was stronger in sprinters ( $0.66 \leq r \leq 0.71$  for 100-400 m specialist) than those specialised in the longer events ( $r = 0.40$  for 5000 m specialists). In contrast, three studies did not find a significant correlation between AOD and athletic performance in 800-m running ( $-0.02 \leq r \leq 0.20$ ; Craig & Morgan, 1998), 600 m skiing cross country time-trials ( $-0.53 \leq r \leq -0.13$ ; McGawley & Holmberg 2014), or and 400- or 800-m runs (Olesen et al., 1996). In summary, AOD has been shown moderate to strong correlations with performance in sporting events that rely on anaerobic energy production in some, though not all, studies.

The third approach used to study the validity of AOD is based on the assumption that AnC is finite. Medbø and co-workers (Medbø et al., 1988; Medbø & Tabata, 1989, 1993) observed that AOD reached a plateau during a CWR test where exhaustion occurred within 2-5 min. Furthermore, Weber and Schneider (2001) determined the AOD from four supramaximal bouts of exercise: two tests at 110%  $\dot{V}O_{2max}$  and two tests at 120%  $\dot{V}O_{2max}$ . The authors reported that AOD was not different between repeated trials at either 110% or 120%. In addition, the mean AOD in the repeated trials at 110%  $\dot{V}O_{2max}$  (~2.62 L) was not different from the average AOD in the repeated at 120%  $\dot{V}O_{2max}$  (~2.54 L) (Weber & Schneider, 2001). Moreover, Craig et al. (1995) reported no differences in AOD during cycling events of fixed duration (70, 120 and 300 s) and exercise to exhaustion at 115%  $\dot{V}O_{2max}$ . However, the authors noted that those cyclists who specialised in the shortest events (1 km/sprint) attained higher AODs in the shortest tests, whilst those specialised in the longest events (4000-m pursuit) attained higher AODs in the longer tests. It is worth noting that the AOD determined in thoroughbred horses also remains consistent during exercise to exhaustion at running speed corresponding to 105, 115% and 125%  $\dot{V}O_{2max}$  (Eaton, Evans, Hodgson, & Rose, 1995). With regards to all-out tests, as discussed above, it appears that AOD increases with duration until a plateau is reached during exercise of 60-90 s, as discussed above. In summary, it appears that the AOD remains largely unaffected by the duration of the exercise, provided this is 2-5 mins and 60-90 s for open-loop and closed-loop tests, respectively.

### ***Reliability***

Ramsbottom et al. (1994) reported that the mean AOD determined in two running tests at 120%  $\dot{V}O_{2max}$  were similar (65.2 and 66.3 mL·kg<sup>-1</sup>·min<sup>-1</sup>) and strongly correlated ( $r = 0.94$ ). Similarly, very strong correlation coefficients have been observed for AOD determined in consecutive tests ( $r \geq 0.95$ ) in cycling (Jacobs et al., 1997; Weber & Schneider, 2001). Whilst these studies suggest that AOD is a highly reliable test, a high correlation coefficient does not necessarily imply good reproducibility (Hopkins, 2000; Weir, 2004). Indeed, Doherty, Smith, and Schroder (2000) studied the reliability of AOD from three running tests to exhaustion at 125%  $\dot{V}O_{2max}$  using CV, intraclass correlation coefficient (ICC), and 95% limits of agreement. They reported a CV, ICC and limits of agreement of 6.8%, 0.91 and 15.1 mL 'O<sub>2</sub> eq'·kg<sup>-1</sup>, respectively. The authors argued that the limits of agreements were too high to deem AOD as a reliable test. Then again, the limits of agreement might be too stringent to assess the reliability in sport science studies (Hopkins, 2000). In summary, there is some controversy in regards to the

reliability of AOD. Whilst simple test-retest correlation coefficient suggests good reliability, the evidence from other statistical approaches is limited.

#### **1.7.4. Limitations**

Despite a theoretically simple approach, the AOD has failed to gain popularity within the sport-science community. Beyond its practical constraints (e.g. number of testing sessions required), the test relies on a number of assumptions that compromise its validity as a measure of AnC. Arguably, the main limitation of AOD is the estimation of oxygen demands (as a proxy measure of energy demand) during supramaximal intensities. Specifically, the AOD relies on two assumptions. i) The  $\dot{V}O_2$ -work rate relationship can be extrapolated to predict supramaximal oxygen demands, typically assuming a linear relationship. ii) The characteristics of this line remain constant during exercise, so the estimated oxygen demand remains constant during a CWR bout of exercise to exhaustion. Both assumptions have been challenged and remain controversial. In addition, the effect that oxygen stores in blood might have on AOD, and whether  $\dot{V}O_2$  (i.e. pulmonary oxygen uptake) represents muscle oxygen uptake, and therefore aerobic energy production in the exercising muscle, are also possible confounding factors.

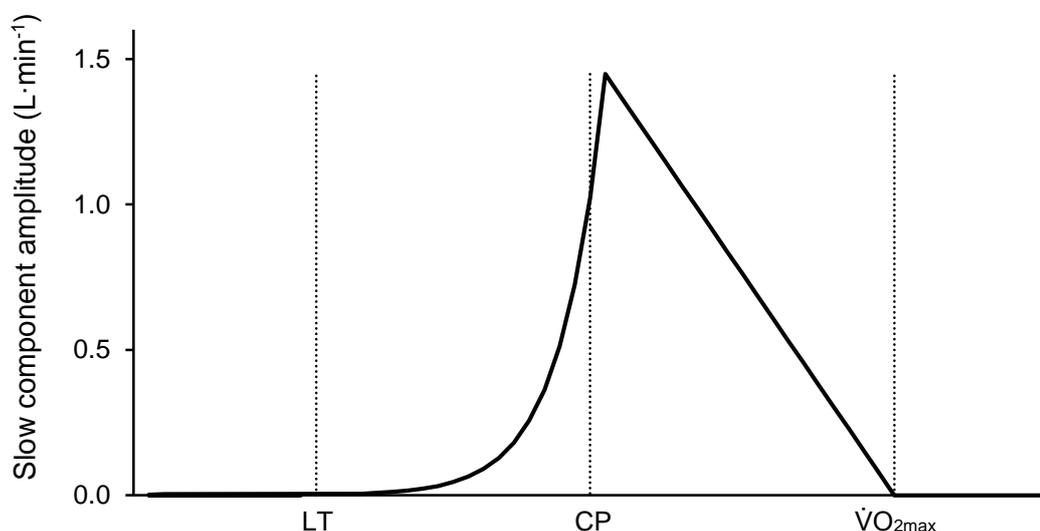
#### ***Prediction of supramaximal energy demands***

An exercise bout aiming to deplete AnC requires somewhere between 60 – 360 s of high intensity exercise, where most (i.e. during an all-out test) or the entire test (i.e. a CWR test to exhaustion) lies within the severe exercise domain, where an estimation of energy demand represents a challenge (Noordhof et al., 2010). At intensities below lactate threshold, in the so called moderate exercise domain,  $\dot{V}O_2$  achieves a steady state 2-3 minutes after the start of exercise. The amplitude of  $\dot{V}O_2$  during steady state exercise depends on the intensity of exercise, so  $\dot{V}O_2$  and work rate display a linear relationship. However, at intensities above LT, the kinetics of  $\dot{V}O_2$  becomes more complex. In the heavy exercise domain (i.e. > lactate threshold but < CP),  $\dot{V}O_2$  continues to rise, after the initial rise (phase I) has been attained. The steady state  $\dot{V}O_2$ , therefore, exceeds values predicted from moderate domain models. The superimposed  $\dot{V}O_2$  in the heavy domain is termed the slow component of  $\dot{V}O_2$ . As a consequence, a steady state of  $\dot{V}O_2$  is delayed for several minutes (Jones et al. 2011). As discussed above, during exercise at intensities greater than CP, in the severe domain, a steady state is not achieved and the slow component of  $\dot{V}O_2$  is not discernable (Figure 1.12). Instead,  $\dot{V}O_2$  increases until  $\dot{V}O_{2max}$  is attained. The presence of the slow component, therefore, challenges the assumption of a

linear relationship between  $\dot{V}O_2$  and work rate. Indeed, a number of studies have shown an upward drift in the relationship at intensities above the LT (Bearden & Moffatt, 2001; Barstow & Molé, 1991; Hughson, O'Leary, Betik, & Hebestreit, 2000; Jones, Carter, & Doust, 1999; Lucia, Hoyos, Santalla, Perez, & Chicharro, 2002; Pedersen, Sørensen, Jensen, Johansen, & Levin, 2002; Zoladz et al., 2002; Zoladz, Rademaker, & Sargeant, 1995). Then, again, others have reported a negligible (though statistically significant) increase in  $\dot{V}O_2$  during high-intensity exercise (Lucia, Hoyos, & Chicharro, 2000), and even a decrease in the  $\dot{V}O_2$ -work rate relationship at intensities above LT (Bickham, Gibbons, & Le Rossignol, 2004).

The slow component of  $\dot{V}O_2$ , broadly, represents a decrease in efficiency. The mechanisms underpinning the slow component of  $\dot{V}O_2$  have received considerable attention, and are reviewed in detail elsewhere (Hughson, 2009; Jones et al., 2011; Poole & Jones, 2012; Poole, Barstow, Gaesser, Willis, & Whipp, 1994; Xu & Rhodes, 1999). In brief, recruitment of type II fibres, increased acidosis, increased levels of circulating catecholamines, increased work from surrounding muscles, and the increased temperature (through the  $Q_{10}$  effect) have all been suggested to contribute to the slow component of  $\dot{V}O_2$  (Poole et al., 1994). Nonetheless, the relative contribution of the above processes to the magnitude of the slow component varies considerably. It is known that over 80% of the slow component is originated in the exercising muscle (Krustrup et al., 2009; Poole et al., 1991; Rossiter et al., 2002). Moreover, an increased recruitment of type II fibres and/or fatigue of already recruited type I fibres are, arguably, the most likely intramuscular cause of the slow component of  $\dot{V}O_2$  (Cannon, White, Andriano, Kolkhorst, & Rossiter, 2011; Copp et al., 2010; Krustrup, Söderlund, Mohr, & Bangsbo, 2004; Poole et al., 1994; Poole & Jones, 2012; Pringle, Doust, Carter, Tolfrey, & Jones, 2003; Zoladz et al., 2008). The remaining magnitude of the slow component of  $\dot{V}O_2$  attributable to extramuscular factors (15-20%) may occur, for example, due to an increased work from muscles other than those involved in the exercise contribute to the development of the slow component of  $\dot{V}O_2$ . For example, it has been shown that oxygenation in the biceps brachii is different during cycling exercise as power output increases below vs. above the LT (Özyener, Whipp, & Ward, 2012). Similarly, respiratory muscle power increases as the intensity of the exercise contributes to the amplitude of the slow component of  $\dot{V}O_2$  (Cross, Winters, Sheel, & Sabapathy, 2014).

There are, however, a few factors that might affect the magnitude of the  $\dot{V}O_2$  slow component, and therefore the  $\dot{V}O_2$ -work rate relationship. On the one hand, beyond the precise mechanism(s) underpinning the slow component of  $\dot{V}O_2$ , it is worth considering the time- and intensity-course of the magnitude of the slow component, and how it is affected by training. On the one hand, the slow component of  $\dot{V}O_2$  is evident at intensities above the LT, where its magnitude increases rapidly until CP is reached. At intensities above  $\dot{V}O_{2max}$ , there cannot be a slow component because the increase in  $\dot{V}O_2$  is truncated at  $\dot{V}O_{2max}$ . It is important to acknowledge that, despite the fact that the slow component of  $\dot{V}O_2$  is not manifest (Figure 1.12); its underpinning mechanisms are likely present at intensities above  $\dot{V}O_{2max}$  (Bearden & Moffatt, 2004; Grassi, Rossiter, & Zoladz, 2015; Vanhatalo, Poole, DiMenna, Bailey, & Jones, 2011b). On the other hand, magnitude of the slow component seems to be related with fitness status, so endurance athletes show a smaller magnitude in the amplitude of slow component than untrained subjects (Koppo, Bouckaert, & Jones, 2004; Lucia et al., 2000; Russell et al., 2002b). Similarly, training has been found to reduce the magnitude of the slow component (Casaburi, Storer, Ben-Dov, & Wasserman, 1987); which in turn reduces the non-linear  $\dot{V}O_2$ -power output relationship (Majerczak et al., 2011).



**Figure 1.12.** Magnitude of the slow component in the continuum of exercise intensities. Below lactate threshold, there is no slow component in  $\dot{V}O_2$ . Above lactate threshold, the slow component increases its amplitude exponentially until CP, where rapidly starts to decrease. At intensities above  $\dot{V}O_{2max}$  there is no slow component, due to  $\dot{V}O_2$  rising inexorably until  $\dot{V}O_{2max}$  has been reached. Constructed from, Jones et al. (2011), Poole and Jones (2012) and Wilkerson et al. (2004).

***The parameters of the  $\dot{V}O_2$ -work rate relationship remain constant during exercise.***

The second main assumption in the AOD method is that oxygen demand remains a constant function of the parameters of the linear relationship between  $\dot{V}O_2$  and work rate, so that if power output remains constant, the oxygen demand remains constant too (e.g. Panel B, Figure 1.4). This implies that the efficiency remains constant throughout high-intensity exercise. However, again because of the slow component of  $\dot{V}O_2$ , the assumption of a constant oxygen demand during a high-intensity CWR exercise bout has been challenged.

Despite the difficulties in assessing *in vivo* energy production, Bangsbo et al. (2001) reported a progressive increase in the rate of ATP turnover during the initial phase (0 – 20 s) of a 3-min CWR exercise. This result has been observed in subsequent studies (Cannon et al., 2014; Krstrup, Ferguson, Kjaer, & Bangsbo, 2003). Grassi et al. (2015) has recently suggested a link between the slow component of  $\dot{V}O_2$  and the loss of efficiency observed during an exercise bout at high-intensity exercise. However, Cannon et al. (2014) observed that the  $\dot{V}O_2$  slow component was not correlated with the increase in ATP turnover ( $R^2 = 0.06$ ), suggesting that the decrease in efficiency during exercise is not solely caused by an increased phosphate cost of power output production. These studies suggest that, although the mechanical power output during a high-intensity CWR bout of exercise is constant, there is a decrease in efficiency and, therefore, the energy demand increases progressively. It has been suggested that the reduction in efficiency and the slow component of  $\dot{V}O_2$  represent the same physiological processes, although this is still debated.

***Further limitations of the maximal accumulated oxygen deficit.***

There two further potential limitations, or confounding factors, in the AOD determination. First, stored oxygen bound to myoglobin is, usually, not taken into consideration in the AOD calculation. Second, the assumption that pulmonary  $\dot{V}O_2$  (i.e. assessed at the mouth) represents muscle  $\dot{V}O_2$  needs to be addressed.

It has long been recognised that oxygen is stored within the body bound to haemoglobin in blood and myoglobin in muscle, dissolved in body fluids, and present in the lungs (Farhi & Rahn, 1955). The oxygen stores in the body, which have been estimated to amount < 10% of AOD (Medbø et al., 1988),

appear to remain consistent within an individual. Moreover, the oxygen stores seem to be unaffected by endurance training, sprint training, and resistance training, though the combination of training and hypoxia might increase myoglobin concentration and, consequently, oxygen storage capacity (Terrados, 1992; Vogt & Hoppeler, 2010). As a result, estimations of AnC derived from the AOD method have been reduced by ~10% in some groups (e.g. Medbø & Tabata, 1993; Scott et al., 1991; Sloniger et al., 1997; Weber & Schneider, 2002). Nonetheless, the majority of studies using AOD have acknowledged this limitation, but have not included any correction for oxygen stores.

Finally, it is important to discuss whether pulmonary  $\dot{V}O_2$  reflects muscle  $\dot{V}O_2$ . Oxygen uptake has been simultaneously measured in the limbs and at the mouth (i.e. muscle and pulmonary  $\dot{V}O_2$ , respectively) during exercise involving a large muscle mass (e.g. Bangsbo et al., 2001, Grassi et al., 1996; Krstrup et al., 2009; Poole et al., 1991). Pulmonary  $\dot{V}O_2$  measurements are characterised by an initial 'cardiodynamic' response, which lasts ~15 s and does not represent a metabolic process but a sudden increase in venous blood flow. After the cardiodynamic phase, pulmonary  $\dot{V}O_2$  increases exponentially. Instead, there appears to be no delay in muscle  $\dot{V}O_2$  (Behnke, Barstow, & Poole, 2004), which might therefore affect AOD estimation. Then again, characterisation of the phase I of  $\dot{V}O_2$  kinetics is difficult and unusual during high intensity exercise, and it is likely to have a relatively small effect on the overall kinetics at supramaximal work rates (e.g. Scheuermann & Barstow, 2003; Wilkerson, Koppo, Barstow, & Jones, 2004).

#### **1.7.5. Summary**

The AOD test estimates AnC as the difference between the accumulated oxygen demand and accumulated oxygen uptake during high-intensity exercise. These exercises are performed at supramaximal intensities, where the oxygen demand exceeds  $\dot{V}O_{2max}$ , and therefore needs to be estimated. At supramaximal intensities, the oxygen demand is estimated from a linear projection of the submaximal  $\dot{V}O_2$ -power output relationship. Unfortunately, the estimation of oxygen demands has been challenged, and it is currently the major limitation of the test. Moreover, the evaluation of AnC via AOD requires a time-consuming protocol.

## 1.8. Summary of the literature review

During high-intensity exercise, such as sporting events that last less than a few minutes, both aerobic and anaerobic energy production contribute to meet the energy demand. Unfortunately, the intramuscular nature of anaerobic metabolism makes it difficult to quantify AnC, which potentially compromises the design and evaluation of training programmes or the analysis of energy demands for a sporting event. Assessment of muscle metabolites from muscle biopsies and  $^{31}\text{P}$ -MRS induced by a bout of exercise may quantify AnC from a single muscle group. However, these techniques are limited for whole-body exercises, invasive and/or technically challenging, and ultimately compromise its applicability. Alternative approaches have attempted to estimate AnC from the assessment of physical (power output, work), kinematic (speed) or physiological ( $\dot{V}\text{O}_2$ ,  $\text{La}^-$ ) variables during or after high intensity exercise with various degrees of success. The curvature constant of the  $P$ - $d$  relationship,  $W'$ , has been proposed to represent a measure of work performed from anaerobic energy sources during exercise above CP. However, the putative mechanisms of  $W'$  likely include other physiological parameters, such as the slow component of  $\dot{V}\text{O}_2$  kinetics and the capacity to acquire and tolerate fatigue, as well as anaerobic energy stores. Currently, the AOD test is considered the best non-invasive approach to estimate AnC. The AOD calculates the difference between energy demand and aerobic energy release (determined as oxygen demand and  $\dot{V}\text{O}_2$ , respectively) during a bout to exhaustion at supramaximal intensities. Unfortunately, the AOD relies on a number of assumptions that have prevented the test from becoming a regular feature of physiological testing.

## **1.9. Aims**

### **1.9.1. General aim**

The overarching aim of this PhD thesis was to investigate the assumptions and limitations surrounding the determination of AnC by means of quantification of AOD and  $W'$ .

### **1.9.2. Specific aims**

1. To establish the validity and reliability of the  $\dot{V}O_2$ -power output relationship to predict supramaximal oxygen demands.
2. To determine the test-retest reliability of AOD.
3. To test the hypothesis that AOD remains constant during constant-load cycling exercise to exhaustion at different supramaximal intensities.
4. To determine whether AOD can be determined in a single-day trial.
5. To establish the correlation between AOD and  $W'$ , and whether the strength of the correlation is affected by the type of exercise.
6. To test the hypothesis that AOD and  $W'$  remain unchanged during a CWR test to exhaustion and an all-out test.
7. To determine the effects of hypoxia and hyperoxia on AOD and  $W'$ .

## **Chapter 2: General methods**

## **2.1. Ethical approval, consent forms and health and safety considerations**

Prior to any data collection, ethical approval was gained from St Mary's University Ethics Committee (see Appendix 1 for an example). Before any test started, participants were informed orally and in writing of the aim, risks and benefits of taking part in each the study, and were given the opportunity to ask any questions. Subsequently, they completed a physical activity readiness questionnaire (Appendix 2) and signed a consent form (see example in Appendix 3).

## **2.2. Participants**

The participants for all experimental studies were recruited from cycling and triathlon clubs based in south-west London (UK). All participants were volunteers and, consequently, did not receive any financial or otherwise incentives to complete each study. However, upon completion of each study, each participant received a personalised report with their results derived from the tests. The physical characteristics of the participants are provided in each study. Common inclusion criteria in all of the studies were: i) being a man, ii) aged 18-50 years, iii) attainment of a cycling  $\dot{V}O_{2max} \geq 45$  mL·kg<sup>-1</sup>·min<sup>-1</sup>, and iv) being injury-free for last three months. There is confusion in the literature with regards to the qualitative descriptors of cycling participants ("trained", "well-trained", "elite", etc.) in research studies (De Pauw, Roelands, Cheung, de Geus, Rietjens, & Meeusen, 2013; Jeukendrup et al., 2000). Following the recent guidelines proposed by de Pauw et al. (2013), participants in all studies were, at least, considered as "recreationally trained", with the majority being considered as "trained" or "well-trained".

## **2.3. Laboratory conditions**

All testing sessions were conducted in one of the exercise laboratories at St Mary's University, Twickenham, UK. The environmental conditions within the laboratory were controlled with an air-conditioned system. Across all testing sessions, the means  $\pm$  standard deviations (*SD*) for temperature, humidity and pressure were  $19 \pm 1$  °C,  $36 \pm 8\%$ , and  $1011 \pm 11$  Pa, respectively.

## 2.4. Measurements

### 2.4.1. Gas exchange

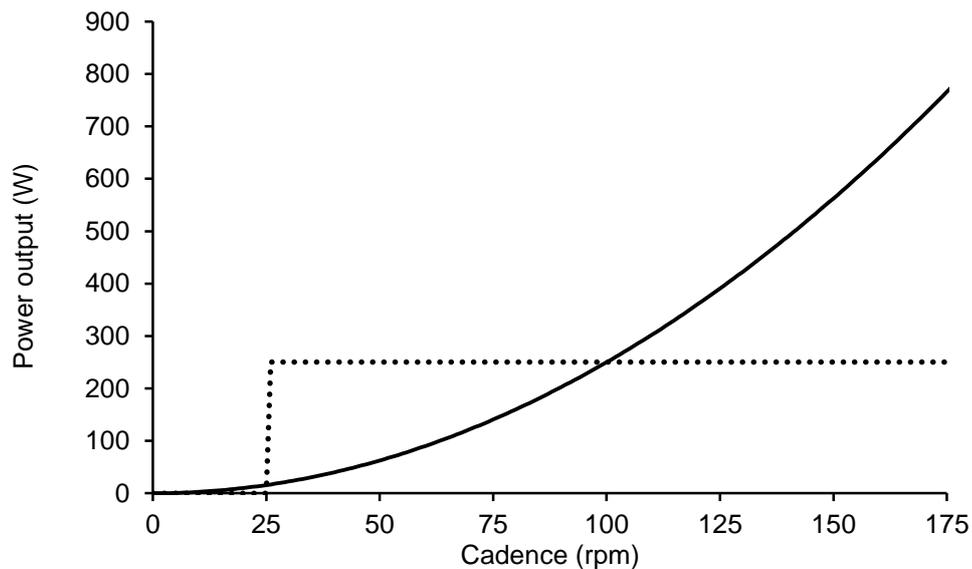
Common to all tests was the measurement of gas exchange using an online, rapid response gas analyser (Oxycon Pro, Jaeger, Hoechberg, Germany). Participants breathed through a silicone facemask (7450, Hans Rudolph Inc., Kansas City, MO) connected to a mouthpiece and impeller and a low resistance ( $0.75 \text{ mmHg}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$ ) turbine assembly (Triple V, Jaeger, Hoechberg, Germany). The combined dead space of the facemask (99 or 125 mL for the small- and medium-size facemask, respectively) and mouthpiece (90 mL) was entered into the gas analyser's software for each participant. Volume ventilation and gas concentrations were continuously sampled at 100 Hz. Expired gas was sampled through a 1.5 m capillary line (0.5 mm internal diameter) connected to the triple-V assembly. Oxygen and  $\text{CO}_2$  concentrations were analysed using differential paramagnetic and infrared absorption analysers, respectively. Oxygen uptake,  $\dot{V}\text{CO}_2$  and minute ventilation were calculated using standard formulae (Beaver, Lamarra, & Wasserman, 1981; Beaver, Wasserman, & Whipp, 1973), and displayed breath-by-breath. The precision of this online gas analyser, as reported by the manufacturer, is 2% for minute ventilation, and 3% for  $\dot{V}\text{O}_2$  and  $\dot{V}\text{CO}_2$  within normal physiological ranges (i.e. minute ventilation  $< 300 \text{ L}\cdot\text{min}^{-1}$ ,  $\dot{V}\text{O}_2$  and  $\dot{V}\text{CO}_2 < 7 \text{ L}\cdot\text{min}^{-1}$ ). Moreover, the validity and reliability of this system has been demonstrated (Carter & Jeukendrup, 2002; Rietjens, Kuipers, Kester, & Keizer, 2001), and the device is commonly used in studies looking at  $\dot{V}\text{O}_2$  kinetics. Prior to any test, the gas analysers were calibrated with gases of known concentration (16%  $\text{O}_2$ ; 5%  $\text{CO}_2$ , 79% nitrogen; Carefusion, Höechberg, Germany) and room air, and the turbine volume transducer was calibrated using a 3 L syringe (Viasys Healthcare, Höechberg, Germany 209 Rudolph, KS).

### 2.4.2. Exercise ergometer

All tests were performed using an electromagnetically braked cycle-ergometer (Lode Excalibur Sport, Groningen, the Netherlands). The cycle-ergometer was set in the hyperbolic mode for all tests (with the exception of the 3AO tests). In the hyperbolic mode, the ergometer exerts a constant resistance (range 8-1500 W) irrespective of the cadence (within the range of 25 – 180 rpm). In Chapters 8 and 9, the cycle-ergometer was also used in the linear mode, where the workload is dependent on the cadence and a linear factor ( $\alpha$ ):

$$\text{Power output (W)} = \text{Cadence}^2 \times \alpha \quad [12]$$

In the linear mode, therefore, power output increases exponentially as a function of cadence (Figure 2.1). According to the manufacturer, the workload accuracy in the hyperbolic mode is 2% for power outputs from 8 W to 1500 W, and 5% for power outputs > 1500 W. The height of the saddle and handle-bar, and the distance between saddle and handle-bar, were individually adjusted for optimal performance. Individual settings were noted and replicated within each study. In all experimental tests, participants wore their own clip-in shoes.



**Figure 2.1.** Exponential increase in power output as function of cadence in the linear mode of the cycle-ergometer (solid line,  $\alpha = 0.030$ ; cadence = 90 rpm). In the hyperbolic function (broken line), power output remains constant irrespective of cadence (providing rpm  $\geq 25$ ).

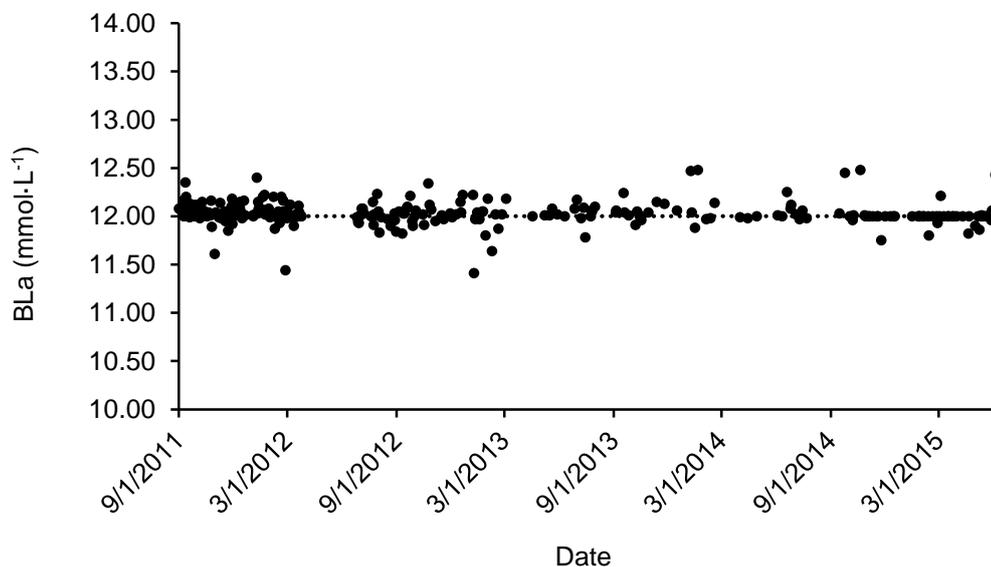
### 2.4.3. Heart rate

Heart rate was recorded continuously in all experiments using a telemetric system. The system consists of a coded strap located on the chest (T31, Polar Electro, Kempele Finland), which detects and transmits the R-R time interval of the electrocardiogram signal to a watch (s610i, Polar Electro, Kempele Finland) via telemetry, where they are recorded at 5 s intervals. As stated by the manufacturer, the accuracy of heart rate measurement using this equipment is  $\pm 1 \text{ b}\cdot\text{min}^{-1}$ , within the measurable range of 15-240  $\text{beats}\cdot\text{min}^{-1}$ .

### 2.4.4. Blood lactate concentration

Capillary blood samples were taken from the earlobe for the determination of BL<sub>a</sub>. The earlobe was initially massaged in order to increase blood flow, cleaned with an alcohol wipe, and punctured using an automatic, single-use lancing device (Safe-T-Pro Plus, Roche Holding, Basel, Switzerland).

Subsequently, 20  $\mu\text{L}$  of blood was collected using end-to-end plastic capillaries tubes heparinised with sodium (Sanguis Counting, Nümbrecht, Germany). Following manufacturer instructions, each whole blood sample was introduced in a plastic tube pre-filled with 1 mL of lactate/glucose haemolysing solution (EKF Diagnostic, Magdeburg, Germany), and subsequently analysed for BL<sub>a</sub> and glucose (not reported) concentration using an enzymatic-amperometric analyser (Biosen C-line, EKF Diagnostic, Magdeburg, Germany). Before any testing session commenced, the system was calibrated automatically following the recommendations of the manufacturer. The test-retest variation of the device at BL<sub>a</sub> concentration typically observed after high-intensity exercise (i.e.  $12 \text{ mmol}\cdot\text{L}^{-1}$ ) is  $\leq 1.5\%$ , as reported by the manufacturer. The BL<sub>a</sub> analyser was regularly calibrated against a sample with a known concentration of  $12 \text{ mmol}\cdot\text{L}^{-1}$  (Figure 2.2). Indeed, the reliability of this lactate gas analyser has been shown to be excellent, both within and between devices (Power, Deacon, Neupert, Ryan, & Jobson, 2014). This device measures BL<sub>a</sub> within the range of  $0.5\text{--}40 \text{ mmol}\cdot\text{L}^{-1}$ , displaying an error if the concentration of BL<sub>a</sub> drops  $< 0.5 \text{ mmol}\cdot\text{L}^{-1}$ . In those instances, a BL<sub>a</sub> of  $0.5 \text{ mmol}\cdot\text{L}^{-1}$  was assumed.



**Figure 2.2.** Measurements of blood lactate concentration, using an enzymatic-amperometric analyser (Biosen C Line, EKF Diagnostic, Magdeburg, Germany), using a standard, calibrated samples of  $12.00 \text{ mmol}\cdot\text{L}^{-1}$  (; denoted by broken line). Data taken from September 2011 to June 2015 ( $n = 422$ ). Mean  $\pm$  SD difference from the calibration sample:  $0.03 \pm 0.14 \text{ mmol}\cdot\text{L}^{-1}$ ; 95% confidence limits: 12.01 to 12.04  $\text{mmol}\cdot\text{L}^{-1}$ .

#### **2.4.5. Anthropometric assessment**

Stature, mass and body composition were determined for all participants at the beginning of each experimental study. Stature was assessed using a stadiometer to the nearest 0.001 m (Seca, Birmingham, UK). Body composition (body mass and fat-free mass) was measured using the air-displacement plethysmography method (BOD-POD, Life Measurement, Inc, Concord, CA). In brief, the BOD-POD first estimates the volume of the person. Subsequently, the mass of the person is measured with an electronic scale. By dividing volume by mass, whole-body density can be estimated. Finally, using Siri's equation (Siri, 1956), whole-body fat mass is estimated from body density. As indicated by the manufacturers, the BOD-POD chamber was calibrated using a ~50 L cylinder and a 20 kg weight. In addition, participants were instructed to wear suitable clothing and a swimming cap. Estimates of body composition derived from BOD-POD are within 1% of those obtained from the hydrostatic weighing and dual-energy X-ray absorptiometry methods (Fields, Goran, & Mccrory, 2002).

**Chapter 3: The effect of the oxygen uptake-power output relationship on the prediction of supramaximal oxygen demands**

A version of this study has been published in the “*Journal of Sports Medicine and Physical Fitness*”.

### **3.1. Abstract**

The aims of this study were to investigate the relationship between oxygen uptake ( $\dot{V}O_2$ ) and power output at intensities below and above the lactate threshold (LT) in cyclists; and to determine the reliability of supramaximal power outputs linearly projected from these relationships. Nine male cyclists (mean  $\pm$  standard deviation age:  $41 \pm 8$  years; mass:  $77 \pm 6$  kg, height:  $1.79 \pm 0.05$  m and  $\dot{V}O_{2max}$ :  $54 \pm 7$  mL·kg<sup>-1</sup>·min<sup>-1</sup>) completed two cycling trials each consisting of a step test (10  $\times$  3 min stages at submaximal incremental intensities) followed by a maximal test to exhaustion. The lines of best fit for  $\dot{V}O_2$  and power output were determined for: the entire step test; stages below and above the LT; and from rolling clusters of five consecutive stages. Lines were projected to determine a power output predicted to elicit 110% peak  $\dot{V}O_2$ . There were strong linear correlations ( $r \geq 0.953$ ;  $P < 0.01$ ) between  $\dot{V}O_2$  and power output using the three approaches; with the slope, intercept, and projected values of these lines unaffected ( $P \geq 0.05$ ) by intensity. The coefficient of variation of the predicted power output at 110%  $\dot{V}O_{2max}$  was 6.7% when using all ten submaximal stages. Cyclists exhibit a linear  $\dot{V}O_2$  and power output relationship when determined using 3 min stages, which allows for prediction of a supramaximal intensity with a 6.7% test-retest reliability.

### **3.2. Introduction**

Anaerobic capacity can be estimated from the maximal accumulated oxygen deficit (MAOD). The MAOD determines the difference between the accumulated oxygen uptake and the accumulated oxygen demand in a supramaximal (i.e. above  $\dot{V}O_{2max}$ ) bout of exercise to exhaustion which lasts two to five min. (Medbø et al., 1988) A power output predicted to elicit supramaximal oxygen demands needs to be estimated, typically, as a projection of the linear relationship between the  $\dot{V}O_2$  and power output. The assumption of a linear relationship between  $\dot{V}O_2$  and power output, however, has been the subject of considerable debate; and is considered to be a major limitation of MAOD (Bangsbo, 1996; Medbø, 1996; Noordhof et al., 2010).

Concerns about the linear relationship between  $\dot{V}O_2$  and power output have mostly derived from differences in the response of  $\dot{V}O_2$  at the onset of exercise below and above the LT (Poole & Jones,

2012). Below LT,  $\dot{V}O_2$  attains a steady state, approximately 2-3 min after the start of the exercise, with a magnitude dictated by power output (Carter et al., 2000). At intensities above LT, but below the CP, a slow component emerges after 2-3 min which elevates  $\dot{V}O_2$  to a steady state value above that predicted from the sub-LT  $\dot{V}O_2$ -power output relationship (Poole et al., 1988). As a consequence, there is a linear relationship between  $\dot{V}O_2$  and power output below LT; but at intensities greater than LT the relationship between  $\dot{V}O_2$  and power output typically exhibits an upward drift (e.g. Barstow & Molé, 1991; Jones et al., 1999; Pedersen et al., 2002; Zoladz et al., 1995, 2002). Crucially, during exercise at intensities above CP, such as those required for MAOD determination, there is no  $\dot{V}O_2$  slow component. Instead,  $\dot{V}O_2$  does not attain a steady state but rises inexorably until  $\dot{V}O_{2max}$  is reached or exhaustion prevents exercise from continuing (Jones et al., 2011; Poole & Jones, 2012).

Before dismissing the potential for MAOD to be used as a means of evaluating AnC, it is important to highlight factors which could solely or interactively serve to influence the slow component of  $\dot{V}O_2$  and the  $\dot{V}O_2$ -power output relationship. First, Noordhof et al. (2010) reported that the submaximal exercise bouts used to construct the  $\dot{V}O_2$ -power output relationship last typically 4 – 10 min. Given the relatively short duration of the MAOD test, and that at intensities above CP there is no  $\dot{V}O_2$  slow component because  $\dot{V}O_2$  rises inexorably until  $\dot{V}O_{2max}$ , the use of  $\dot{V}O_2$  values to construct the  $\dot{V}O_2$ -power output relationship derived from submaximal bouts longer than the typical duration of the test are likely to limit the predictive ability of the resultant relationship. Moreover, extended bouts of submaximal cycling exercise may increase the magnitude of any slow component of  $\dot{V}O_2$  and thereby magnify any deviation from linearity of the  $\dot{V}O_2$ -power output relationship (Green & Dawson, 1996). Secondly, the slow component of  $\dot{V}O_2$  has been shown to be reduced following relatively short (2-8 weeks) periods of endurance training (Carter et al., 2000; Casaburi et al., 1987; Majerczak et al., 2008; Saunders et al., 2003; Womack et al., 1995). Although information on the effects of long-term periods of endurance training on the  $\dot{V}O_2$  slow component is somewhat limited, Lucía et al. (2000) reported that the slow component of  $\dot{V}O_2$  in professional cyclists exercising at ~80% of their  $\dot{V}O_{2max}$  for 20 min was only 0.13 L. Moreover, in well-trained endurance athletes some authors have observed a reduction in the slope of the  $\dot{V}O_2$ -power output relationship at intensities above LT (Bickham et al., 2004; Lucia et al., 2002).

Given that in trained healthy individuals  $\dot{V}O_2$  reaches a steady state in approximately 2–3 min (Carter et al., 2000; Koppo et al., 2004) and that at supramaximal intensities, as required to determine MAOD,

there is no discernable slow component (Jones et al., 2011), consecutive exercise bouts of 3 min at increasing power outputs in endurance-trained athletes may result in a linear relationship between  $\dot{V}O_2$  and power output, as assumed in the MAOD test. The purpose of this study, therefore, was to investigate the  $\dot{V}O_2$ -power output relationship below and above LT in trained cyclists and whether a linear projection of this line can predict supramaximal intensities. Specifically, the aims were to determine a) whether the relationship between  $\dot{V}O_2$  and power output is affected by exercise intensity (below vs. above LT); and b) whether supramaximal power outputs can be reliably predicted from the protocol proposed.

### **3.3. Methods**

#### **3.3.1. Participants**

Nine male cyclists were recruited from local cycling clubs to participate in this study. Means  $\pm$  SD for age, mass and height were  $42 \pm 8$  years,  $77 \pm 6$  kg and  $1.79 \pm 0.05$  m, respectively. All participants met the inclusion criteria of being involved in cycling training for at least the last two years. On a representative week, they completed  $12 \pm 4$  hours of cycling training. Having been informed of the purpose, and possible risks and benefits, participants gave written consent to take part in the study, which was approved by the institutional Ethics Committee.

#### **3.3.2. Experimental overview**

Each participant completed a familiarization trial and two main trials on the same electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The familiarization trial consisted of a cycling ramp test to exhaustion to provide data to assist in the design of the subsequent step incremental tests. Trials 2 and 3, the main trials, were identical, with participants first completing a  $10 \times 3$  min step test, to determine the relationship between  $\dot{V}O_2$  and power output, followed, 5 min later, by a continuous test to exhaustion (maximal test) to determine peak  $\dot{V}O_2$ . All tests were performed at the same time of the day ( $\pm 1$  h) and participants were required to follow the same diet 24 h before every test. In addition, they were requested to refrain from alcohol, caffeine, and strenuous exercise in the 12 h before each trial. All trials were interspersed with at least 48 h for recovery.

### 3.3.3. Procedures

In the familiarization trial, prior to the start of the ramp test, the handlebar and saddle height of the cycle ergometer were individually adjusted, and the settings were noted for replication in subsequent trials. All the participants used clip-in pedals and their own cycling shoes. After 3 min of seated rest, participants started to cycle at 100 W for 3 min. The cadence was freely chosen by each participant and kept constant ( $\pm 5$  rpm) throughout the entire test. The resistance increased continuously thereafter at a constant rate of  $15 \text{ W}\cdot\text{min}^{-1}$  until volitional exhaustion (identified by a decrease  $\geq 10$  rpm for  $\geq 5$  s despite strong verbal encouragement). Preferred cadence was noted for subsequent replication.

In each of the step tests during the main trials, participants firstly rested for 3 min on the cycle ergometer, followed by  $10 \times 3$  min exercise bouts at increasing power outputs interspersed with 30 s of passive rest. The initial power output was 100 W for all the participants, with increases of 15 - 20 W in each subsequent stage. The increases in power output were individually adjusted, based on the results from the ramp test, with the aim of obtaining five stages below and above LT (defined for the purposes of this investigation as an increase of  $0.4 \text{ mmol}\cdot\text{L}^{-1}$  above resting levels). Once the last 3 min stage was completed, participants rested for 5 min before resuming cycling to complete the maximal test. In the first minute of the maximal test, the power output corresponded to the third completed stage of the previous step test, and increased thereafter every minute using same increment as before (i.e. 15 W or 20 W) until volitional exhaustion. Gas exchange was measured breath-by-breath using an online gas analyzer (Oxycon Pro, Jaeger Ltd. Höechberg, Germany). The gas analyser was calibrated before each test in accordance with manufacturer's instructions using a 3 L syringe (Viasys Healthcare, Höechberg, Germany) and gases of known concentrations (5%  $\text{CO}_2$ , 16%  $\text{O}_2$ , 79%  $\text{N}_2$ ; Carefusion, Höechberg, Germany). Heart rate was measured throughout and recorded every 5 s using a telemetric system (Polar S610, Polar Electro, Finland). Capillary blood samples (20  $\mu\text{L}$ ) were collected from the earlobe at rest and in the 30 s of passive rest between each submaximal stage during the step test for the determination of blood lactate concentration (BLa) using an enzymatic-amperometric analyser (Biosen C-line, EKF Diagnostic, Germany). The relationship between BLa and power output was plotted for each individual and LT was determined using software (Lactate-E) specifically developed for the purpose (Newell et al., 2007).

### 3.3.4. Statistical analyses

Data were analysed using IBM SPSS 21.0 software (IBM Corp, Armonk, NY) and are presented as mean  $\pm$  SD. For the step tests, breath-by-breath  $\dot{V}O_2$  was filtered to exclude values outside 4 SD from a local (5-breath) rolling average (Lamarra, Whipp, Ward, & Wasserman, 1987). For each stage,  $\dot{V}O_2$  was estimated as the highest value obtained in the last 60 s of each stage from a breath-by-breath 30 s rolling average. Peak  $\dot{V}O_2$  in the maximal tests was considered as the highest 30 s rolling average attained in each trial.  $\dot{V}O_{2max}$  was defined as the average peak  $\dot{V}O_2$  attained in trials 2 and 3. In both step tests, the relationship between steady state  $\dot{V}O_2$  and power output was studied using three approaches. First, the line-of-best-fit and the Pearson product-moment correlation coefficient were calculated using all 10 submaximal exercise intensities. Secondly, two lines-of-best-fit were fitted to values lying at intensities below and above LT for each trial. Thirdly,  $\dot{V}O_2$  values were grouped into rolling five-point clusters from the start of each test, thereby providing the characteristics of the linear  $\dot{V}O_2$ -power output relationship from the initial five stages of the test (1-5), stages two to six (2-6), and subsequent consecutive five-stage clusters (i.e. 3-7, 4-8, 5-9 and 6-10). The characteristics of the lines-of-best-fit (slope, intercept and Pearson product-moment coefficient) and the power output predicted to elicit 110% of peak  $\dot{V}O_2$  as a linear projection of the line-of-best-fit for values below and above LT were analysed, separately for each trial, using a paired samples t-test. Similarly, the characteristics of the lines and the corresponding supramaximal power output predictions at 110% peak  $\dot{V}O_2$  from each of the rolling clusters were analysed for trials 2 and 3 using repeated measures analysis of variance (ANOVA). Furthermore, the characteristics of all lines and the predicted supramaximal power output from the three approaches were compared using repeated measures ANOVA. Significant effects were investigated using Bonferroni post hoc tests as appropriate. Significance was accepted at  $P < 0.05$ . Test-retest reliability for peak  $\dot{V}O_2$ , LT and predicted intensities at 110% peak  $\dot{V}O_2$  were determined as coefficient of variation (CV) and intraclass correlation coefficient (ICC). The CV was determined from the typical error of the difference expressed as percentage of the mean as described by Hopkins (2000). The ICC was determined using the 3,1 approach, as proposed by Weir (2005)

## 3.4. Results

Peak  $\dot{V}O_2$  in trials 2 and 3 were  $4.12 \pm 0.40$  L $\cdot$ min $^{-1}$  and  $4.21 \pm 0.40$  L $\cdot$ min $^{-1}$  respectively.  $\dot{V}O_{2max}$  was therefore  $4.17 \pm 0.38$  L $\cdot$ min $^{-1}$  ( $54 \pm 7$  mL $\cdot$ kg $^{-1}$  $\cdot$ min $^{-1}$ ). In the step tests, the initial power output of 100 W

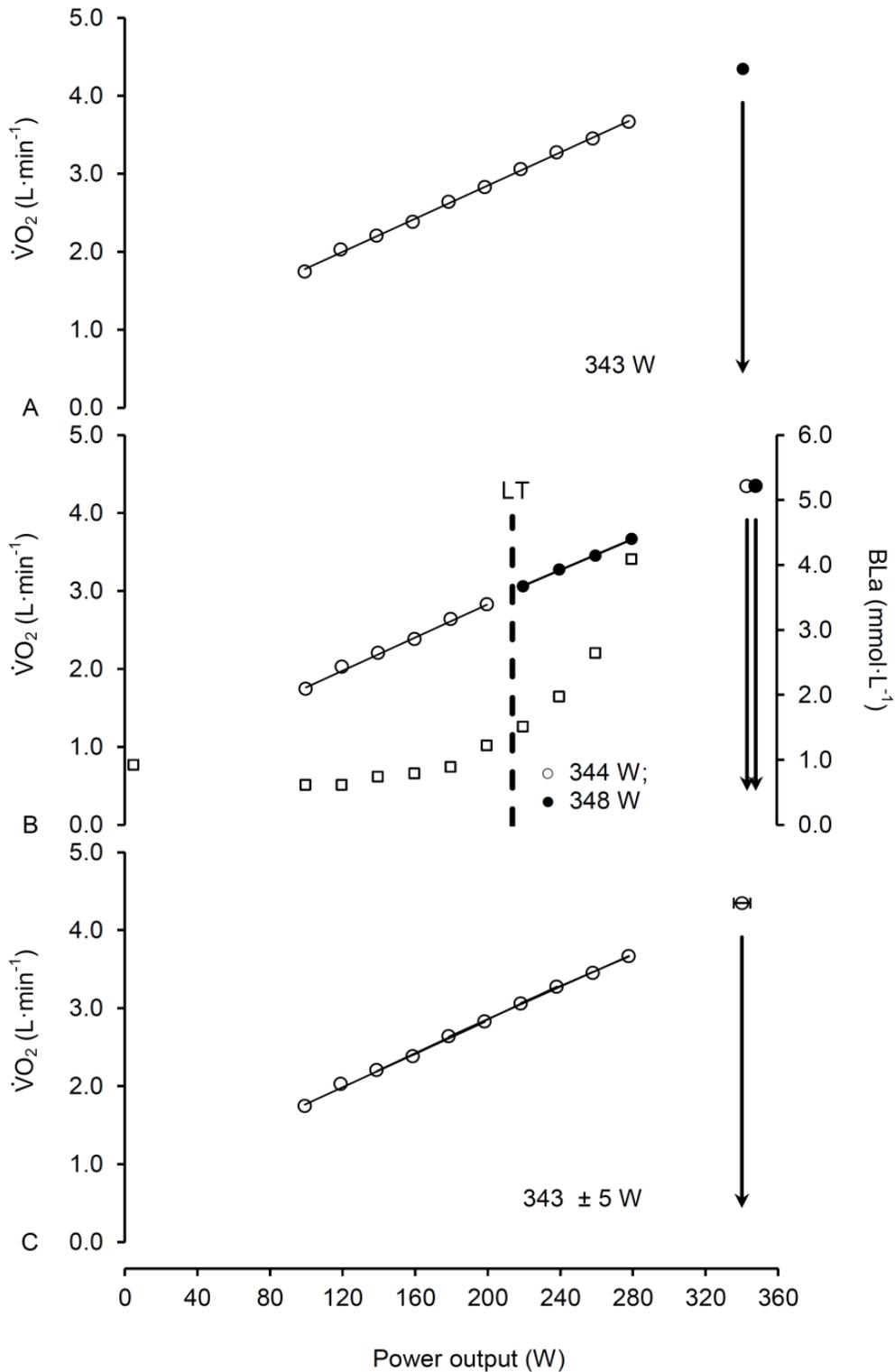
in the first stage corresponded to  $46 \pm 3\%$  and  $44 \pm 3\%$  of  $\dot{V}O_{2max}$  in trials 2 and 3 respectively; whilst the corresponding final stages were at  $96 \pm 2$  and  $94 \pm 6\%$  of  $\dot{V}O_{2max}$ . Peak heart rate in two main trials was  $177 \pm 16$  and  $176 \pm 17$  beats·min<sup>-1</sup>, respectively. In Trial 2, BLa increased from a resting value of  $0.98 \pm 0.29$  to  $5.36 \pm 2.54$  mmol·L<sup>-1</sup> after the tenth stage; whilst the corresponding values for Trial 3 were  $0.82 \pm 0.25$  mmol·L<sup>-1</sup> and  $4.81 \pm 3.17$  mmol·L<sup>-1</sup>, respectively.

### **3.4.1. Linear relationship between $\dot{V}O_2$ -power output**

There was a linear relationship between  $\dot{V}O_2$  and power output in all participants as denoted by the strength of the lines-of-best-fit derived from all three approaches ( $r \geq 0.953$ ;  $P < 0.001$ ). The  $\dot{V}O_2$  and power output for a representative participant is presented in Figure 3.1, along with lines-of-best-fit for the three approaches used to construct the  $\dot{V}O_2$ -power output relationship. Table 3.1 shows the group mean characteristics of the lines-of-best-fit for the entire step test, for values below and above the LT, and for the 5-data point rolling clusters. In trials 2 and 3, the LT was estimated to occur at  $74 \pm 4\%$  and  $74 \pm 9\%$   $\dot{V}O_{2max}$ , respectively; which corresponded to power outputs of  $209 \pm 29$  W and  $206 \pm 35$  W. There were no differences between the slopes, intercepts, or correlation coefficients of the lines-of-best-fit for both Trial 2 ( $P = 0.101$ ;  $P = 0.225$  and  $P = 0.410$ , respectively) and Trial 3 ( $P = 0.601$ ;  $P = 0.852$  and  $P = 0.378$ , respectively) for values below versus above LT. In Trial 2, the predicted power output at 110% peak  $\dot{V}O_2$  from values below LT was lower than that predicted from above LT ( $P = 0.033$ ), whilst in Trial 3 there were no such differences ( $P = 0.470$ ). There were no within-trials differences ( $P \geq 0.05$ ) between five-stage cluster measures of slope, intercept, correlation coefficient, or predicted supramaximal intensity.

### **3.4.2. Test-retest reliability**

The CVs for peak  $\dot{V}O_2$  and LT were 4.8% (95% confident limits (CL): 3.2, 9.3) and 4.3% (2.9, 8.3), respectively; whilst the ICCs were 0.812 (0.317, 0.959) and 0.887 (0.540, 0.976), respectively. The CV and ICC of the power outputs predicted to elicit 110% peak  $\dot{V}O_2$  determined from the three approaches to study the  $\dot{V}O_2$ -power output relationship are presented in Table 3.2.



**Figure 3.1.** Power output predicted to elicit 110% of peak  $\dot{V}O_2$  in a representative participant. In panel A, the power output has been determined as a projection of the  $\dot{V}O_2$ -power output relationship for the entire step test. In panel B, the power output was determined as a linear projection for values below (○) and above (●) LT (□ represent BLa). LT was determined as an increase of 0.4 mmol·L<sup>-1</sup> above resting BLa. In panel C, supramaximal power output was predicted from the consecutive rolling cluster of 5-values (denoted by dotted lines). Note that projections from the three approaches produced similar estimations of supramaximal power output ( $P \geq 0.05$ ).

**Table 3.1.** Characteristics of the lines-of-best-fit for the relationship between  $\dot{V}O_2$  and power output for: i) the entire 10 × 3 min step test, ii) for values below and above LT, and iii) for a 5-value rolling cluster.

	Trial	Entire test	< LT	> LT	1 - 5	2 - 6	3 - 7	4 - 8	5 - 9	6 - 10
Slope (mL·min <sup>-1</sup> ·W <sup>-1</sup> )	2	11.11 ± 1.40	11.52 ± 1.48	9.80 ± 2.66	11.10 ± 1.92	12.01 ± 1.82	11.94 ± 0.85	11.33 ± 1.68	10.84 ± 1.74	10.37 ± 2.37
	3	12.02 ± 1.04	11.60 ± 0.85	12.03 ± 1.64	11.75 ± 1.16	11.74 ± 1.46	12.01 ± 1.52	12.16 ± 1.34	12.47 ± 1.23	12.04 ± 1.59
Intercept (L)	2	0.77 ± 0.22	0.79 ± 0.39	1.11 ± 0.53	0.77 ± 0.28	0.63 ± 0.30	0.63 ± 0.13	0.75 ± 0.33	0.85 ± 0.33	0.96 ± 0.45
	3	0.61 ± 0.21	0.66 ± 0.12	0.63 ± 0.39	0.64 ± 0.17	0.65 ± 0.24	0.61 ± 0.29	0.58 ± 0.31	0.51 ± 0.29	0.61 ± 0.37
Correlation (r value)	2	0.995 ± .005	0.990 ± .011	0.985 ± .015	0.986 ± .012	0.993 ± .006	0.993 ± .007	0.994 ± .006	0.994 ± .005	0.990 ± .009
	3	0.997 ± .001	0.993 ± .006	0.988 ± .014	0.994 ± .002	0.994 ± .003	0.994 ± .004	0.995 ± .002	0.992 ± .004	0.991 ± .006

**Table 3.2.** Power output predicted to elicit 110% of peak  $\dot{V}O_2$  as a projection of the linear relationship between  $\dot{V}O_2$  and power output. Predictions from the 10 × 3-min step test, from values below and above LT, and from 5-value rolling clusters.

	Entire test	< LT	> LT	1 - 5	2 - 6	3 - 7	4 - 8	5 - 9	6 - 10
Trial 2 (W)	341 ± 40	328 ± 38	357 ± 45	347 ± 61	331 ± 49	327 ± 34	337 ± 38	342 ± 36	350 ± 40
Trial 3 (W)	336 ± 38	343 ± 37	336 ± 41	341 ± 42	342 ± 43	338 ± 42	336 ± 41	333 ± 39	337 ± 40
CV (%)	6.65	7.40	7.90	11.41	7.96	6.49	7.11	7.56	7.96
ICC	0.660	0.528	0.613	0.407	0.638	0.688	0.649	0.559	0.563

CV: coefficient of variation. ICC: intraclass correlation coefficient.

### 3.5. Discussion

This study investigated whether the relationship between  $\dot{V}O_2$  and power output remains linear at intensities below and above LT in trained cyclists performing consecutive 3-min stages, and the reliability of those lines to predict a supramaximal intensity. The main finding of the present study was that trained cyclists exhibit a linear relationship between  $\dot{V}O_2$  and power output at intensities below and above LT. The three approaches used to analyse the data resulted in predicted power outputs with acceptable test-retest reliability. The highest test-retest reliability (i.e. lowest CV and highest ICC) appears to be best when using all ten stages to construct the  $\dot{V}O_2$ -power output relationship. These results support the assumption of a linear relationship between  $\dot{V}O_2$  and power output in trained individuals using 3-min stages, as required to predict supramaximal oxygen demands to determine MAOD.

The linear  $\dot{V}O_2$ -power output relationship reported in the current study conflicts with the upward drift in the  $\dot{V}O_2$  above the LT observed in previous studies (e.g. Barstow & Molé, 1991; Jones et al., 1999; Pedersen et al., 2002; Zoladz et al., 1995, 2002). The protocol employed in the present study may, at least partly, explain the different results between studies. In contrast to some research (Barstow & Molé, 1991; Hughson et al., 2000; Jones et al., 1999), participants in the current study performed consecutive stages of a relatively short duration, aiming to attain a steady state in  $\dot{V}O_2$ , but preventing the appearance of a  $\dot{V}O_2$  slow component, as recommended for the prediction of supramaximal intensities (Noordhof et al., 2010). Then again Pedersen et al. (2002) and Zoladz et al. (2002) reported an upward drift of the  $\dot{V}O_2$ -power output relationship above LT using stages of the same duration to those of the current study. Given that the slow component of  $\dot{V}O_2$  might be affected by training status, and that the  $\dot{V}O_{2max}$  values of the participants in both the Pedersen et al. (2002) and Zoladz et al. (2002) studies were comparable to those of the present study, it is difficult to explain this discrepancy. However, a possible contributory factor could be the lack of mode-specific training in those studies. Indeed, endurance-trained runners present no changes (Shaw, Ingham, & Folland, 2014) or even a decrease in the slope of the  $\dot{V}O_2$ -running speed relationship above LT (Bickham et al., 2004).

Whilst endurance training appears to reduce the slow component of  $\dot{V}O_2$ , the mechanisms to explain this response have yet to be elucidated. Nevertheless, it has been suggested that several years of endurance training might change cell myosin heavy chains from type II to I (Wilson, Loenneke, Jo,

Wilson, Zourdos, & Kim, 2012; Yan, Okutsu, Akhtar, & Lira, 2011), which in turn can minimize the slow component of  $\dot{V}O_2$  (Lucía et al., 2000; Majerczak et al., 2008). Indeed, there is a strong negative relationship ( $r = 0.75$ ) between the magnitude of the slow component and the percentage of type I muscle fibres (Poole & Jones, 2012). It seems reasonable to assume, therefore, that the participants in the present study exhibited a large percentage of type I fibres, since mature and more experienced athletes have a higher percentage these fibres than untrained individuals or less experienced athletes (Hopker et al., 2013). Moreover, endurance training has been shown to reduce muscle activation (Saunders et al., 2003), most likely due to a reduced recruitment of type II fibres (Krustrup et al., 2004). Unfortunately, it is not possible to determine the extent to which muscle fibre composition and muscle activation accounted for the results of the present study.

The second aim of this study was to investigate the reliability of a supramaximal power output linearly projected from the  $\dot{V}O_2$ -power output relationship. The results showed a 6.7% test-retest reliability for the predicted power output at 110% of peak  $\dot{V}O_2$ . Moreover, the reliability of LT and peak  $\dot{V}O_2$  was comparable to that of previous research (Barbosa, Montagnna, Denadai, & Greco, 2014). The test-retest reliability of the power output predicted to elicit 110% of peak  $\dot{V}O_2$  was similar from all of the linear regression fits. However, in agreement with the recommendations previous research (Buck & McNaughton, 1999b; Noordhof et al., 2010), the reliability was generally better when using data from all ten submaximal exercise bouts than from smaller datasets.

Although the present study observed a linear relationship between  $\dot{V}O_2$  and power output, it is important to acknowledge that the predicted value at 110% of peak  $\dot{V}O_2$  assumes that this relationship holds true at supramaximal intensities. Indeed, it is possible that although there is no slow component evident in supramaximal exercise (Jones et al., 2011) the mechanisms that underpin the slow component could still be influencing the oxygen demand. Then again, given that the highest submaximal intensities used in the present study of  $\sim 95\% \dot{V}O_{2max}$  showed no deviation from linearity, suggest that the predictive ability of the regression fit is likely to hold true at the supramaximal intensities required to establish MAOD (Shaw, Ingham, & Folland, 2014; Stuart, Howley, Gladden, & Cox, 1981).

In the current study, LT was defined as an increase in BL<sub>a</sub> of  $0.4 \text{ mmol}\cdot\text{L}^{-1}$  above resting values. The nomenclature and methodological approaches surrounding LT determination remains controversial (for reviews, see Binder et al., 2008; Meyer et al., 2005). The purpose of this investigation was to investigate whether the  $\dot{V}O_2$ -power output relationship shifted upwards at intensities above the LT, when BL<sub>a</sub>

accumulates. Mean BLa reached  $\sim 4.8 \text{ mmol L}^{-1}$  at the end of the tenth stage, and  $\dot{V}O_2$  was  $\sim 95\%$  of  $\dot{V}O_{2\max}$ , suggesting that the workrate towards the end of the step test was above LT, irrespective of the method used for LT estimation (Binder et al., 2008; Meyer et al., 2005).

### **3.5.1. Practical Applications**

The present study provides a practical framework to determine supramaximal oxygen demands, as required for those wishing to estimate AnC by means of the AOD protocol. Consecutive 3-min stages allows single-day determination of the LT,  $\dot{V}O_{2\max}$ , and to construct the  $\dot{V}O_2$ -power output relationship, which in turn can be used to calculate power outputs at supramaximal intensities. Athletes and coaches should consider that this method to determine LT,  $\dot{V}O_{2\max}$ , and a supramaximal power output have a test-retest reliability of 4.3, 4.8, and 6.6%, respectively.

### **3.5.2. Conclusion**

In conclusion, this study demonstrates that trained cyclists present a linear increase in  $\dot{V}O_2$  over power outputs from  $\sim 45\%$  to  $\sim 95\%$   $\dot{V}O_{2\max}$  using consecutive 3-min stages. The use of consecutive 3 min bouts of exercise, which minimise the slow component of  $\dot{V}O_2$ , is therefore recommended to predict supramaximal oxygen demands as required in the assessment of MAOD. In addition, consecutive 3-min stages at increasing intensities followed by an incremental test allow reliable determination of LT,  $\dot{V}O_{2\max}$  and prediction of supramaximal intensities.

**Chapter 4: Accumulated oxygen  
deficit during exercise to  
exhaustion determined at different  
supramaximal work-rates**

A version of this study has been accepted, pending minor revisions, in the "*International Journal of Sports Physiology and Performance*".

#### **4.1. Abstract**

The aim of the study was to determine the variability and test-retest reliability of the accumulated oxygen deficit (AOD) during exhaustive cycling exercise at different supramaximal intensities. Twenty one male cyclists and triathletes (mean  $\pm$  standard deviation for age, height and mass were  $41 \pm 7$  years,  $1.82 \pm 0.08$  m and  $79.6 \pm 7.5$  kg, respectively) performed initial tests to determine the linear relationship between oxygen uptake ( $\dot{V}O_2$ ) and power output and maximum  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ). In subsequent trials, AOD was determined from exhaustive square-wave cycling exercise at 105, 112.5 (in duplicate), 120 and 127.5%  $\dot{V}O_{2max}$ . The intensity of the exercise had an effect ( $P = 0.011$ ) on the AOD ( $3.84 \pm 1.11$ ,  $4.23 \pm 0.96$ ,  $4.09 \pm 0.87$  and  $3.93 \pm 0.89$  L at 105, 112.5, 120 and 127.5%  $\dot{V}O_{2max}$ , respectively). Specifically, AOD at 112.5%  $\dot{V}O_{2max}$  was greater than at 105%  $\dot{V}O_{2max}$  ( $P = 0.033$ ); and at 127.5%  $\dot{V}O_{2max}$  ( $P = 0.022$ ); but there were no differences between the AOD at 112.5% and 120%  $\dot{V}O_{2max}$ . In 78% of the participants, the maximal AOD occurred at 112.5 or 120%  $\dot{V}O_{2max}$ . The reliability of AOD at 112.5%  $\dot{V}O_{2max}$ , determined as intraclass correlation coefficient and coefficient of variation, were 0.927 and 8.72% respectively. The AOD, determined from square-wave cycling bouts to exhaustion, peaks at intensities of 112.5-120%  $\dot{V}O_{2max}$ .

#### **4.2. Introduction**

During high-intensity exercise, both aerobic and anaerobic energy systems contribute to meet the energy demands (Gastin, 2001). Aerobic energy production is easily quantified as  $\dot{V}O_2$  (Poole et al., 1991). However, the quantification of anaerobic energy production represents a challenge in exercise physiology (Noordhof et al., 2010, 2013) Moreover, anaerobic energy production is finite, so if high-intensity exercise is sustained to exhaustion, anaerobic energy production is considered to reach its maximum value and represent AnC (Green & Dawson, 1993) Since direct methods to quantify AnC are expensive and/or invasive, indirect approaches such as the accumulated oxygen deficit (AOD) have been developed (Medbø et al., 1988; Noordhof et al., 2010). The AOD is determined as the difference between the sudden increase in oxygen demand and the exponential increase  $\dot{V}O_2$  at the onset of exercise. The quantification of AnC via the AOD relies on a number of assumptions which might compromise the validity of the test (Noordhof et al., 2010).

First, determination of AnC requires exercising at intensities that exceed the  $\dot{V}O_{2max}$  (Medbø et al., 1988; Noordhof et al., 2010; Özyener et al., 2003). The oxygen demands at supramaximal intensities need to be estimated, typically from a linear projection of the relationship between steady-state  $\dot{V}O_2$  and power output at submaximal intensities. The assumption of a linear relationship between  $\dot{V}O_2$  and power output, in turn, is compromised due to the emergence of the slow component of  $\dot{V}O_2$ , which may increase the slope of the  $\dot{V}O_2$ -power output relationship at intensities above the GET. Since at intensities greater than  $\dot{V}O_{2max}$  there is no slow component of  $\dot{V}O_2$  (i.e.  $\dot{V}O_2$  increases inexorably towards  $\dot{V}O_{2max}$ ; Jones et al., 2011). Noordhof et al. (2010) recommended using relatively short exercise bouts to construct the  $\dot{V}O_2$ -power output relationship. Secondly, as a measure of AnC, the AOD is assumed to remain constant at any supramaximal intensity lasting 2-5 minutes (Medbø & Tabata, 1993; Medbø et al., 1988; Noordhof et al., 2010). Whilst consistent AODs have been reported in cycling at 110% and 120%  $\dot{V}O_{2max}$  (Weber & Schneider, 2001) whether the AOD remains consistent determined from CWR at intensities outside the range of 110 – 120%  $\dot{V}O_{2max}$ , but within the range of 2-5 min, remains unknown.

In addition to the methodological issues described above, the reliability of the AOD remains controversial. It is important for athletes and coaches to know the test-retest reliability of a measurement (Hopkins, 2000), but unfortunately only two studies have quantified the test-retest reliability of the AOD (Doherty et al., 2000; Weber & Schneider, 2001). Moreover, the results of these studies were inconsistent. Doherty et al. (2000) concluded that the AOD determined during running exercise was not a reliable test; whilst Weber and Schneider (Weber & Schneider, 2001) reported good test-retest reliability of the AOD in cycling tests at both 110 and 120% of  $\dot{V}O_{2max}$ .

The purpose of this study was to address the above limitations by studying the variability and test-retest reliability of the AOD during cycling at CWRs to exhaustion. Specifically, the primary aim of the study was to determine whether the AOD remains constant during cycling exercise to exhaustion at four supramaximal CWR intensities. The secondary aim of the study was to determine the test-retest reliability of the AOD during identical supramaximal CWRs tests. It was hypothesized that, as an estimate of AnC, supramaximal exhaustive exercise at different supramaximal intensities would result in similar AODs. It was also hypothesised that the AOD would exhibit acceptable test-retest reliability.

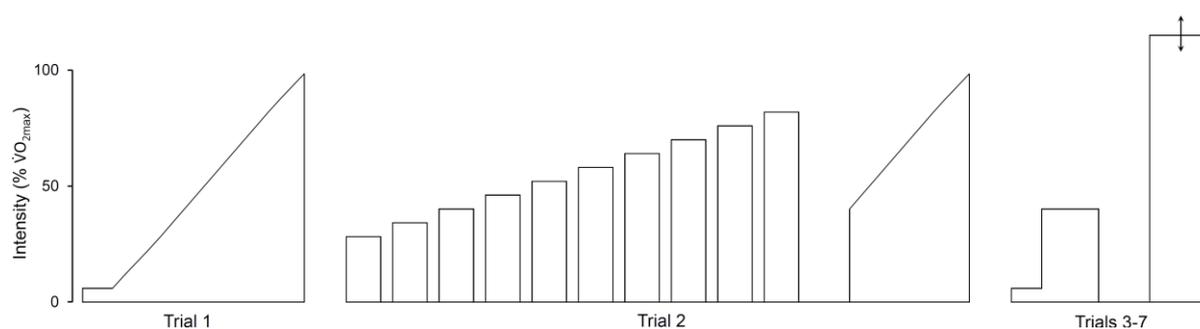
## 4.3. Methods

### 4.3.1. Participants

Twenty-one trained male cyclists and triathletes voluntarily participated in this study. Their mean  $\pm$  SD for age, height and mass were  $41 \pm 7$  years,  $1.82 \pm 0.08$  m and  $79.6 \pm 7.5$  kg, respectively.

### 4.3.2. Experimental overview

Each participant was required to complete seven visits to the physiology laboratory, typically once a week ( $7 \pm 2$  days between trials), with each trial separated by at least 48 h. All trials were conducted on the same individually-adjusted, electromagnetically braked cycle-ergometer (Lode Excalibur Sport, Groningen, the Netherlands) at a similar time of the day ( $\pm 2$  h) and under controlled ambient conditions ( $19 \pm 1$  °C and  $33 \pm 5\%$  humidity). After two preliminary trials to determine GET,  $\dot{V}O_{2\max}$ , and the  $\dot{V}O_2$ -power output relationship, participants completed five experimental trials, each consisting of a CWR to exhaustion at 105, 112.5, 120 or 127.5% of  $\dot{V}O_{2\max}$ . The 112.5%  $\dot{V}O_{2\max}$  trial was repeated to determine test-retest reliability. The order of the experimental trials was randomised, with the exception of the identical trials at 112.5% of  $\dot{V}O_{2\max}$ , which were performed consecutively. Participants were provided with a food record diary and instructed to follow a similar diet and to refrain from strenuous exercise in the 24 h before each trial. In addition, they were instructed to refrain from caffeine and alcohol ingestion 12 h prior to each trial. Figure 4.1 schematically outlines the protocol.



**Figure 4.1.** Outline of the experimental approach.

### 4.3.3. Procedures

Initially, participants completed the preliminary trials. First, a ramp test to exhaustion was used to determine the GET. After three minutes of unloaded pedalling, the resistance increased continuously

at a rate of  $0.5 \text{ W}\cdot\text{s}^{-1}$  (i.e.  $30 \text{ W}\cdot\text{min}^{-1}$ ) until exhaustion, defined by a decrease  $> 10 \text{ rpm}$  for  $> 5 \text{ s}$  despite strong verbal encouragement. The cadence for this trial was freely chosen by each participant ( $87 \pm 8 \text{ rpm}$ ), and remained constant throughout this and subsequent tests. Two researchers independently determined the GET for each participant using the V-slope method (Beaver et al., 1986; Schneider & Phillips, 1993). On a separate day, participants performed a submaximal step test to determine the relationship between  $\dot{V}\text{O}_2$  and power output followed by a ramp to exhaustion to determine  $\dot{V}\text{O}_{2\text{max}}$ . The submaximal step test consisted of  $10 \times 3 \text{ min}$  stages at increasing intensities. The test started at an intensity that corresponded to 50% GET and increased by 10% GET in each subsequent 3 min stage, so that the tenth 3-min stage was completed at 140% GET. There were 30 s of passive recovery between stages to allow a capillary sample to be collected (see below). After completion of the tenth 3 min stage, participants remained seated on the cycle ergometer for five minutes before completing the ramp test to exhaustion. The starting intensity in the ramp test corresponded to 70% GET and increased continuously at a rate of  $15\% \text{ GET}\cdot\text{min}^{-1}$  until exhaustion.  $\dot{V}\text{O}_{2\text{max}}$  was calculated as the highest value derived from a 30-s rolling average; excluding  $\dot{V}\text{O}_2$  values  $\pm 4 \text{ SD}$  outside a local 5-breath average (Lamarra, et al., 1987). Approximately 20 min after the completion of the test, participants performed a supramaximal CWR test to exhaustion for familiarization purposes.

The five experimental trials started with 3 min of unloaded cycling immediately followed by 5 minutes at 70% GET. After a further 5 min of passive rest, participants were instructed to attain their preferred cadence as soon as possible ( $\leq 5 \text{ s}$ ) and to maintain that cadence for as long as possible. The intensity of the trials were 105, 112.5, 120 and 127.5% of  $\dot{V}\text{O}_{2\text{max}}$ . This range of supramaximal intensities (105% - 127.5%  $\dot{V}\text{O}_{2\text{max}}$ ) encompasses the range of typical intensities used during AOD determination, and was intended to cause exhaustion between  $\sim 2$  and  $\sim 5 \text{ min}$  (Hill, Poole, & Smith, 2002; Medbø et al., 1988; Weber & Schneider, 2001). Subjects were unaware of the power output (or percentage of  $\dot{V}\text{O}_{2\text{max}}$ ), elapsed time or expected time to exhaustion (TTE). Capillary blood samples ( $20 \mu\text{L}$ ) were collected 1, 3 and 5 min after exhaustion. The AOD was determined as the difference between the accumulated oxygen demand and accumulated oxygen uptake (Medbø et al., 1988).

#### **4.3.4. Measurements**

During all trials, participants breathed room air through a facemask (Hans Rudolph, Kansas City, MO, USA). Gas exchange samples were collected and analysed breath-by-breath using an open spirometric

system (Oxycon Pro, Jaeger Ltd. Höechberg, Germany). The gas analyser was calibrated before each test accordingly to manufacturer instructions with gases of known concentrations (5% CO<sub>2</sub>, 16% O<sub>2</sub>, 79% nitrogen; Carefusion, Höechberg, Germany) and a 3 L syringe (Viasys Healthcare, Höechberg, Germany). Blood samples were analysed for BLa using the enzymatic-amperimetric method (Biosen C-line, EKF Diagnostic, Germany). Heart rate was measured using a telemetric monitor (Polar S610, Polar Electro, Finland) at 5 s intervals. Breath-by-breath  $\dot{V}O_2$  was filtered (see above) and, subsequently, linearly interpolated to produce second by second data. The accumulated oxygen uptake was determined as the integrated  $\dot{V}O_2$  values from the onset of exercise until exhaustion (recorded to the nearest second). The accumulated oxygen demand was determined as the product of the oxygen demand and TTE. Oxygen demand, in turn, was determined as a linear projection of the  $\dot{V}O_2$ -power output relationship. In the experimental trials, peak heart rate and peak BLa were determined as the highest value recorded during exercise, and the highest post-exercise BLa concentration, respectively. End-exercise  $\dot{V}O_2$  corresponded to the average  $\dot{V}O_2$  during the last 10 s of exercise before exhaustion.

#### **4.3.5. Statistical Analysis**

Data were analysed using IBM SPSS 21 (IBM Corp, Armonk, NY) and presented as mean  $\pm$  SD. Differences between AOD at 105%  $\dot{V}O_{2max}$  (AOD<sub>105</sub>), AOD<sub>112.5</sub>, AOD<sub>120</sub> and AOD<sub>127.5</sub>, alongside other physiological variables (power output, TTE, accumulated oxygen demand and oxygen uptake, peak BLa, peak heart rate and end-exercise  $\dot{V}O_2$ ), were determined using repeated measures ANOVA. The presence of a training or learning effect in the AOD was evaluated by studying the difference between AOD in consecutive trials using repeated measures ANOVA. A *post hoc* Bonferroni t-test was conducted to locate differences between trials if a significant *F* value was detected. Pearson's product-moment correlation coefficients for the relationship between AOD and TTE and between AOD and peak BLa were determined for each supramaximal exercise bout. The test-retest reliability of the AOD was determined as coefficient of variation (CV) and intraclass correlation coefficient (ICC). The CV was determined from the typical error expressed as percentage of the mean (Hopkins, 2000); whilst the ICC was calculated from the standard error of measurement derived from the ANOVA using the 3,1 ICC(Weir, 2005). 95% CL were determined for both measures of reliability. Significance was accepted at  $P < 0.05$ .

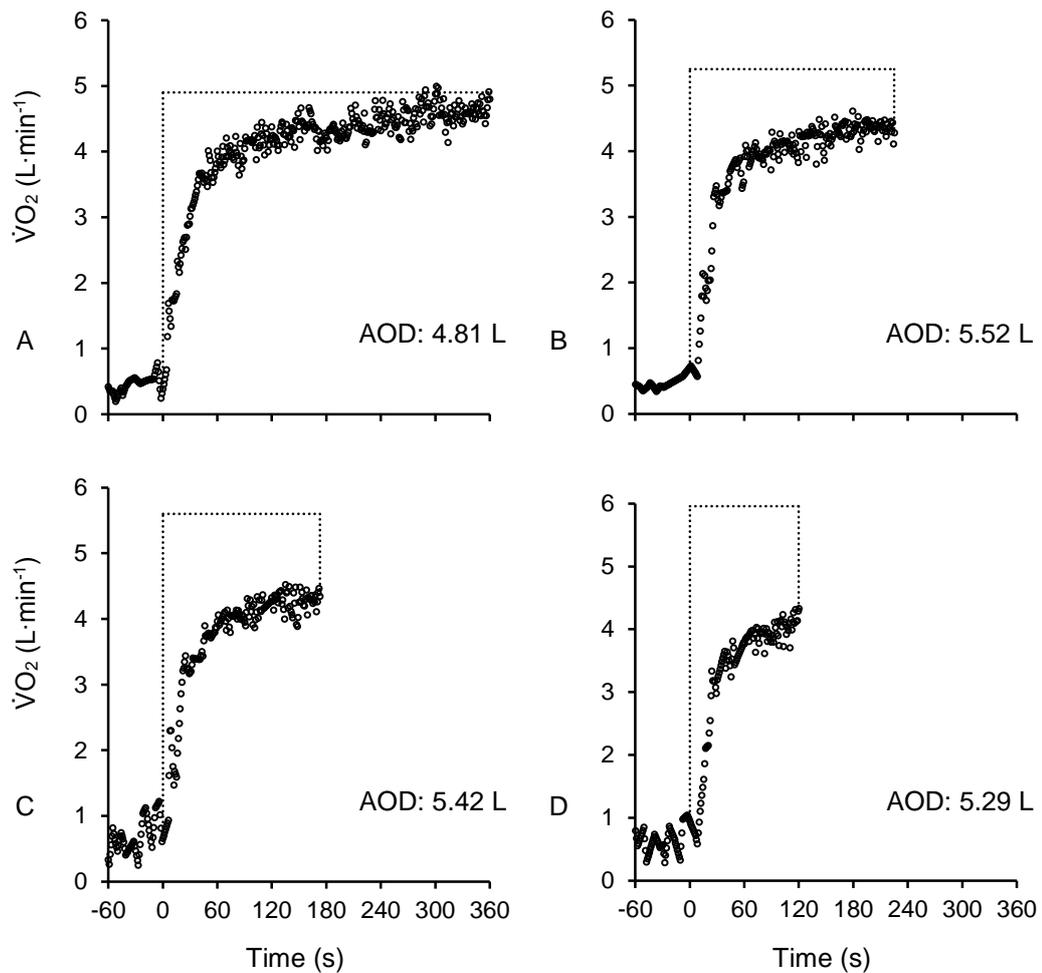
## 4.4. Results

### 4.4.1. Preliminary trials

The GET and  $\dot{V}O_{2max}$  corresponded to  $2.60 \pm 0.33$  L·min<sup>-1</sup> ( $189 \pm 25$  W) and  $4.53 \pm 0.54$  L·min<sup>-1</sup> ( $57 \pm 6$  mL·kg<sup>-1</sup>·min<sup>-1</sup>), respectively. The power output for the initial 3 min stage in the step test was  $95 \pm 13$  W, and increased by  $19 \pm 3$  W in each subsequent stage until the tenth stage, which was completed at  $265 \pm 36$  W. These workloads corresponded to intensities from  $42 \pm 4\%$  to  $85 \pm 6\%$   $\dot{V}O_{2max}$  and were accompanied by increases in BLa from  $0.97 \pm 0.22$  mmol·L<sup>-1</sup> at the end of the first stage to  $4.01 \pm 1.73$  mmol·L<sup>-1</sup> at the end of the tenth stage. There was a strong linear relationship between  $\dot{V}O_2$  and power output ( $P < 0.001$  for all the subjects;  $r = 0.995 \pm 0.005$ ).

### 4.4.2. Experimental trials

One participant experienced technical problems during the supramaximal test at 105%  $\dot{V}O_{2max}$ , and his data were removed from the analysis. Data presented in Table 4.1, therefore, summarises the result for the rest of participants ( $n = 20$ ). The intensity of the supramaximal CWR tests had a significant effect on TTE, accumulated oxygen demand and accumulated oxygen uptake (all  $P < 0.001$ ; Table 4.1). *Post-hoc* tests revealed that TTE, accumulated oxygen demand and accumulated oxygen uptake decreased with each increase in oxygen demand (all  $P < 0.001$ ; Table 4.1). There was no training effect on AOD, as no differences were observed between the AOD during consecutive supramaximal trials ( $P = 0.563$ ). The AOD, however, was affected by the intensity of the supramaximal exercise ( $P = 0.011$ ). *Post-hoc* tests revealed that  $AOD_{112.5}$  was greater than  $AOD_{105}$  ( $P = 0.033$ ) and  $AOD_{127.5}$  ( $P = 0.022$ ). There were no differences ( $P \geq 0.05$ ) between  $AOD_{105}$ ,  $AOD_{120}$  and  $AOD_{127.5}$ . The maximal AOD (MAOD) corresponded to  $4.46 \pm 0.96$  L (or  $56.1 \pm 11.1$  mL·kg<sup>-1</sup>). Ten percent of the participants achieved their MAOD in at 105%  $\dot{V}O_{2max}$ , 48% at 112.5%  $\dot{V}O_{2max}$ , 28% at 120%  $\dot{V}O_{2max}$  and 14% at 127.5%  $\dot{V}O_{2max}$ . The determination of the AOD for a representative subject at each supramaximal intensity is presented in Figure 4.2.



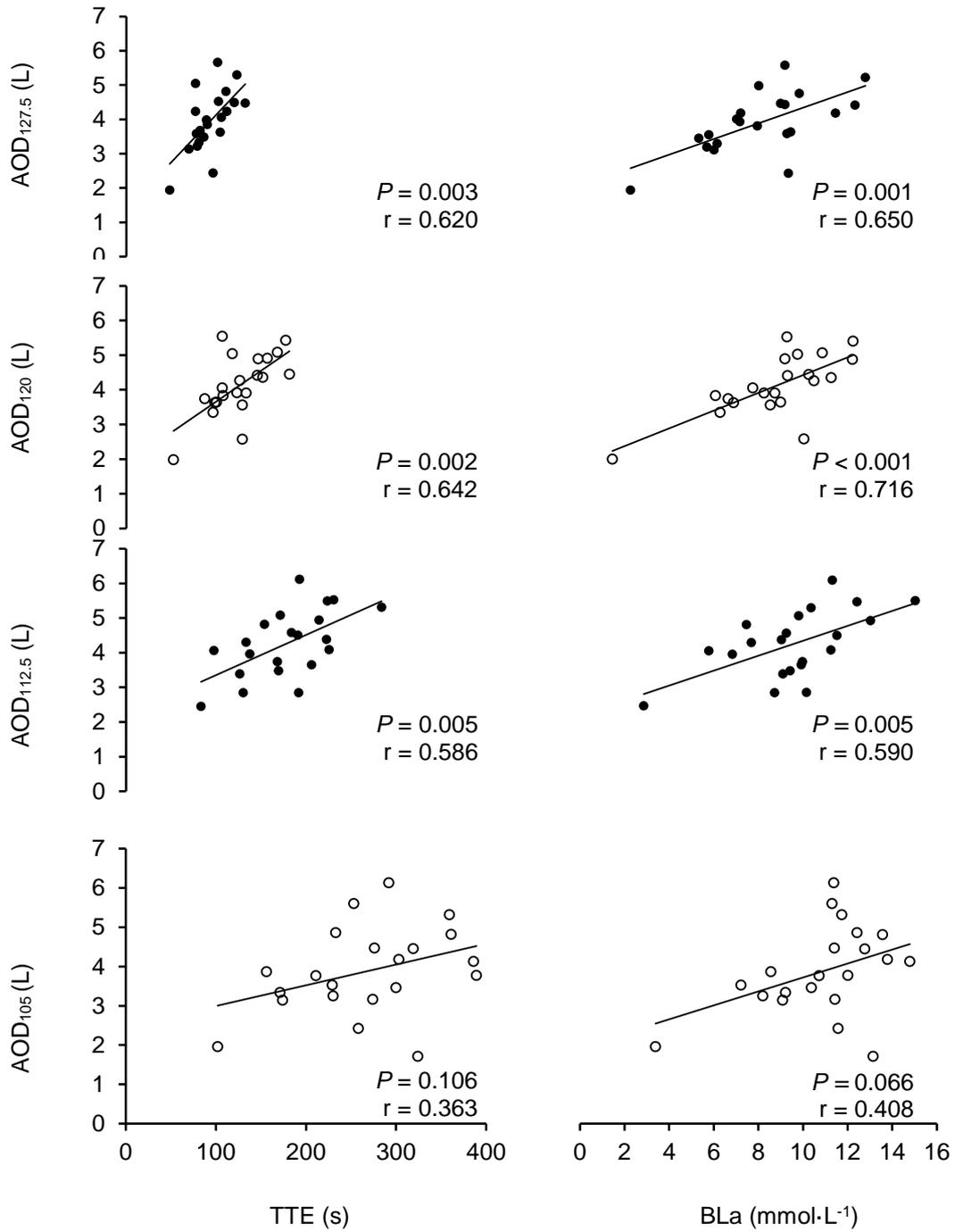
**Figure 4.2.** Determination of the AOD in a representative subject during cycling exercise to exhaustion at 105 (Panel A), 112.5 (Panel B), 120 (Panel C) and 127.5%  $\dot{V}O_{2max}$  (Panel D). Dotted lines represent oxygen demand and open circles  $\dot{V}O_2$ .

#### 4.4.3. Test-retest reliability

One participant did not perform the retest trial at 112.5%  $\dot{V}O_{2max}$  due to training commitments and in another subject there were technical problems during data collection. Therefore, results presented in Table 4.2 correspond to test-retest bouts to exhaustion of the remaining participants ( $n = 19$ ). The test-retest ICC and CV of the AOD were 0.869 [0.691, 0.947] and 8.72% [6.52, 13.16], respectively.

#### 4.4.4. Relationship between accumulated oxygen deficit, time to exhaustion and blood lactate

The relationship between AOD and both TTE and peak BLa at supramaximal intensities are presented in Figure 4.3. AOD and TTE were significantly correlated at 112.5, 120 and 127.5%  $\dot{V}O_{2max}$ , but not at 105%  $\dot{V}O_{2max}$ . Similarly, AOD and peak BLa were significantly correlated in the trials at 112.5, 120 and 127.5%  $\dot{V}O_{2max}$ , but not at 105%  $\dot{V}O_{2max}$ .



**Figure 4.3.** Correlation between accumulated oxygen deficit (AOD) and time to exhaustion (TTE; left panels) and between AOD and blood lactate concentration (BLa; right panels).

**Table 4.1.** Characteristics and physiological responses for cycling bouts to exhaustion at A: 105; B: 112.5; C: 120; and C: 127.5% of  $\dot{V}O_{2max}$  ( $n = 20$ ).

	105%	112.5%	120%	127.5%
Power output (W) <sup>#</sup>	341 ± 48	370 ± 52	399 ± 56	428 ± 59
Duration (s) <sup>#</sup>	267 ± 78	173 ± 48	123 ± 31	91 ± 20
Acc O <sub>2</sub> demand (L) <sup>#</sup>	21.28 ± 6.69	14.81 ± 4.37	11.15 ± 2.95	8.83 ± 2.10
Acc O <sub>2</sub> uptake (L) <sup>#</sup>	17.40 ± 6.02	10.55 ± 3.62	7.03 ± 2.21	4.88 ± 1.33
End-exercise $\dot{V}O_2$ (L·min <sup>-1</sup> ) <sup>‡</sup>	4.50 ± 0.53	4.30 ± 0.63	4.20 ± 0.56	4.12 ± 0.55
AOD (L) <sup>§</sup>	3.87 ± 1.13	4.26 ± 0.97	4.12 ± 0.88	3.96 ± 0.90
AOD (mL·kg <sup>-1</sup> ) <sup>§</sup>	48.52 ± 12.83	53.65 ± 11.86	51.90 ± 11.14	49.74 ± 10.82
Anaerobic contribution (%) <sup>#</sup>	19.1 ± 5.0	29.9 ± 6.0	37.8 ± 5.0	45.1 ± 4.6
Peak BLa (mmol·L <sup>-1</sup> ) <sup>§</sup>	11.67 ± 2.58	10.92 ± 2.48	10.24 ± 2.38	9.56 ± 2.58
Peak HR (beats·min <sup>-1</sup> )	169 ± 13	168 ± 11	166 ± 12	162 ± 11

Acc O<sub>2</sub> demand/uptake: accumulated oxygen demand/uptake; EE: end-exercise; AOD: accumulated oxygen deficit. HR: heart rate. #: Denotes significant differences between all trials. ‡: trial at 105%  $\dot{V}O_{2max}$  was greater than all others. §: trial at 105%  $\dot{V}O_{2max}$  significantly different than at 120 and 127.5; and 112.5% was different than the 127.5% trial.

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**Table 4.2.** Characteristics and physiological responses to two identical cycling trials to exhaustion at 112.5%  $\dot{V}O_{2max}$  ( $n = 19$ ).

	Trial 1	Trial 2	Trial 1 – Trial 2 [95% CL]	ICC [95% CI]	CV [95% CI]
Duration (s)	168 ± 44	160 ± 49	-7 [-22, 7]	0.792 [0.537, 0.914]	14.31 [10.63, 21.87]
Acc $\dot{V}O_2$ (L)	10.11 ± 3.37	9.56 ± 3.47	-0.55 [-1.93, 0.45]	0.735 [0.433, 0.889]	18.79 [13.90, 29.00]
End-exercise $\dot{V}O_2$ (L·min <sup>-1</sup> )	4.27 ± 0.66	4.27 ± 0.55	0.00 [-0.11, 0.11]	0.927 [0.822, 0.971]	3.78 [2.84, 5.64]
AOD (L)	4.19 ± 0.99	4.09 ± 0.98	-0.10 [-0.56, 0.38]	0.869 [0.691, 0.947]	8.72 [6.52, 13.16]
AOD (mL·kg <sup>-1</sup> )	52.3 ± 11.7	51.1 ± 11.8	-1.2 [-6.5, 4.8]	0.866 [0.685, 0.946]	8.72 [6.52, 13.16]
Anaerobic contribution (%)	30.3 ± 6.1	31.0 ± 5.1	0.7 [-1.3, 5.1]	0.669 [0.320, 0.858]	10.68 [7.97, 16.19]
Peak BLa (mmol·L <sup>-1</sup> )	10.88 ± 2.60	10.41 ± 2.75	-0.37 [-1.16, 0.42]	0.818 [0.587, 0.926]	14.16 [10.45, 21.97]
Peak HR (beats·min <sup>-1</sup> )	167 ± 11	165 ± 11	-2 [-5, 1]	0.896 [0.751, 0.959]	2.26 [1.66, 3.51]

ICC: intraclass correlation coefficient; CV: coefficient of variation. 95% CL: 95% confidence limits.

## 4.5. Discussion

The main aim of this study was to determine whether AOD, as a means to quantify AnC, remains constant during exercise to exhaustion at constant supramaximal intensities from 105 to 127.5%  $\dot{V}O_{2max}$ . The secondary aim of the study was to determine the test-retest variability of AOD. The main original finding of the study is that, contrary to the hypothesis, cycling AOD determined from exhaustive CWR supramaximal exercise is affected by the intensity of exercise. Specifically, the AOD at supramaximal intensities followed an inverted U-shape with highest values attained at 112.5% and 120%  $\dot{V}O_{2max}$ . Moreover, at 112.5%  $\dot{V}O_{2max}$ , the AOD has acceptable test-retest reliability. These results suggest that AnC should be determined from a supramaximal CWR to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ . In addition, athletes and coaches need to consider the test-retest reliability of the AOD when using the AOD as a means to quantify AnC.

Part of the variation observed in AOD can be explained by the range of TTEs during the exhaustive CWR tests. Medbø et al. (Medbø & Tabata, 1993; Medbø et al., 1988) reported increases in the AOD concurrent with increases in TTE during CWR to exhaustion shorter than 2 min, possibly because shorter bouts did not allow a full depletion of AnC. Since the CWR test at 127.5%  $\dot{V}O_{2max}$  lasted ~1.5 min, it is possible that AnC was not fully depleted at the time of exhaustion. The finding of a lowered AOD<sub>105</sub> compared to AOD<sub>112</sub> was, however, somewhat unexpected. There are various plausible reasons to explain the reduced AOD observed at the lowest supramaximal intensity. First, exhaustion in the AOD<sub>105</sub> trial occurred in ~4.44 min. Early studies reported a constant AOD during square-wave-exercise bouts lasting up to 15 min (Karlsson & Saltin, 1970; Medbø et al., 1988), although neither of these studies (Karlsson & Saltin, 1970; Medbø et al., 1988) reported the actual intensity as a percentage of  $\dot{V}O_{2max}$ . Moreover, Karlsson and Saltin (1970) used a slightly different protocol to determine AOD and a small number of participants ( $n = 3$ ); and Medbø et al. (1988) used running exercises of up to ~5 min. Secondly, it has been suggested that the MAOD is reached during an exercise protocol that best simulates the athlete's actual competitive event instead of CWR tests to exhaustion (Craig et al., 1995; Noordhof et al., 2010). Using time-trials to determine AOD, however, there might be changes in pacing that, in turn, affect AnC (Jones et al., 2008b). Thirdly, we assumed a linear relationship between  $\dot{V}O_2$  and power output and that the parameters of this relationship remain unaffected during exercise, which, in turn, implies that the oxygen cost for a given supramaximal power output remains constant, provided there is no change in the workrate. However, there is evidence that gross efficiency decreases during

a CWR bout of high intensity exercise (Cannon et al., 2013; Mulder, Noordhof, Malterer, Foster, & De Koning, 2015). Indeed, Mulder et al. (2015) reported a decrease in the AOD during time-trials of increasing duration, which was not observed if the decrease in efficiency was incorporated in AOD calculations. Nevertheless, the relationship between  $\dot{V}O_2$  and power output in the present study was very strong for all participants. Whilst, unfortunately, the data presented herein cannot explain the lowered AOD<sub>112.5</sub>, the present study suggests that supramaximal intensities of 110 to 120%  $\dot{V}O_{2max}$  should be used in order to estimate AnC by means of the AOD method.

The second aim of the present study was to determine the test-retest reliability of AOD at 112.5%  $\dot{V}O_{2max}$ . Similar to the present study, Weber and Schneider (Weber & Schneider, 2001) reported high correlation coefficients ( $\geq 0.95$ ) and low CVs ( $\leq 7\%$ ) for AOD determined at both 110% and 120%  $\dot{V}O_{2max}$ . Doherty et al. (Doherty, Smith, & Schroder, 2000) concluded that the AOD determined from three running tests to exhaustion at 125%  $\dot{V}O_{2max}$  was unreliable; despite an ICC and CV of 0.91 and 6.8% respectively, because of large 95% limits of agreement. The limits of agreement, in turn, have been disregarded by some authors because they are too stringent (Hopkins, 2000; Weir, 2005). It is important to note that the variability in the measurement of AOD reported in the present and previous studies (Doherty et al., 2000; Weber & Schneider, 2001) is still greater than the ~5% test-retest variability typically observed in other physiological parameters such as  $VO_{2max}$  or LT (Barbosa et al., 2014).

The larger variability in AOD compared with other physiological measures can be explained by the protocol employed in the current study to quantify AOD. Open-loop tests have more variation than closed-loop tests (i.e. tests where the duration, distance or work to be completed is known). Although Currell and Jeukendrup (2008) suggested that the greater variability in open-loop tests compared to time trials is augmented at submaximal intensities, open-loop tests performed at high intensities still have more variability than closed-loop tests. The variability in 1.5 km and 5 km running time trials (2.0% and 3.3%, respectively), for instance, is greater than tests at constant speed of similar durations (15.1% and 13.2%, respectively; Laursen, Francis, Abbiss, Newton, & Nosaka, 2007). The latter values approximate the test-retest variability in TTE reported in the present study, despite different modes of exercise (cycling vs. running). Moreover, in cycling, there is a 6–10% variability in TTE during exercise at intensities at or close to  $\dot{V}O_{2max}$  (Barbosa et al., 2014; Laursen, Shing, & Jenkins, 2003). Interestingly,

the curvature constant of the power-duration relationship, which can be considered as a means at estimating anaerobic work capacity (Morton, 2006), also presents high test-retest variability (Johnson, et al., 2011; Simpson et al., 2015). It is therefore plausible that the large test-retest variability of the measurement in the AOD represents the large variability of AnC itself.

Another important finding of the present study was that, in accordance with previous research (Faina et al., 1997), there were moderately strong relationships between AOD and TTE, and between AOD and BLA in three of the four supramaximal intensities tested. Assuming BLA represents a broad estimate of anaerobic glycolysis (Faude et al., 2009), these results suggest that a greater AOD (i.e. greater AnC) allows supramaximal intensities to be sustained for longer.

#### **4.5.1. Practical Applications**

Athletes wishing to determine their AnC by means of the AOD method typically use a single supramaximal exercise bout to exhaustion at constant intensity. The present study demonstrates that the intensity of the supramaximal exercise does affect AOD. It is suggested, therefore, that the determination of AnC using the AOD method is performed from a CWR to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ , where it peaks for 77% of the participants. Moreover, athletes and coaches using the AOD to evaluate AnC should consider that the measurement has 8.72% test-retest variability.

#### **4.5.2. Conclusion**

This study demonstrates that the AOD determined from cycling CWR to exhaustion is affected by the intensity of the exercise (and, consequently, TTE). The AOD followed an inverted U-shape, with 77% of subjects reaching its peak (i.e. MAOD) at either 112.5% or 120%  $\dot{V}O_{2max}$ . The AOD can be used to estimate AnC during a CWR test to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ . At supramaximal intensities, the reliability of AOD is 8.7%.

**Chapter 5. The recovery of  
anaerobic capacity: Practical  
implications for the maximal  
accumulated oxygen deficit**

A version of this study is currently under review in the “*Journal of Strength and Conditioning Research*”.

## 5.1. Abstract

The purpose of this study was to assess whether the accumulated oxygen deficit (AOD), which estimates AnC, could be determined within a single trial. The AOD was determined using a single-trial approach and, subsequently on a separate day, using a traditional approach in 20 trained male cyclists and triathletes (mean  $\pm$  standard deviation age:  $41 \pm 7$  years; peak oxygen uptake ( $\dot{V}O_{2peak}$ ):  $4.57 \pm 0.62$  L $\cdot$ min $^{-1}$ ). In the single-trial approach, subjects performed a submaximal ( $10 \times 3$  min at increasing intensities) and maximal (ramp test to exhaustion) test, followed by 25 min of rest and a supramaximal test to exhaustion at  $112.5\% \dot{V}O_{2max}$  ( $373 \pm 56$  W). The traditional AOD consisted of repeating the supramaximal test to exhaustion on a separate day. The AOD was determined as the difference between the accumulated oxygen demand and the accumulated oxygen uptake. There were no differences between the single-trial and traditional approach for peak  $\dot{V}O_2$ , peak heart rate and peak blood lactate. However, in the single-trial approach, AOD and time to exhaustion were reduced compared to the traditional approach by 17% ( $4.20 \pm 3.40$  vs.  $3.40 \pm 0.70$  L;  $P < 0.001$ ) and 16% ( $168 \pm 53$  vs.  $137 \pm 41$  s;  $P < 0.001$ ), respectively. In conclusion, the single-trial approach underestimates AOD by 17%.

## 5.2. Introduction

Physiological determinants of a relatively short cycling event ( $< 16.1$  km) include the characteristics of oxygen uptake ( $\dot{V}O_2$ ) kinetics, its maximum value ( $\dot{V}O_{2max}$ ), CP, and the capacity to produce energy from anaerobic pathways (Black, Durant, Jones, & Vanhatalo, 2014; Craig & Norton, 2001; Jones & Burnley, 2009). However, whilst there are standard procedures to quantify  $\dot{V}O_2$  kinetics,  $\dot{V}O_{2max}$ , and CP; determination of the finite capacity to produce energy or work from non-oxidative energy systems (i.e. AnC; Green & Dawson, 1993) remains problematic. Given the difficulty of directly quantifying AnC, it is most commonly estimated using indirect approaches. The accumulated oxygen deficit (AOD) was developed by Medbø et al. (1988), and it is currently considered the best non-invasive approach to estimate AnC (Noordhof et al., 2010).

The MAOD test estimates AnC as the difference between the predicted oxygen demand and the accumulated oxygen uptake, typically during a constant work-rate (CWR) exercise bout to exhaustion at a supramaximal intensity (i.e. the energy demand is greater than  $\dot{V}O_{2max}$ ). Among limitations and assumptions discussed elsewhere (Noordhof et al., 2010), this is a time consuming procedure as it requires athletes to perform at least two trials in the laboratory (Medbø et al., 1988; Noordhof et al., 2010). First, the oxygen demand during a supramaximal CWR exercise needs to be estimated, typically as a linear projection of the  $\dot{V}O_2$ -power output relationship. Estimation of that supramaximal oxygen demand requires the athlete to perform several exercise bouts at increasing submaximal intensities to construct a robust  $\dot{V}O_2$ -power output relationship, followed by incremental exercise to exhaustion to determine  $\dot{V}O_{2max}$  (Noordhof et al., 2010). Subsequently, in a separate trial, a bout exercise at a supramaximal CWR to exhaustion is performed. Consecutive submaximal bouts of relatively short duration, as recommended to construct the  $\dot{V}O_2$ -power output relationship (Buck & McNaughton, 1999b; Noordhof et al., 2010), do not appear to affect the  $\dot{V}O_{2max}$  attained in a subsequent maximal test (Scharhag-Rosenberger, Carlsohn, Cassel, Mayer, & Scharhag, 2011). However, it remains unknown whether AOD can be determined as part of the same trial.

In order to determine AOD in a single trial, enough time needs to be provided to ensure a complete recovery between the submaximal and maximal tests to construct the  $\dot{V}O_2$ -power output relationship and  $\dot{V}O_{2max}$ , respectively, and the CWR test to exhaustion to determine MAOD. Unfortunately, the recovery kinetics of AOD has not been studied, which makes it difficult to determine which duration allows a full recovery of AOD. Moreover, a long recovery might compromise practical application of the test. Other approaches used to estimate AnC can help to gain an insight into the recovery kinetics of AnC. The curvature constant of the hyperbolic power-duration relationship ( $W'$ ) represents the finite amount of work that can be performed above CP (i.e. the asymptote of the hyperbola), and was previously considered to be a measure of anaerobic work capacity (Morton, 2006). Although it is currently thought that factors other than AnC determine  $W'$  (Broxterman et al., 2015a,b; Chidnok et al., 2013b,c; Johnson et al., 2014), the strong correlation between AOD and  $W'$  (Chatagnon et al., 2005; Miura et al., 2002) suggests that both measures are underpinned by anaerobic energy production. After exhaustive exercise, the recovery of  $W'$  is non-linear, and appears to follow exponential-like kinetics (Ferguson et al., 2010; Skiba et al., 2012, 2014, 2015). For cycle-ergometer exercise, the time constant has been estimated at ~6.5 min (Ferguson et al., 2010; Skiba et al., 2012), which in turn suggests that

~98% of  $W'$  would be restored after ~25 min. Indeed, estimations of  $W'$  derived from the 3 min all-out test has been shown to be unaffected when 20 min of rest are provided between an incremental test to exhaustion and the 3 min all-out test (Constantini et al., 2014).

Assuming AOD and  $W'$  are determined by a shared factor, it can be hypothesised that MAOD could, with sufficient recovery, be determined in a single trial. The aim of this study was, therefore to test the hypothesis that AOD can be determined in a single trial providing that 25 min of rest are given between the end of the incremental test to determine  $\dot{V}O_{2max}$  and the subsequent CWR test to exhaustion.

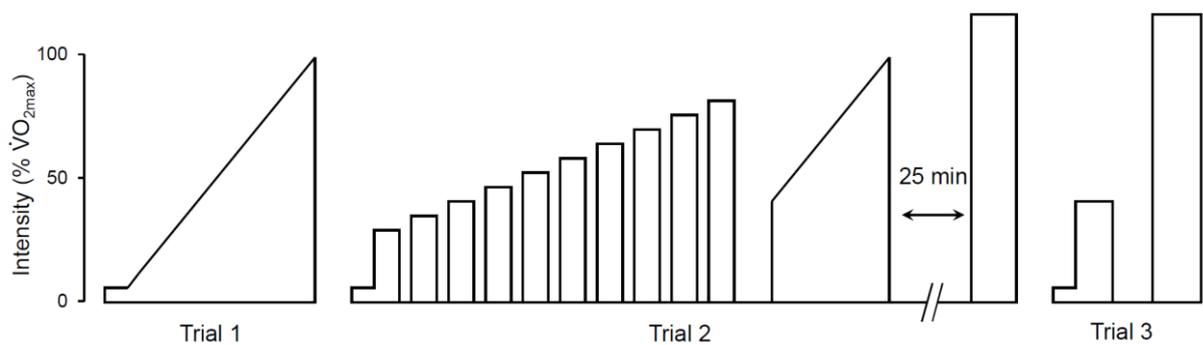
## **5.3. Methods**

### **5.3.1. Participants**

Twenty male trained cyclists and triathletes volunteered to participate in the study. Their means  $\pm$  *SD* for age, mass and stature were  $41 \pm 7$  years,  $79.9 \pm 7.5$  kg and  $1.82 \pm 0.07$  m, respectively. The participants were informed of the aim, and possible risks and benefits associated with the study, and signed a consent form. The study was approved by St Mary's University Ethics Committee.

### **5.3.2. Experimental overview**

Each subject completed three trials in the human performance laboratory. Trial 1, consisted of a ramp test to exhaustion to determine the gas exchange threshold (GET). In Trial 2, participants completed an incremental test (with stages above and below the GET) in order to construct the  $\dot{V}O_2$ -power output relationship, followed by an exhaustive ramp test to determine  $\dot{V}O_{2max}$ . After 25 min of rest, a CWR test to exhaustion at 112.5% of  $\dot{V}O_{2max}$  was performed to determine AOD in a single-day trial ( $AOD_{single}$ ). In Trial 3, the CWR exercise bout was repeated to determine AOD using the traditional approach ( $AOD_{trad}$ ). As per the design, in all participants the  $AOD_{single}$  was determined before the  $MAOD_{trad}$ . All trials were performed at approximately the same time of the day ( $\pm 1$  h). In addition, participants were instructed to follow a similar diet, to refrain from strenuous exercise 24 h before each trial, and to refrain from caffeine and alcohol in the 12 h before each trial. A schematic outline of the protocol is presented in Figure 5.1.



**Figure 5.1.** Outline of the protocol design.

### 5.3.3. Procedures

*Ramp test to exhaustion.* The ramp test to exhaustion started with 3 min of unloaded cycling on a cycle-ergometer. The resistance then increased at a constant rate of  $0.5 \text{ W}\cdot\text{s}^{-1}$  ( $30 \text{ W}\cdot\text{min}^{-1}$ ) until volitional exhaustion, which was defined for this and subsequent trials as a decrease  $> 10 \text{ rev}\cdot\text{min}^{-1}$  for  $> 5 \text{ s}$ . The GET was determined using the V-slope method (Beaver et al., 1986) by two researchers independently, and the average value was used for analysis.

*Single-day trial AOD.* In Trial 2, the linear  $\dot{V}O_2$ -power output relationship was determined from ten consecutive 3 min stages comprising intensities from 50% to 140% GET, with a 10% GET increase in each stage. Stages were interspersed with 30 s of passive rest. After completion of the last stage, participants were given 5 min of passive rest before completing a ramp test to exhaustion. In the ramp test, the initial resistance was 70% GET and increased at a constant rate of  $15\% \text{ GET}\cdot\text{min}^{-1}$  until exhaustion. After completion of the exhaustive ramp test, the participants performed 10 min of unloaded cycling followed by a further 15 min of passive rest. Water was provided *ad libitum* during the recovery period, but ingestion of food or carbohydrate beverages were not permitted. At the end of the recovery period, the CWR exercise bout to exhaustion was conducted. The participants commenced cycling from a stationary start, and were instructed to attain their preferred cadence as quickly as possible (typically  $< 3 \text{ s}$ ) and to continue cycling for as long as possible. Time to exhaustion (TTE) was recorded to the nearest second.

*Traditional AOD.* In Trial 3,  $AOD_{\text{trad}}$  was determined from a CWR exercise bout to exhaustion. After 3 min of rest on the cycle-ergometer, subjects performed an 8 min warm-up consisting of 3 min of

unloaded cycling followed by 5 min at 70% GET. After 5 min of subsequent passive rest, the subjects performed the exhaustive CWR exercise bout as described above.

*Equipment.* All tests were conducted on an electromagnetically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The settings of the ergometer were adjusted in the first trial and replicated in subsequent trials. Subjects were allowed to cycle at their freely chosen cadence in Trial 1 ( $87 \pm 8$  rpm), but instructed to repeat that same cadence in subsequent tests. During all tests, subjects wore a sealed face-mask (Hans Rudolph Inc, Kansas City, MO, USA) that was fitted with a low-resistance volume transducer. Expired gases were analysed using an online gas analyser (Oxycon Pro, Jaeger Ltd. Höechberg, Germany). The gas analyser was calibrated before all tests, in accordance with manufacturer recommendations, with gases of known concentration (16% O<sub>2</sub> and 5% CO<sub>2</sub>; 79% N<sub>2</sub>; Carefusion, Höechberg, Germany) and ambient air. The volume transducer was calibrated using a 3-L syringe (Viasys Healthcare, Höechberg, Germany). Heart rate was recorded at 5 s intervals during all trials with a telemetric heart rate monitor (Polar S610, Polar Electro, Kempele, Finland). In both CWR tests to exhaustion, capillary blood samples (20 µL) were collected from the earlobe at rest (~1 min before the start of the test) and 1, 3 and 5 min after exhaustion. Whole blood samples were analysed for blood lactate (BLa) concentration using a lactate/glucose enzymatic-amperometric analyser (Biosen C-line, EKF Diagnostic, Magdeburg, Germany).

#### **5.3.4. Data analyses**

In Trial 2, to construct the  $\dot{V}O_2$ -power output relationship, breath-by-breath  $\dot{V}O_2$  data were initially filtered, whereby  $\dot{V}O_2$  values outside 4 SD from a 5-breath rolling average were excluded. Subsequently, the highest  $\dot{V}O_2$  attained within the last minute of each stage was determined from a 30 s rolling average. The  $\dot{V}O_2$ -power output relationship was linearly extrapolated to predict power output corresponding to an oxygen demand of 112.5%  $\dot{V}O_{2max}$ . During the CWR tests to exhaustion, the O<sub>2</sub> demand was assumed to remain constant, so the accumulated O<sub>2</sub> demand was calculated as the product of the oxygen demand (i.e. 112.5%  $\dot{V}O_{2max}$ ) and TTE. To calculate the accumulated oxygen uptake, further to the filtering described above, breath-by-breath  $\dot{V}O_2$  values were linearly interpolated to produce second by second data. The accumulated oxygen uptake was determined by integration of the  $\dot{V}O_2$  data. The aerobic energy production was compared in the two CWR tests to exhaustion as the accumulated oxygen uptake in the first minute (isotime). In both CWR tests, resting  $\dot{V}O_2$  and heart rate

were determined as the mean value between 90 s and 30 s before the start of each test. Peak  $\dot{V}O_2$  was determined as the mean  $\dot{V}O_2$  during the last 10 s before exhaustion, and peak heart rate was considered as the highest heart rate value recorded. Peak BLa was determined as the highest BLa value. Data were analysed using IBM SPSS 21 (IBM Corp, Armonk, NY), and presented as mean  $\pm$  SD. Physiological responses to the supramaximal CWR tests to exhaustion in the single-trial approach and the traditional approach were compared using paired samples t-tests. Significance was accepted at  $P \leq 0.05$ .

## 5.4. Results

### 5.4.1. Preliminary testing

The GET occurred at  $2.60 \pm 0.33$  L $\cdot$ min $^{-1}$  ( $189 \pm 25$  W), whilst  $\dot{V}O_{2max}$  was  $4.57 \pm 0.62$  L $\cdot$ min $^{-1}$  or  $57 \pm 6$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ . The stages in the step test increased from  $94 \pm 13$  W ( $1.89 \pm 0.26$  L $\cdot$ min $^{-1}$ ) at 50% GET to  $263 \pm 36$  W ( $3.85 \pm 0.52$  L $\cdot$ min $^{-1}$ ) at 140% GET. There was a strong linear relationship between  $\dot{V}O_2$  and power output ( $P < 0.001$  for all the subjects;  $r = 0.995 \pm 0.005$ ). Projection of the linear  $\dot{V}O_2$ -power output relationship resulted in a CWR intensity predicted to elicit 112.5%  $\dot{V}O_{2max}$  of  $373 \pm 56$  W.

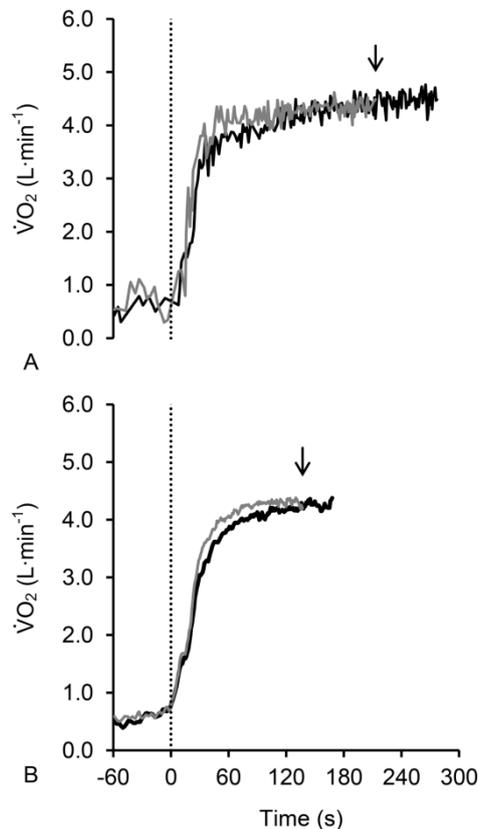
### 5.4.2. Traditional vs. single trial

The characteristics and physiological responses to both exercise bouts at 112.5%  $\dot{V}O_{2max}$  are presented in Table 5.1. MAOD<sub>single</sub> was  $0.80 \pm 0.62$  L lower than MAOD<sub>trad</sub> ( $P < 0.001$ ). Similarly, TTE was  $31 \pm 29$  s shorter in the single-day trial compared to the control trial ( $P < 0.001$ ). Resting  $\dot{V}O_2$  was not significantly different immediately before the supramaximal tests used to determine AOD in the traditional vs. single-day approaches. Similarly, there were no differences in peak  $\dot{V}O_2$ , heart rate or BLa between both trials; but resting BLa was  $4.14 \pm 2.06$  mmol $\cdot$ L $^{-1}$  higher in the single-trial CWR test (Table 5.1). The  $\dot{V}O_2$  response during both CWR tests to exhaustion from a representative subject and the group mean are presented in Figure 5.2.

**Table 5.1.** Physiological responses to a constant work-rate exercise bout to exhaustion at 112.5%  $\dot{V}O_{2max}$  ( $373 \pm 56$  W).

	Traditional	Single trial	Traditional- Single trial difference	<i>P</i> value
TTE (s)	168 ± 53	137 ± 41	31 ± 29	< 0.001
Acc O <sub>2</sub> demand (L)	14.29 ± 4.69	11.64 ± 3.46	2.65 ± 2.60	< 0.001
Acc O <sub>2</sub> uptake (L)	10.10 ± 3.93	8.24 ± 2.91	1.85 ± 2.27	0.002
Acc O <sub>2</sub> uptake isotime (L)	2.66 ± 0.39	2.89 ± 0.39	-0.22 ± 0.23	0.001
AOD (L)	4.20 ± 1.00	3.40 ± 0.70	0.80 ± 0.62	< 0.001
AOD (mL·kg <sup>-1</sup> )	53 ± 13	43 ± 9	10 ± 7	< 0.001
Rest $\dot{V}O_2$ (L·min <sup>-1</sup> )	0.55 ± 0.11	0.60 ± 0.10	-0.05 ± 0.11	0.083
Peak $\dot{V}O_2$ (L·min <sup>-1</sup> )	4.25 ± 0.62	4.26 ± 0.62	-0.01 ± 0.028	0.865
Work (kJ)	61.6 ± 19.1	50.3 ± 13.6	11.4 ± 11.3	< 0.001
Rest BLa (mmol·L <sup>-1</sup> )	0.81 ± 0.22	4.97 ± 1.98	-4.14 ± 2.06	< 0.001
Peak BLa (mmol·L <sup>-1</sup> )	10.69 ± 2.61	11.09 ± 3.56	-0.40 ± 3.09	0.573
Rest HR (beat·min <sup>-1</sup> )	77 ± 10	88 ± 12	11 ± 11	< 0.001
Peak HR (beat·min <sup>-1</sup> )	167 ± 11	167 ± 11	0 ± 5	0.932

TTE: time to exhaustion. Acc: accumulated. MAOD: maximal accumulated oxygen deficit.  $\dot{V}O_2$ : rate of oxygen uptake. BLa: blood lactate concentration. HR: heart rate.



**Figure 5.2.** Oxygen uptake during the single trial (grey line) and the traditional (black lines) approach to determine MAOD for a representative subject (Panel A) and for the mean group (Panel B). Vertical arrows indicate exhaustion during the single-trial approach.

## 5.5. Discussion

This study investigated whether the AOD, a measure that can be used to estimate AnC (Noordhof et al., 2010), could be determined in a single-day trial. The main original finding was that  $AOD_{\text{single}}$  was reduced by 17% in compared to  $AOD_{\text{trad}}$ , with a comparable 16% decrease in TTE. The mechanisms underpinning this reduction in AOD and TTE were beyond the scope of this study, but likely relate to residual fatigue from the submaximal and maximal exercise performed before the CWR test to exhaustion in the single trial. Irrespective of the mechanism underpinning the reduced MAOD, these results demonstrate that, in order to determine MAOD in a single trial, a resting period of more than 25 min should be provided between the maximal test to determine  $\dot{V}O_{2\text{max}}$  and the CWR. Unfortunately, increasing the recovery time would compromise the practicality of determining AOD in a single trial.

The traditional and single-trial approaches used in the present study differed in the exercise performed immediately before the supramaximal bout used to determine AOD. Whilst  $AOD_{\text{single}}$  was preceded by ten 3-min stages at moderate and heavy intensities followed by a maximal test;  $AOD_{\text{trad}}$  was determined after a warm-up at a moderate intensity. Prior exercise at intensities in the heavy exercise domain increases the aerobic contribution in the primed (i.e. second) exercise bout, which results in an increased performance (i.e. longer TTE) (Jones, Wilkerson, Burnley, & Koppo, 2003). The kinetic parameters of  $\dot{V}O_2$  were not characterized in the present study because only one rest-to-exercise transition was performed in each trial. Nonetheless, the increased accumulated oxygen uptake at isotime (Table 5.1, Figure 5.2) suggests that a priming effect was present in the CWR test to exhaustion using the single-trial approach. Immediately before  $AOD_{\text{single}}$  was determined,  $BLa$  was  $\sim 5 \text{ mmol}\cdot\text{L}^{-1}$ , which lies at the upper limit of resting  $BLa$  values for prior exercise to elicit a positive effect on subsequent exercise, possibly by facilitating oxygen availability through vasodilation and a Bohr shift in the oxygen-dissociation curve (Bailey, Vanhatalo, Wilkerson, Dimenna, & Jones, 2009). The effects of prior high intensity exercise seem to be determined by the intensity of the priming exercise and the duration of the rest period between the two exercise bouts (Bailey et al., 2009; Burnley, Davison, & Baker, 2011). Importantly, the exercise conducted prior to  $AOD_{\text{single}}$  was not intended to cause a priming effect, and was substantially different from the typical 6 min non-exhaustive CWR exercise bout at a heavy/severe intensity used to induce a priming effect (Bailey et al., 2009; Burnley et al., 2011; Jones et al., 2003; Jones, Fulford, & Wilkerson, 2008a). It is possible, therefore, that prior exercise increased the aerobic contribution during the CWR to exhaustion of the single-trial approach, but residual fatigue

from the ramp test to exhaustion compromised anaerobic energy production (i.e. AOD), which caused an overall reduction in performance (i.e. TTE).

Anaerobic capacity derives energy provision from intramuscular phosphates and anaerobic glycolysis (Green & Dawson, 1993; Noordhof et al., 2010). During exhaustive exercise, phosphocreatine (PCr) decreases until a critical threshold is attained at exhaustion, where the concentration of PCr corresponds to 5–30% of that at rest (Cannon et al., 2011; Karatzaferi et al., 2001; Vanhatalo et al., 2010; Wackerhage, Hoffmann, Essfeld, Leyk, Mueller, Zange, 1998). However, PCr is rapidly restored after exhaustion, so that within the first ~10 min of recovery PCr is restored to > 90% of its resting value (McMahon & Jenkins, 2002; Skiba, Fulford, Clarke, Vanhatalo, & Jones, 2015). Therefore it is expected that PCr would be fully restored at the start of the single-trial CWR test. The other major component of anaerobic energy production is anaerobic glycolysis, whose main substrate is muscle glycogen. The prior submaximal and maximal tests performed prior the CWR in the single-trial approach might have reduced glycogen content in type I and II fibres, respectively (Carter et al., 2004). Whilst glycogen depletion might not be a chief cause of exercise intolerance during a supramaximal CWR exercise bout to exhaustion (Bangsbo et al., 1992), it is plausible that low glycogen in selected fibres, or selected pools within the muscle fibre (Ørtenblad et al., 2013), contributed to the impaired performance observed in the single-trial.

In CWR tests to exhaustion, such as those performed in the present study, the cause of fatigue (i.e. exercise intolerance) is complex and, likely, multifactorial. Further to depletion of energy stores, the accumulation of metabolites derived from anaerobic energy production such as adenosine diphosphate, inorganic phosphate or hydrogen ions (i.e. low pH) have been suggested to disturb muscle homeostasis and, ultimately, cause exercise intolerance (Kent-Braun et al., 2012). The recovery kinetics of these metabolites (adenosine diphosphate and inorganic phosphate), however, suggests that the 25 min would be enough to restore muscle homeostasis between the maximal and CWR tests to exhaustion in the single-trial approach (Chidnok, Fulford, et al., 2013; Wackerhage et al., 1998). Nonetheless, as discussed above, intramuscular pH was likely decreased immediately before the CWR test to exhaustion in the single-trial (McCormick, Meijen, & Marcora, 2015; Wackerhage et al., 1998), as denoted by the higher resting BLa. Further evidence of a complete recovery of AnC can be derived from  $W'$ , which was traditionally considered as another paradigm to estimate AnC. Whilst current evidence

suggests  $W'$  appears to be recovered in ~25 min (Constantini, Sabapathy, & Cross, 2014; Ferguson et al., 2010; Skiba et al., 2012, 2015), the present study suggests the same is not true for MAOD. There are other models of fatigue that incorporate psychological and psychobiological factors (McCormick, Meijen, & Marcora, 2015; Noakes, 2000), that might explain the reduced performance during the CWR to exhaustion in the single-trial approach despite a theoretical complete recovery of AnC. The current study did not investigate the mechanisms underpinning exercise intolerance, so it is not possible to determine which, if any, of the mechanisms discussed above caused the reduced MAOD and TTE observed during the single-trial approach.

Lastly, it is worth considering whether a learning effect may have influenced these results, since the order of the single-trial and traditional approaches were not randomized and all subjects completed first the single trial. If the reduced TTE and MAOD observed in the single-trial were due to a learning effect, a familiarization of the CWR to exhaustion test would be required. However, adding a familiarization test would hamper the potential for MAOD to be determined in a single trial. Moreover, whilst the possibility of the learning effect cannot be ruled out, the results of the current study suggest that subjects were exercising maximally given there were no differences in peak  $\dot{V}O_2$ , heart rate or BLa between the CWR tests to exhaustion during the single-trial and traditional approaches.

### **5.5.1. Practical applications**

This study provides evidence that a CWR test to exhaustion performed 25 min after submaximal and maximal tests to construct the  $\dot{V}O_2$ -power output relationship and determine  $\dot{V}O_{2max}$ , respectively, underestimates AnC, as evidenced by a reduced MAOD. Whilst increasing the recovery period between the ramp and CWR tests to exhaustion over 25 min might allow MAOD to be determined in a single trial, it would result in a long, non-practical testing protocol. Therefore, the present study suggests that estimations of AnC via MAOD should not be determined using the single-trial approach. Instead, based on the present study, it is recommended that MAOD is determined in a separate trial, using the traditional approach.

### **5.5.2. Conclusion**

The estimation of AnC by means of AOD determination currently requires two laboratory visits. This study investigated whether a more time-efficient approach could be used to determine AOD in a single-

day trial. The main finding of the study was that AOD (as well as TTE, and work done) were reduced in the single-day trial, compared to a traditional approach.

**Chapter 6. Accumulated oxygen deficit and  $W'$  during all-out and constant intensity exercise**

## 6.1. Abstract

The aims of this study were to i) investigate whether estimates of AnC as the accumulated oxygen deficit (AOD) and the curvature constant of the power-duration relationship ( $W'$ ) remain constant during supramaximal exercise to exhaustion at constant work-rate (CWR) and all-out exercises; and ii) determine the relationship between AOD and  $W'$  during CWR and all-out exercises. Twenty-one male cyclists completed preliminary testing to determine the gas exchange threshold,  $\dot{V}O_2$ -power output relationship and  $\dot{V}O_{2max}$ . Subsequently, AOD and  $W'$  were determined during a CWR to exhaustion at 112.5%  $\dot{V}O_{2max}$  and a 3-min all-out (3AO) test. In both tests, AOD was determined as the difference between the accumulated oxygen demand and oxygen uptake; and  $W'$  as work completed above CP. There were no differences between CWR and the 3AO tests for duration ( $164 \pm 46$  s vs.  $180 \pm 0$  s, respectively;  $P = 0.127$ ) or average power output ( $376 \pm 55$  W vs.  $376 \pm 55$  W, respectively;  $P = 0.882$ ). However, there were differences between the CWR and 3AO tests for AOD ( $4.18 \pm 0.95$  L vs.  $3.68 \pm 0.98$  L respectively;  $P = 0.004$ ) and  $W'$  ( $9.55 \pm 4.00$  kJ vs.  $11.37 \pm 3.84$  kJ respectively;  $P = 0.010$ ). AOD and  $W'$  were correlated in CWR ( $P < 0.001$ ,  $r = 0.654$ ) and 3AO ( $P < 0.001$ ,  $r = 0.664$ ). Despite the correlation between AOD and  $W'$ , CWR and 3AO affected the magnitude of AOD and  $W'$ , suggesting that the mechanisms underpinning AOD and  $W'$  may be different, and affected by factors other than AnC.

## 6.2. Introduction

At the onset of exercise, ATP stores in skeletal muscle are continuously resynthesised by the complex and closely integrated interaction of aerobic and anaerobic energy pathways (Gastin, 2001). However, whilst aerobic energy production is relatively easy to quantify as  $\dot{V}O_2$ , quantification of AnC remains challenging (Noordhof et al., 2010, 2013). Direct methods for quantifying AnC are invasive and/or expensive, and, as a consequence, AnC is more commonly estimated using indirect tests (Noordhof et al., 2010).

The AOD, as proposed by Medbø et al. (1988), is a common approach to estimate AnC by determination of the difference between the accumulated oxygen demand and the accumulated oxygen uptake. The AOD can be determined from a CWR exercise bout to exhaustion at supramaximal intensity (i.e. above  $\dot{V}O_{2max}$ ); or an all-out test of known duration. In order to be considered as a measure of AnC, the AOD

needs to reach its maximum value. Using a supramaximal CWR test to exhaustion, it has been shown that the highest AOD is attained in tests lasting 2-5 min, which corresponded to intensities of 110-120% of  $\dot{V}O_{2max}$  (Medbø et al. 1988; Weber & Schneider, 2001). The AOD determined during all-out efforts also appears to be sensitive to the duration of the test. All-out tests shorter than 60 s seem to underestimate AnC (Calbet et al., 1997). Instead, if the all-out effort lasts 60-90 s, the AOD plateaus and reaches its maximum value (Gastin et al., 1995; Whitters et al., 1991; 1993). The effect of all-out efforts longer than 90 s on the AOD has not been studied. It is important to note that the AOD relies on the assumptions that i) the oxygen demand can be extrapolated from the  $\dot{V}O_2$ -power output relationship determined at submaximal intensities; and ii) for a given power output, the required oxygen demand is not altered during high-intensity exercise. Whilst both assumptions have been questioned, the AOD continues to be considered the best non-invasive test to estimate AnC (Noordhof et al., 2010).

Another approach to estimate AnC has been derived from the parameters of the hyperbolic power-duration relationship. The first component is the asymptote of the hyperbola, termed CP, which represents the boundary between the heavy and severe exercise domains (Morton, 2006, Walsh, 2000). The second component is the curvature constant ( $W'$ ), which represents a fixed amount of work that can be performed above CP. Traditionally,  $W'$  has been known as anaerobic work capacity, and thought to represent work produced using anaerobic energy sources (Hill, 1993; Morton, 2006). However, it has been recently suggested that the precise aetiology of  $W'$  might be more complex than simply anaerobic energy production, and its underpinning mechanisms remains to be determined (Broxterman et al., 2015; Murgatroyd et al., 2011; Murgatroyd & Wylde, 2011; Simpson et al., 2015). Nonetheless,  $W'$  is affected by glycogen content (Miura et al., 2000) and creatine supplementation (Smith et al., 1998). Moreover,  $W'$  depletion results in the build-up of fatigue-inducing by-products from anaerobic energy production (Jones et al., 2008c; Poole et al., 1988), and the rate of accumulation of these metabolites is proportional to the rate of  $W'$  depletion (Vanhatalo et al., 2010b). As a result, the magnitude of  $W'$  typically remains constant irrespective of its rate of depletion (Chidnok et al., 2013a; Fukuba et al., 2003).

Since AOD and  $W'$  might represent AnC, it is not surprising that there is a strong correlation observed between them (Chatagnon et al., 2005; Miura et al., 2002). In the studies reporting a correlation between AOD and  $W'$ , the latter was determined by modelling 4-6 CWR tests to exhaustion. Aiming to reduce

such a time consuming protocol, Vanhatalo et al. (2007c) reported that the end-power output during a 3AO test corresponded to critical power; whilst the work performed above end-power output corresponded to  $W'$ . It remains unknown whether the previously reported correlation between  $W'$  and AOD holds true when  $W'$  is determined from a 3AO test.

The aims of this study, therefore, were i) to determine whether AOD and  $W'$  remain constant irrespective of rate of depletion (i.e. CWR vs. 3AO); and ii) to investigate the relationship between AOD and  $W'$  during an exhaustive CWR to exhaustion and a 3AO test. It was hypothesised that AOD and  $W'$  would not be affected by the exercise mode. It was also hypothesised that  $W'$  and AOD would be strongly and positively correlated in both the CWR and 3AO test.

## **6.3. Methods**

### **6.3.1. Participants**

Twenty-one trained male cyclists and triathletes volunteered to participate in this study, which was approved by St Mary's University Ethics Committee. Their mean  $\pm$  standard deviation (*SD*) for age, height and mass were  $40 \pm 6$  years,  $1.81 \pm 0.08$  m and  $79.8 \pm 7.5$  kg, respectively. The participants were recruited from local cycling and triathlete clubs. All participants voluntarily signed a consent form after being informed orally and in written of the aim, benefits and possible risks of taking part in the study.

### **6.3.2. Equipment**

All tests were performed on an electromagnetically braked cycle-ergometer (Lode Excalibur Sport, Groningen, Netherlands). The saddle height and handle bar position of the cycle-ergometer were individually adjusted for comfort in the first trial, and replicated in all subsequent trials. During all trials, participants breathed room air through a facemask (Hans Rudolph, Kansas City, MO). Gas exchange samples were drawn continuously through a 1.5 m sampling line and analysed for  $O_2$  and  $CO_2$  concentrations (Jaeger Oxycon Pro, Jaeger Ltd., Höechberg, Germany). The gas analyser was calibrated before each test according to manufacturer instructions using gases of known concentrations (Carefusion, Höechberg, Germany) and room air, and the turbine was calibrated using a 3 L syringe (Viasys Healthcare, Höechberg, Germany). Blood samples were drawn from the earlobe using 20  $\mu$ L tubes (EKF Diagnostics, Barleben, Germany) for analysis of blood lactate concentration (BLa) at several

points (see below) using an enzymatic-amperometric BLa/glucose analyser (Biosen C-line, EKF Diagnostic, Germany). Heart rate was measured using a telemetric monitor (Polar S610, Polar Electro, Finland) at 5 s intervals during all trials.

### **6.3.3. Procedures**

The study consisted of four trials in an exercise physiology laboratory. All tests were performed at approximately the same time of the day ( $\pm 1$  h). After two preliminary trials to determine the GET, the  $\dot{V}O_2$ –power output relationship, and  $\dot{V}O_{2max}$ ; participants completed a CWR test to exhaustion at 112.5% of  $\dot{V}O_{2max}$  and a 3AO test. All trials were separated by at least 48 h to allow complete recovery. The participants were provided with a food record diary and were advised to follow a similar diet and to avoid strenuous exercise in the 24 h before each trial. Similarly, they were requested to avoid caffeine and alcohol ingestion 12 h before each trial.

#### ***Preliminary tests***

The preliminary tests included two trials. In Trial 1, participants completed a ramp test to exhaustion. The test started with 3 min of unloading cycling. The resistance of the flywheel increased thereafter at a constant rate of  $30 \text{ W}\cdot\text{min}^{-1}$  until exhaustion, defined in this study as a decrease in cadence of  $> 10$  rpm for  $> 5$  s despite strong verbal encouragement. The cadence was freely chosen by each participant and kept constant throughout the test. The preferred cadence was annotated and replicated in subsequent trials. The GET was independently identified by two investigators using the V-slope method (3), and the average of the two values was used for subsequent calculations. In instances where GET estimates differed by  $> 10\%$ , a third investigator determined the GET, and the average of the two closest estimates was used for analysis. Trial 2 consisted of  $10 \times 3$ -min consecutive steps to determine the relationship between  $\dot{V}O_2$  and power output, followed by a ramp test to exhaustion to determine  $\dot{V}O_{2max}$ . The first step was performed at 50% GET and the intensity increased by 10% GET in each subsequent step, so that the final work rate corresponded to 140% GET. Steps were interspersed with 30 s of rest to allow a capillary blood sample to be drawn for BLa analysis. After completion of the final step, participants were allowed 5 min of stationary rest on the ergometer. Cycling was resumed at 70% GET, and increased at a rate of 15% GET every minute until volitional exhaustion (as defined above).  $\dot{V}O_{2max}$  was determined as the highest  $\dot{V}O_2$  obtained from a 30-s rolling average, which excluded breath-by-breath values outside 4 *SD* from a local (5-breath) average (Beaver et al., 1986).

### ***Constant work-rate test***

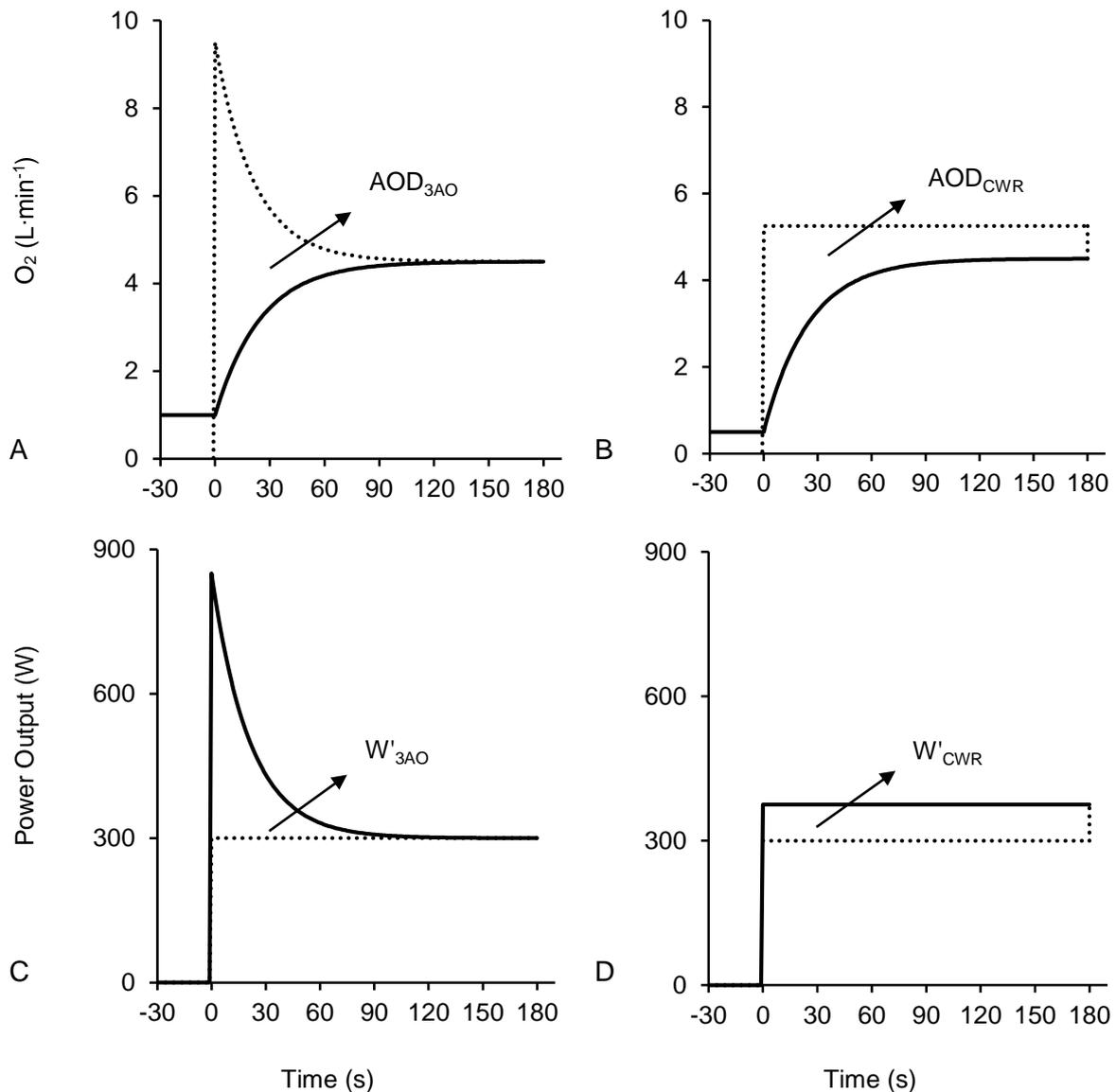
The CWR test commenced with 3 min of unloading cycling followed by 5 min at 70% GET. Then, after 5 min stationary rest on the cycle-ergometer, participants were instructed to attain their preferred cadence after a 5-second countdown. The power output during the CWR corresponded to 112.5%  $\dot{V}O_{2max}$ , determined as a linear extrapolation of the relationship between  $\dot{V}O_2$  and power output.  $\dot{V}O_2$  values to construct the  $\dot{V}O_2$ -power output relationship were determined from each stage as the highest  $\dot{V}O_2$  value derived from a 30 s rolling average (see above). Participants were instructed before and encourage throughout the test to exercise for as long as they possibly could, but were unaware of elapsed time or expected duration. Capillary blood samples were drawn 1, 3 and 5 min after exhaustion for BLa determination.

### ***3-min all-out test***

The 3AO test was performed as outlined by Vanhatalo et al. (2007). The trial commenced with 5 min cycling at 70% GET and a further 5 min resting on the cycle ergometer. Then, participants completed 3 min of unloaded pedalling at their preferred cadence. In the last 10 seconds of the unloaded phase, participants were instructed to increase their cadence up to 110 – 120 rpm. At the start of the 3AO test, the cycle-ergometer switched to linear mode, so the resistance (i.e. the power output) represented a function of the cadence. The alpha factor of the linear mode was determined to elicit a power output at each participant's preferred cadence corresponding to 50% of the difference between the intensity at GET and that at the end of the ramp test (i.e. 50% $\Delta$ ). The subjects were instructed before the test to attain peak power (i.e. highest cadence) as soon as possible and to maintain the highest possible cadence throughout the test. Strong verbal encouragement was provided by the same investigator. As in the CWR test, time cues were removed from the area to prevent pacing. All participants completed one familiarization trial of the 3AO test that was not included in data analysis. The criteria to deem a 3AO test as valid is yet to be established. Nevertheless, it has been reported that, during a 3AO test: i) peak power is typically attained within the first 10 s (Vanhatalo et al., 2007); ii) mean  $\dot{V}O_2$  corresponds to 97-99%  $\dot{V}O_{2max}$  (Burnley et al., 2006; Vanhatalo et al., 2007); iii)  $W'$  is depleted to ~5% of its initial value within the first 90 s (Vanhatalo et al., 2008b); and iv) end-test cadence should be within  $\pm 10$  rpm of each participant's preferred cadence, as otherwise might affect  $W'$  (Vanhatalo et al., 2008b). As in the CWR test, capillary BLa was determined 1, 3 and 5 min after the 3AO test.

#### 6.3.4. Statistical analyses

The AOD was determined as the difference between the estimated oxygen demand and accumulated oxygen uptake (Medbø et al., 1988). In the CWR test, the oxygen demand was assumed to remain constant during the test (i.e. 112.5%  $\dot{V}O_{2max}$ ), so the accumulated oxygen demand was estimated as the product of oxygen demand and TTE. In the 3AO test, raw recording of power output (6 Hz) were averaged at 1-second intervals to produce second-by-second values. The second-by-second oxygen demand was calculated from a linear projection of the  $\dot{V}O_2$ -power output relationship. Subsequently, the accumulated oxygen demand was determined as the integral of second-by-second oxygen demand. Breath-by-breath  $\dot{V}O_2$  values were filtered (as described above) and linearly interpolated to produce second-by-second  $\dot{V}O_2$  data. The accumulated oxygen uptake was determined as the integral of second-by-second  $\dot{V}O_2$ . End-exercise  $\dot{V}O_2$  and oxygen demand were determined in CWR and 3AO tests as the average  $\dot{V}O_2$  and oxygen demand, respectively, in the last 10 s of the CWR and 3AO tests. In the 3AO test, CP was considered as the average power output in the last 30 s of the test.  $W'$  was determined from the 3AO test ( $W'_{3AO}$ ) as the integral of power output above CP. Assuming CP remained consistent throughout the study (Chidnok et al., 2013),  $W'_{CWR}$  was determined as the work completed above CP during CWR. Figure 6.1 outlines the protocol to determine  $AOD_{CWR}$ ,  $AOD_{3AO}$ ,  $W'_{CWR}$ , and  $W'_{3AO}$ . Data are presented as mean  $\pm$  SD. Using IBM SPSS 21 (IBM Corp, Armonk, NY), physiological responses to the CWR and 3AO tests were compared using paired samples t-tests. Pearson product-moment correlations were determined between  $AOD_{3AO}$  and  $W'_{3AO}$ , and between  $AOD_{CWR}$  and  $AOD_{3AO}$ . In all instances, significance was accepted at  $P < 0.05$ .



**Figure 6.1.** Schematic representation for determination of the accumulated oxygen deficit (AOD) and  $W'$  during a 3-min all-out (3AO) test and a constant work-rate test to exhaustion. Top panels: AOD is determined as the difference between oxygen demand (dotted lines) and estimated oxygen uptake (solid lines) during 3AO test and CWR test (Panels A and B, respectively). Bottom panels:  $W'$  is determined as the area between power output (solid line) and the estimated CP (dotted line) during a 3AO test and CWR test (Panels C and D, respectively).

## 6.4. Results

### 6.4.1. Preliminary tests

In the ramp test, GET occurred at  $188 \pm 25$  W and peak power output corresponded to  $397 \pm 46$  W, so  $50\% \Delta$  was  $293 \pm 34$  W. For the  $10 \times 3$  min step test, the intensity at  $50\%$  GET was  $94 \pm 13$  W and increased by  $19 \pm 3$  W in each step, so the final intensity was  $263 \pm 36$  W. These work rates corresponded to intensities from  $41 \pm 4\%$  to  $84 \pm 7\%$   $\dot{V}O_{2max}$ , and raised BLa from  $0.97$   $\text{mmol}\cdot\text{L}^{-1}$  at the

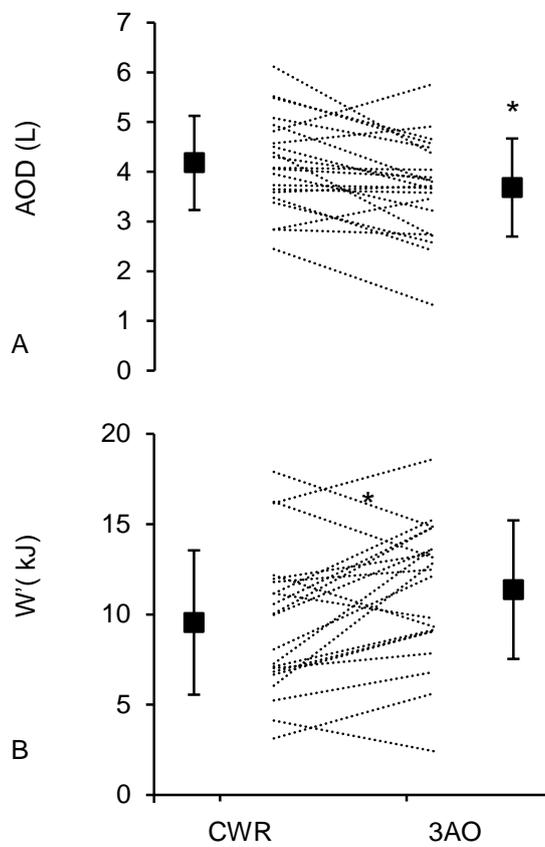
end of the first stage to  $3.93 \pm 1.72$  mmol·L<sup>-1</sup> in the last stage. There was a strong linear relationship between  $\dot{V}O_2$  and power output for all participants ( $P < 0.001$ ;  $r = 0.995 \pm 0.004$ ). In the maximal test,  $\dot{V}O_{2max}$  was  $4.60 \pm 0.61$  L·min<sup>-1</sup> ( $58 \pm 7$  mL·kg<sup>-1</sup>·min<sup>-1</sup>).

#### **6.4.2. Constant-load to exhaustion and 3-min all-out tests**

The results from the CWR and 3AO tests are presented in Table 6.1. The 3AO was deemed as valid for all participants given that: i) peak power ( $645 \pm 127$  W) was attained at the beginning of the test ( $6 \pm 4$  s); ii) peak  $\dot{V}O_2$  was  $> 95\%$   $\dot{V}O_{2max}$  ( $98 \pm 5\%$   $\dot{V}O_{2max}$ ); iii)  $W'$  was depleted to  $\sim 5\%$  of its initial value after 90 s ( $6 \pm 4\%$ ); and iv) the end-test cadence was within 10 rpm of the preferred cadence ( $4 \pm 4$  rpm). The CP and  $W'$ , determined from the 3AO test, were  $316 \pm 50$  W ( $67 \pm 8\%\Delta$ ) and  $11.37 \pm 3.84$  kJ, respectively.

#### **6.4.3. Estimation of anaerobic capacity**

Estimations of AnC from AOD and  $W'$  were significantly correlated in the CWR ( $r = 0.654$ ;  $P < 0.001$ ) and 3AO ( $r = 0.664$ ;  $P < 0.001$ ) tests. There were no differences between CWR and 3AO for duration, average power output and work completed (Table 6.1). However, there were differences for both estimations of AnC between CWR and 3AO tests. Specifically,  $W'_{3AO}$  was greater than  $W'_{CWR}$  whilst  $AOD_{CWR}$  was greater than  $AOD_{3AO}$  (Table 6.1; Figure 6.2). The estimates of anaerobic energy contribution during CWR were different between AOD vs.  $W'$  (Table 6.1; mean difference  $14 \pm 10\%$ ;  $P < 0.001$ ). In contrast, there were no differences between estimations of the anaerobic energy contribution in the 3AO test derived from AOD and  $W'$  (Table 6.1; mean difference  $3 \pm 11\%$ ;  $P = 0.175$ ).



**Figure 6.2.** Accumulated oxygen deficit (AOD) and  $W'$  during a constant work-rate (CWR) exercise to exhaustion and a 3-min all-out (3AO) test. Individual responses (dotted lines) and group means and standard deviations are shown. \* denotes difference from the CWR test ( $P < 0.05$ ).

**Table 6.1.** Physiological responses to a constant-work rate (CWR) test to exhaustion and a 3-min all-out (3AO) test.

	CWR	3AO	3AO-CWR difference	<i>P</i> value
Duration (s)	164 ± 46	180 ± 0	-16 ± 46	0.127
Power output (W)	376 ± 55	376 ± 55 <sup>#</sup>	-1 ± 23	0.882
Work (kJ)	60.85 ± 17.30	67.72 ± 9.84	-6.88 ± 15.63	0.057
W' (kJ)	9.55 ± 4.00	11.37 ± 3.84	-1.82 ± 2.93	0.010
W' (%)	20 ± 12	17 ± 6	-3 ± 9	0.116
Acc. O <sub>2</sub> demand (L)	14.08 ± 4.14	15.55 ± 2.14	1.48 ± 3.56	0.071
Acc. O <sub>2</sub> uptake (L)	9.90 ± 3.46	11.87 ± 1.48	1.97 ± 3.34	0.013
AOD (L)	4.18 ± 0.95	3.68 ± 0.98	0.50 ± 0.71	0.004
AOD (%)	31 ± 7	23 ± 5	8 ± 9	0.001
End-exercise $\dot{V}O_2$ (L·min <sup>-1</sup> )	4.29 ± 0.63	4.48 ± 0.61	-0.20 ± 25	0.002
End-exercise O <sub>2</sub> demand (L·min <sup>-1</sup> )	5.17 ± 0.69	4.49 ± 0.61	0.68 ± 25	<0.001
Peak BLa (mmol·L <sup>-1</sup> )	10.70 ± 2.57	11.77 ± 2.94	-1.07 ± 1.85	0.015
Peak HR (beats·min <sup>-1</sup> )	166 ± 11	165 ± 11	2 ± 7	0.131

<sup>#</sup> : average power output during the 3AO test. EE: end-exercise. HR: heart rate.

## 6.5. Discussion

The aims of the present study were to determine whether AOD and  $W'$  remain constant irrespective of rate of depletion (i.e. CWR vs. 3AO); and to investigate the relationship between AOD and  $W'$  during an exhaustive CWR to exhaustion and a 3AO test. The main findings of the study were that i) there is a positive correlation between AOD and  $W'$ ; ii) the strength of the correlation between AOD and  $W'$  is not affected by the mode of exercise (CWR vs. 3AO); iii) AOD and  $W'$ , however, are sensitive to the mode of exercise and were different between the CWR and 3AO tests; iv) the differences observed between CWR and 3AO in AOD and  $W'$  followed inverse directions: whilst AOD was greatest in CWR,  $W'$  was greatest in 3AO. These results suggest that ~43% of the variance of AOD and  $W'$  is determined by a shared factor, most likely related to anaerobic energy production. However, since both estimates of AnC were affected by the mode of exercise, factors other than anaerobic energy production might determine the magnitude of AOD and/or  $W'$ .

The strong correlation between AOD and  $W'$  is consistent with previous research in healthy adults (Chatagnon et al., 2005; Leclair et al., 2010; Miura et al., 2002). Moreover, the relationship between  $D'$ , the running equivalent of  $W'$ , and AOD has also been shown to be moderate, irrespective of the mathematical model used to determine  $D'$  in some (Bosquet et al., 2007), but not all (Zagatto et al., 2013), studies. Whilst in the above studies  $W'$  (or  $D'$ ) was determined from several CWRs to exhaustion, the present study demonstrates that this correlation holds true when  $W'$  is determined from the time-efficient 3AO test. Moreover, the strength of the correlation between AOD and  $W'$  ( $0.48 \leq r \leq 0.76$ ) reported in the above studies (Chatagnon et al., 2005; Leclair et al., 2010; Miura et al., 2002) compares well with the results of the present study. Overall, present and previous studies suggest that some 23-58% of the variance of AOD and  $W'$  is underpinned by a shared mechanism, likely related to anaerobic energy production.

Another important finding of the present study was that both AOD and  $W'$  were sensitive to power output profile (i.e. CWR vs. 3AO tests), as denoted by the differences between both estimates of AnC during the CWR and 3AO tests. However, these differences followed opposite directions. Previous research have shown that  $W'$  remains unaffected irrespective of its rate of depletion (Chidnok et al., 2013a, Fukuba et al., 2003). In order to accept that  $W'$  remains constant irrespective of its rate of depletion, one needs to assume that aerobic energy production supplies power output at intensities below CP

from the onset of the exercise (Murgatroyd et al., 2011), which, in turn, implies infinitely fast  $\dot{V}O_2$  kinetics (Panel D, Figure 6.1). Despite this limitation (Jones et al., 2008b), it is assumed that  $W'$  remains constant during a CWR test lasting > 3 min (Chidnok et al., 2013a). Indeed, Chidnok et al. (2013a) observed constant  $W'$  irrespective of power output profile during a 3AO test and a CWR test of ~3.1 min. In the current study, CWR fell slightly short of 3 min (~2.7 min), which might not allow for a complete a depletion of  $W'$ .

The AOD is thought to reach its peak value, and therefore providing an estimate AnC, during CWRs in which exhaustion occurs within 2-5 min (Medbo et al., 1988; Weber & Schneider, 2001) or during all-outs test of at least 60 s (Calbet et al., 1997; Gastin et al., 1995; Withers et al., 1991, 1993). In the present study, despite CWR and 3AO meeting those two conditions,  $AOD_{CWR}$  was 12% greater than  $AOD_{3AO}$ . It is possible that, given the progressive increase in  $\dot{V}O_2$  and decrease in power output observed during an all-out effort,  $\dot{V}O_2$  at the end of the 3AO test was greater than the oxygen demand, decreasing  $AOD_{3AO}$ . However,  $\dot{V}O_2$  and oxygen demand at the end of the 3AO test were similar (Table 6.1), and most of the AOD occurs at the onset of all-out tests (see Figure 6.1). Alternatively, at the onset of the 3AO there is a higher demand of ATP turnover which can accelerate kinetics of  $\dot{V}O_2$  and, possibly, reduce the anaerobic contribution (Bailey, Vanhatalo, DiMenna, Wilkerson, & Jones, 2011; Bishop, Bonetti, & Dawson, 2002; Chidnok et al., 2013a; Jones et al., 2008b). However, studies looking at the effects of pacing strategies during 2-6 min trials have reported that an all-out start has no effect on the AOD (Aisbett, Lerossignol, McConell, Abbiss, & Snow, 2009a, b; Bishop et al., 2002). Moreover, BLa and pH, which can also be considered markers of anaerobic energy production, also remain unaffected by an all-out start (Aisbett et al., 2009a, b; Bishop et al., 2002; Chidnok et al. 2013a). In contrast, the higher BLa observed in 3AO in the current study may be indicative of a greater perturbation in the muscular milieu during the 3AO, which might trigger a cascade of processes that ultimately caused exercise intolerance before AOD had reached its maximal value. Then again, whilst the increased BLa might suggest higher metabolic disturbance during the 3AO, there is evidence that all-out and CWR tests result in similar intramuscular metabolic perturbation (Burnley et al., 2010). Intramuscular metabolites were not quantified in the present study, and therefore it is difficult to account for the effect that possible differences in the metabolic milieu between CWR and 3AO tests might contribute to explain the observed difference between  $AOD_{3AO}$  and  $AOD_{CWR}$ .

During a high-intensity bout of exercise at intensities above CP, peak  $\dot{V}O_2$  has been shown to attain  $\dot{V}O_{2max}$ , irrespective of the pacing strategy adopted, by some (Aisbett et al., 2009a, b; Bishop et al., 2002; Burnley et al., 2006; Chidnok et al. 2013a; James, Sandals, Draper, & Wood, 2007; Jones et al., 2008b; Simpson et al., 2015), but not all (Bailey et al., 2011; Sawyer, Morton, Womack, & Gaesser, 2012; Vanhatalo 2008b), studies. In the present study peak  $\dot{V}O_2$  during the 3AO test was  $\sim 98\%$   $\dot{V}O_{2max}$ , but it only attained  $\sim 94\%$   $\dot{V}O_{2max}$  in the CWR test, despite the intensity being  $119 \pm 8\%$  of CP. It is possible that the relatively short duration combined with slow  $\dot{V}O_2$  kinetics during the CWR test to exhaustion (see discussion above) incurred in a larger reliance on anaerobic energy production, as denoted by greater  $AOD_{CWR}$  (Table 6.1). As a result, exercise might have been terminated before  $\dot{V}O_{2max}$  was reached in the CWR test.

### **6.5.1. Conclusion**

In conclusion, this is the first study to compare two approaches to estimate AnC ( $AOD$  and  $W'$ ) during a CWR to exhaustion and a 3AO test. The correlation between  $AOD$  and  $W'$  during CWR and 3AO suggests that  $\sim 43\%$  of the magnitude of  $AOD$  and  $W'$  is determined by a shared factor, likely linked to anaerobic energy production. Moreover, the strength of the correlation between  $AOD$  and  $W'$  seems to be consistent irrespective of the type of exercise. However, contrary to the assumption of a constant AnC, we observed that  $AOD_{CWR}$  and  $W'_{3AO}$  were greater than  $AOD_{3AO}$  and  $W'_{CWR}$ , respectively. These results suggest that anaerobic energy production is not the solely factor contributing to  $AOD$  and  $W'$ . Moreover, this study suggests that factors other than anaerobic energy production contributing to  $AOD$  and  $W'$  are different between a CWR test to exhaustion and a 3AO test.

**Chapter 7. Accumulated oxygen deficit and  $W'$  during constant-load and all-out exercise in hypoxia and hyperoxia**

## 7.1. Abstract

The aim of this study was to determine the effect of acute hypoxia and hyperoxia exposure on the accumulated oxygen deficit (AOD) and the curvature constant of the power output-duration relationship ( $W'$ ). Ten male cyclists and triathletes completed, in a randomised order, constant work-rate (CWR) bouts to exhaustion at 112.5% maximal oxygen uptake ( $\dot{V}O_{2max}$ ) and 3-min all-out (3AO) tests in hypoxia (15% oxygen), normoxia (21% oxygen) and hyperoxia (35% oxygen). AOD was determined as the difference between the accumulated oxygen demand and the accumulated oxygen uptake, whereas  $W'$  was determined as the work completed above critical power. There were no differences in AOD between conditions of hypoxia ( $4.15 \pm 2.05$  L), normoxia ( $3.41 \pm 1.29$  L), or hyperoxia ( $2.70 \pm 2.04$  L). However, AOD was greater in CWR tests compared to 3AO tests ( $3.90 \pm 1.83$  vs.  $2.94 \pm 1.86$  L). In contrast,  $W'$  was not affected by condition ( $12.84 \pm 5.05$ ,  $11.43 \pm 6.15$ , and  $16.65 \pm 5.38$  kJ for hypoxia, normoxia, and hyperoxia, respectively) or type of test ( $11.49 \pm 6.70$  and  $13.13 \pm 3.85$  kJ for CWR and 3AO tests, respectively). Critical power and  $\dot{V}O_{2max}$  decreased in hypoxia compared to normoxia ( $232 \pm 38$  vs.  $277 \pm 47$  W and  $3.81 \pm 0.64$  vs.  $4.24 \pm 0.65$  L $\cdot$ min $^{-1}$  for critical power and  $\dot{V}O_{2max}$ , respectively). There was no effect of hyperoxia on critical power or  $\dot{V}O_{2max}$  ( $285 \pm 38$  W and  $4.55 \pm 0.63$  L $\cdot$ min $^{-1}$ , respectively) compared to normoxia. Although AOD and  $W'$  were unaffected by condition, large between- and within-subject variability warrants caution to consider either test as AnC. Moreover, due to AOD being different between CWR and 3AO tests, the present study challenges the assumption that it is the best non-invasive method to estimate AnC.

## 7.2. Introduction

In a high-intensity bout of exercise, the demand for ATP exceeds the highest rate of aerobic ATP resynthesis, so in order to meet the energy demands, ATP resynthesis necessitates of both aerobic and anaerobic metabolic pathways. The capacity to resynthesise ATP and generate work using anaerobic metabolic pathways is finite. Unfortunately, direct quantification of AnC requires invasive and/or technically challenging procedures, and it is typically limited during whole body exercise. Alternatively, various indirect approaches have been developed, chiefly AOD and  $W'$  (Noordhof et al., 2010, 2013). Both approaches, however, rely on a number of assumptions which have compromised the adoption of either test as part of regular physiological testing.

For instance, AOD is determined as the difference between the estimated oxygen demand and the accumulated oxygen uptake. Whilst the accumulated oxygen uptake can be easily assessed via  $\dot{V}O_2$  measurements, the oxygen demand during high-intensity exercise cannot be determined. Instead, it is estimated from a projection of the linear relationship between  $\dot{V}O_2$  and power output. Indeed, the assumptions that  $\dot{V}O_2$  and power output exhibit a linear relationship and that such a relationship remains constant during exercise are major drawbacks for AOD calculation. Although the physiological bases of these two assumptions have been challenged (Bangsbo, 1998; Barstow & Mole, 1991; Cannon et al., 2013; Grassi et al., 2015; Jones et al., 2010, 2011), the AOD has been suggested to be the best non-invasive approach to estimate AnC (Noordhof et al., 2010).

The  $W'$ , on the other hand, represents the finite amount of work that can be performed above CP, and has been traditionally considered as anaerobic work capacity (Hill, 1993; Jones et al., 2010; Morton, 2006). This traditional view of  $W'$  as anaerobic work capacity, however, has been recently challenged and it is currently hypothesised that  $W'$  also represents the ability to acquire and tolerate fatigue (Broxterman et al., 2015a, b; Johnson et al., 2013; Skiba et al., 2015). Moreover, the use of  $W'$  as a proxy measure of anaerobic work capacity (i.e. AnC), is limited due to the assumption that  $W'$  is only tagged at intensities above CP, which in turn implies infinitely fast  $\dot{V}O_2$  on-kinetics (Jones et al., 2010). Nonetheless, a consistent  $W'$  has been observed as long as exercise within the severe exercise domain lasts  $\geq 3$  min (Chidnok et al., 2013a, b; Fukuba et al., 2003; Whipp et al., 2005).

Since there is no gold-standard method to determine AnC during whole-body exercise, it is difficult to establish whether any of the above approaches truly represent anaerobic energy production. A paradigm that has been repeatedly used to assess the validity of tests aiming to quantify AnC has been derived by changes in oxygen availability. It is well known that reducing oxygen availability impairs aerobic metabolism, as indicated by reductions in CP and  $\dot{V}O_{2max}$  (e.g. Dekerle et al., 2012; González-Álonso & Calbet, 2003; Simpson et al., 2015; Wehrin & Hallén, 2005). In contrast, increasing the oxygen delivery augments CP and  $\dot{V}O_{2max}$  (Peltonen, Tikkanen, & Rusko, 2001; Vanhatalo et al., 2010). However, it is assumed that AnC is not affected by oxygen availability, and therefore any test aiming to quantify AnC should not be affected by hypoxic or hyperoxic exposure (Medbø et al., 1988).

Both AOD and  $W'$  have been studied in conditions where oxygen delivery was either compromised (Broxterman et al., 2015a; Dekerle et al., 2012; Feriche et al., 2007; Friedmann et al., 2007; Medbø et

al., 1988; Morales-Alamo et al., 2012; Ogura et al., 2006; Simpson et al., 2015; Valli et al., 2011) or augmented (Macdonald et al., 1997; Vanhatalo et al., 2010). Interestingly, although both approaches attempt to measure the same physiological variable (i.e. AnC) and are strongly correlated during cycling exercise (Chatagnon et al., 2005; Miura et al., 2002), they appear to respond differently to changes in oxygen delivery. Compared to a control condition, AOD appears to be unaffected by hypoxia (Feriche et al., 2007; Friedmann et al., 2007; Medbø et al., 1988; Morales-Alamo et al., 2012; Ogura et al., 2006). The effect of hyperoxia on AOD has not been studied, though the oxygen deficit determined from the parameters of  $\dot{V}O_2$  kinetics have been shown to decrease in hyperoxia due to accelerated  $\dot{V}O_2$  kinetics (Macdonald et al., 1997). The effect of hypoxia and hyperoxia on  $W'$  seems to be less consistent. When oxygen delivery has been decreased by hypoxia or blood flow occlusion,  $W'$  has been shown to decrease (Valli et al., 2011), remain unaffected (Dekerle et al., 2012; Simpson et al., 2015), and increase (Broxterman et al., 2015a). If oxygen delivery is augmented by breathing a hyperoxic mixture of gas, the magnitude of  $W'$  has been shown to remain unaffected (Vanhatalo et al., 2010). However, there is growing evidence suggesting a negative relationship between the change, relative to normoxia, in the magnitude of  $W'$  and CP during acute hypoxia or hyperoxia (Dekerle et al., 2012; Simpson et al., 2015; Vanhatalo et al., 2010). In other words, those participants whose CP is more severely decreased by hypoxia have their  $W'$  increased; and those whose CP increased to a larger extent in hyperoxia, had their  $W'$  decreased. Interestingly, some authors have suggested that hypoxia might promote anaerobic energy production during a sprint exercise (Calbet et al., 2003; Ogura et al., 2006); with the opposite being true in hyperoxia (Linossier et al., 2000).

Given that no study has simultaneously investigated the effects of hypoxia and hyperoxia on both AOD and  $W'$ , the effect of oxygen availability on AnC is not yet completely understood. The aim of the present study was to investigate the effects of hypoxia and hyperoxia on AOD and  $W'$  during whole-body exercise. It was hypothesised that, i) whilst indices of aerobic metabolism such as GET, CP and  $\dot{V}O_{2max}$  would be sensitive to hypoxia and hyperoxia, both AOD and  $W'$  would remain unaffected by environmental conditions; ii) AOD and  $W'$  would not be affected by power output profile during a CWR test to exhaustion and a 3AO test; and iii) that the changes in CP and  $W'$  during hypoxic and hyperoxic exercise (compared to normoxia) would be negatively correlated. Similarly, it was hypothesised that changes in  $\dot{V}O_{2max}$  and AOD during hypoxic and hyperoxic conditions would exhibit a negative correlation.

## **7.3. Methods**

### **7.3.1. Participants**

Ten male cyclists and triathletes, residents at sea level and nonacclimatise to altitude, volunteered to take part in this study. Their mean  $\pm$  SD for age, stature, and mass were  $36 \pm 9$  years,  $1.78 \pm 0.06$  m, and  $71 \pm 7$  kg, respectively. Participants were informed of the aims, methods and procedures of the study and signed a consent form. The study was approved by St Mary's University Ethics Committee.

### **7.3.2. Experimental overview**

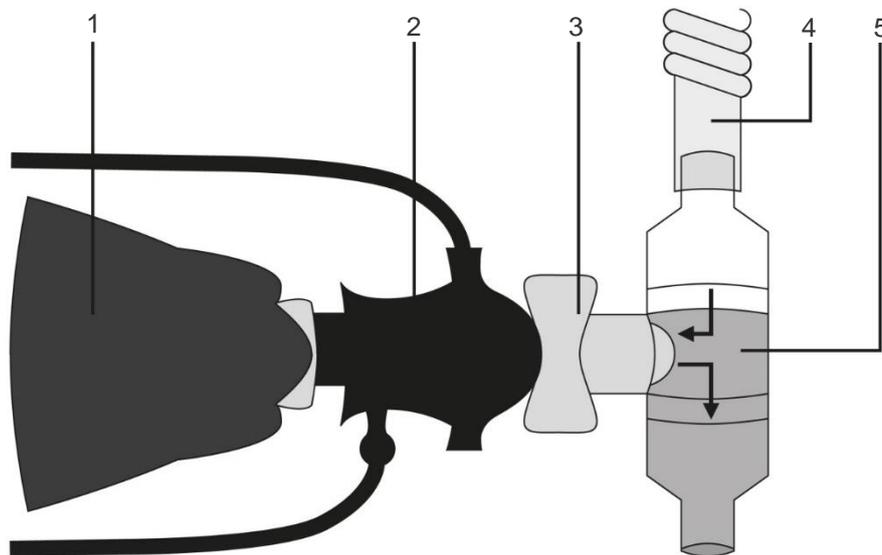
In a single-blinded, semi-randomised, cross-over study design, each participant completed four trials in three different conditions (normoxia, hypoxia and hyperoxia) in an exercise physiology laboratory at sea level (Twickenham, UK). Within each condition, participants first completed a ramp test to exhaustion to determine GET and peak  $\dot{V}O_2$ , followed on a separate day by a step test and a ramp test to exhaustion to determine the  $\dot{V}O_2$ -power output relationship and peak  $\dot{V}O_2$ , respectively. Subsequently and in a randomised order, participants completed a CWR exercise bout to exhaustion and a 3AO test. All tests were conducted at the same time of the day ( $\pm 2$  h), and interspersed with at least 24 h of rest. Participants were instructed to follow a similar diet 24 h before each trial, and to refrain from caffeine and alcohol 12 h before each test. The protocol was completed over the course of  $9 \pm 2$  weeks.

### **7.3.3. Equipment and measurements**

*Exercise testing.* All exercise tests were performed on an electronically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). The handle bar and saddle height were individually adjusted at the beginning of the study, and the settings replicated in subsequent tests. All participants used clip-in pedals and wore their own cycling shoes.

*Gas exchange.* In all tests, participants breathed through a facemask (7450, Hans Rudolph Inc., Kansas City, MO) connected to a low-resistance mouthpiece and impeller turbine transducer assembly (Jaeger 203 Triple V, Jaeger GmbH, Hoechburg, Germany). The gases were continuously sampled and analysed for oxygen and carbon dioxide concentrations using an online gas analyser (Oxycon Pro, Jaeger Ltd. Höechberg, Germany), which was calibrated following the recommendations from the manufacturer. The gas analyser was calibrated in normoxia, irrespective of the test condition, using gases of known concentration (Carefusion, Höechberg, Germany) and ambient air. The volume meter

was calibrated using a 3 L syringe (Viasys Healthcare, Höechberg, Germany). The distal part of the turbine was attached to a two-way non-rebreathing valve (Hans Rudolph Inc., Kansas City, MO). The inlet port of the mouthpiece was connected to a rack holding four Douglas bags (Cranlea Ltd, Birmingham, UK) by a 1.2 m plastic tube, and the outlet port allowed expired air to escape the system (Figure 7.1). There were eight Douglas connected in series, each containing ~125 L of air, so there was ~1000 L available at the onset of each test. Whilst room air was used as normoxic air, hypoxic and hyperoxic mixtures were obtained from two hypoxic units (Cloud 9, Sporting Edge, Sherfield on Loddon, UK) and an air generator (Sporting Edge, Sherfield on Loddon, UK), respectively. The concentration of oxygen was  $15.14 \pm 0.21\%$  (CV: 2.25%) in hypoxia,  $20.79 \pm 0.05\%$  (CV: 0.53%) in normoxia, and  $35.29 \pm 0.46\%$  (CV: 2.75%) in hyperoxia. An online gas analyser (Servomex Ltd., Crowborough, UK) was used to monitor  $\text{FiO}_2$  throughout all tests. Irrespective of the condition, participants started to breathe the assigned gas mixture 5 min before the start of the protocol to allow gas store equilibration. Upon completion of the study, participants were asked whether they could identify which trials were performed in each condition. Five out of 10 participants were unable to differentiate between experimental conditions. The remaining five participants successfully identified the condition in 20% of tests.



**Figure 7.1.** Set up for oxygen uptake measurement in hyperoxia. 1. Silicone face mask. 2. mouthpiece and impeller turbine transducer assembly for gas exchange analysis. 3. Connection piece. 4. Plastic tube to connect Douglas bags. 5. Two-way non-rebreathing valve. Arrows indicate air flow direction within the two-way valve.

*Near infra-red spectroscopy.* During all CWR and 3AO tests, muscle oxygenation was continuously monitored using a wireless, spatially resolved near-infrared spectroscopy (NIRS) device (Portamon, Artinis Medical Systems, BV, The Netherlands). Changes in tissue saturation index (TSI, expressed as a %), oxygenated haemoglobin and myoglobin concentration ( $[O_2Hb]$ ), and deoxygenated haemoglobin ( $[HHb]$ ) were measured using two wavelengths of 750 and 850 nm and an arbitrary value for the differential path length of 3.83 (Patterson, Bezodis, Glaister, & Pattison, 2015). The NIRS device was located on the vastus lateralis muscle of the right leg, two thirds along the line from the anterior spina illaca superior to the lateral side of the patella. The area was shaved and swabbed with alcohol, and covered with transparent adhesive dressing to protect the NIRS device from sweating. The device was secured in place with elasticated adhesive tape and covered with a black light-absorbing cloth to prevent external light from affecting NIRS measurement. The position of the NIRS device was marked with a permanent-ink pen before and after each test, and participants were encouraged to re-mark the position, if necessary, between tests. The NIRS device was connected via Bluetooth to a personal computer, which recorded continuously at a rate of 10 Hz. After completion of the tests, NIRS data was downloaded for analysis at 1 Hz. Skinfold thickness was measured at the site where the NIRS probe was located using Harpenden skinfold calipers (British Indicators Ltd, UK). For all participants, the calculated value of skin and subcutaneous tissue thickness was less than half of the distance between the source and the detector (Ferrari, Mottola, & Quaresima, 2004; Patterson et al., 2015).

*Heart rate and oxygen saturation.* Heart rate was measured throughout all tests using a telemetric monitor (Polar Electro, Kempele, Finland). In each test, the highest heart rate recorded was considered to be the peak heart rate. Oxygen saturation ( $SaO_2$ ) was recorded on the index finger of the right hand at the point of exhaustion using a pulse oximeter (Nonin Medical Inc., Plymouth, MN).

*Blood lactate.* Capillary blood samples were collected from the right earlobe at rest, and 1, 3, and 5 min after completion of the CWR and 3AO tests. Whole capillary blood samples (20  $\mu$ l) were haemolysed in a pre-filled tube and analysed using a blood lactate analyser (Biosen C Line, EKF Diagnostics, Barleben, Germany). The highest BL<sub>a</sub> recorded in each CWR and 3AO test was considered as peak BL<sub>a</sub>.

#### **7.3.4. Procedures**

##### ***Ramp test to exhaustion.***

The ramp test to exhaustion (Ramp 1) started with 3 min of unloaded cycling. Subsequently, the resistance increased by  $30 \text{ W}\cdot\text{min}^{-1}$  until exhaustion, defined for this investigation as a decrease  $> 10$  rpm for  $> 5$  seconds despite strong verbal encouragement. Two investigators blindly determined the GET using the V-slope method (Beaver et al, 1986). The CV for each of these estimates was determined ( $4 \pm 3\%$ ). In instances where the coefficient of variation was  $> 10\%$  ( $n = 1$ ), the GET was determined from a third investigator. The closest two estimates were averaged and used for subsequent analysis.

##### ***Step test and ramp test to exhaustion.***

The step test consisted of  $10 \times 3$  min stages at increasing intensities. The resistance was individually set based on the ramp test, and intended to elicit intensities from  $\sim 40$  to  $\sim 80\%$  peak  $\dot{V}O_2$  for each condition. Stages were interspersed with 30 s of rest for a capillary blood samples to be collected. After the 10<sup>th</sup> stage was completed, participants were given 5 min of rest before another ramp test (Ramp 2) was completed. Ramp 2 started at the intensity of GET, and increased at a constant rate of  $15\% \text{ GET}\cdot\text{min}^{-1}$  until exhaustion.

##### ***Constant-work rate tests to exhaustion.***

The CWR tests to exhaustion started with a 5-min warm-up at  $90\% \text{ GET}$ , followed by a 5 min resting period. Subsequently, after a 5 s countdown and from a stationary position, participants started to cycle. They were instructed before, and encouraged throughout the test to attain their preferred cadence as soon as possible (typically  $\leq 3$  sec) and to continue pedalling for as long as possible. The intensity corresponded to  $112.5\%$  peak  $\dot{V}O_2$  attained in Ramp 2.

##### ***3-min all-out tests.***

Participants completed 3AO tests as described by Vanhatalo et al (2007). In brief, after a 5 min warm-up at  $90\% \text{ GET}$ , and a further 5 min rest, participants started pedalling at their preferred cadence for 3 min, whilst no resistance was applied. In the last 10 s of the unloaded phase, the participants increased their cadence to 110-120 rpm. At the start of the 3AO test, the cycle-ergometer switched to linear mode,

where the resistance is determined by cadence. Within each condition, the alpha factor was set so that, at the participant's preferred cadence, the resistance corresponded to 50% of the difference between the GET and the peak power output attained in Ramp 1. To prevent pacing, participants were instructed before, and verbally encouraged throughout the test, to maintain the highest possible cadence, and were unaware of elapsed time. All participants completed a familiarization 3AO test, which was not included in the subsequent statistical analysis. A 3AO test was deemed as valid if at least two of the following criteria were met: i) peak power was attained within the first 10 s (Vanhatalo et al., 2007); ii) Peak  $\dot{V}O_2$  corresponded to  $\geq 97$ -99%  $\dot{V}O_{2max}$  (Burnley et al., 2006; Vanhatalo et al., 2007); iii)  $W'$  was depleted to  $\sim 5\%$  of its initial value within the first 90 s (Vanhatalo et al., 2008b); and iv) end-test cadence was within  $\pm 10$  rpm of the participant's preferred cadence (Vanhatalo et al., 2008b). Moreover, in instances where pacing was observed ( $n = 2$ ), the participant repeated the test.

### **7.3.5. Statistical analysis**

Changes in  $FiO_2$  and  $SaO_2$  were compared using a two-way ANOVA (condition [i.e. hypoxia, normoxia, and hyperoxia]  $\times$  test [i.e. Ramp 1, Ramp 2, CWR and 3AO tests]). In order to determine  $\dot{V}O_2$  at each submaximal stage of the step tests as well as peak  $\dot{V}O_2$  during Ramp 1 and Ramp 2, breath-by-breath  $\dot{V}O_2$  data were filtered, whereby values  $> 4 SD$  from a 5-breath rolling average were excluded. Using a 30 s rolling average, the  $\dot{V}O_2$  at each submaximal stage was determined as the highest  $\dot{V}O_2$  value within the last 60 s of each stage, whereas peak  $\dot{V}O_2$  was determined as the highest value attained during the ramp tests (Ramp 1 and Ramp 2). The effect of condition on peak  $\dot{V}O_2$  attained in Ramp 1 and Ramp 2 was determined using a two-way ANOVA. The effect that  $FiO_2$  had on the duration, accumulated oxygen uptake and demand, the AOD (absolute and percentage of total oxygen demand),  $W'$  (absolute and percentage of total work done), and peak  $\dot{V}O_2$ , heart rate and  $BLa$  during the CWR and 3AO tests was compared using two-way ANOVAs. Further to the filtering described above, breath-by-breath  $\dot{V}O_2$  data were linearly interpolated on a second-by-second basis. The oxygen demand was determined using a linear extrapolation of the  $\dot{V}O_2$ -power output relationship from the step test, so the accumulated oxygen demand was determined for the CWR and 3AO tests. The highest  $\dot{V}O_2$  attained in the CWR and 3AO tests was calculated as the highest 10-s rolling average. In the 3AO test, since power output is variable, raw power output measurements (recorded at 6 Hz by default), were subsequently averaged to produce 1 Hz data. The accumulated oxygen demand and uptake, therefore, were calculated as the area under the curve for oxygen demand and  $\dot{V}O_2$  over the duration of the test, respectively. AOD was

then determined as the difference between the accumulated oxygen demand minus the accumulated oxygen uptake. A resting value for TSI, O<sub>2</sub>Hb and HHb was obtained in each condition. The difference between end-exercise (last 10 s before exhaustion) and rest ( $\Delta$ ) was determined for each variable, and the effects of differences between condition and test (CWR and 3AO) were determined using a two-way ANOVA. Pearson's product-moment correlation coefficients were determined between the change, expressed as percentage of normoxia, of W' and CP in hypoxia and hyperoxia. Similarly, the percentage of change in AOD and  $\dot{V}O_{2max}$  between hypoxia and hyperoxia were determined. All analyses were conducted using IBM SPSS (Version 21.0, Armonk, NY), and significance was accepted at  $P < 0.05$ . In instances where a significant effect was detected, specific differences were located using Bonferroni-adjusted *post-hoc* tests.

## 7.4. Results

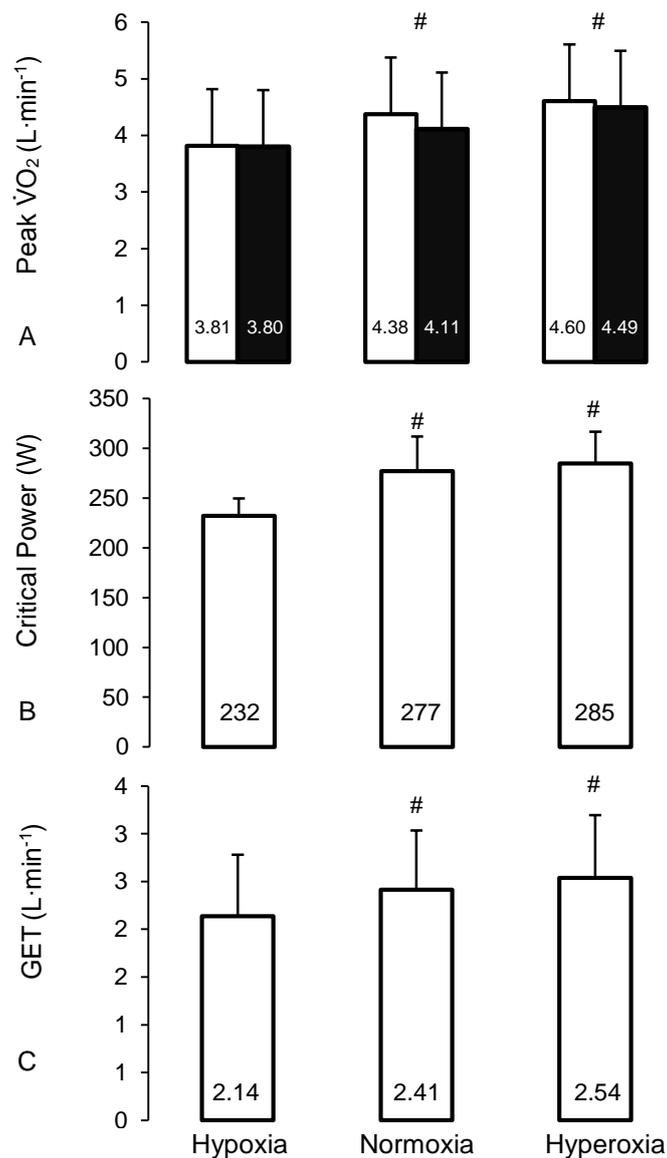
Similarly, there were no differences in SaO<sub>2</sub> between trials within the same condition ( $P = 0.222$ ), but SaO<sub>2</sub> was lowest in hypoxia ( $80.4 \pm 2.9\%$ ), intermediate in normoxia ( $91.5 \pm 2.9\%$ ), and highest in hyperoxia ( $96.1 \pm 2.3\%$ ) (all  $P < 0.001$ ).

### 7.4.1. Step test and ramp tests to exhaustion

Figure 7.2 shows peak  $\dot{V}O_2$  from Ramp 1 and Ramp 2, CP, and the GET for each condition. GET and CP were affected by the condition ( $P = 0.003$  and  $P < 0.001$ , respectively). Specifically, both GET and CP were lowest in hypoxia, intermediate in normoxia, and highest in hyperoxia; though there were no significant differences between normoxia and hyperoxia ( $P = 0.550$  and  $P = 0.974$ , respectively). There was an effect of condition on peak  $\dot{V}O_2$  ( $P = 0.001$ ). *Post-hoc* tests revealed that peak  $\dot{V}O_2$  was lower in hypoxia than normoxia ( $P = 0.016$ ) and hyperoxia ( $P = 0.012$ ). However, there were no differences in peak  $\dot{V}O_2$  between normoxia and hyperoxia ( $P = 0.197$ ). There were no differences in peak  $\dot{V}O_2$  between ramp tests conducted within the same condition i.e. Ramp 1 vs. Ramp 2;  $P = 0.084$ ). For simplicity, therefore,  $\dot{V}O_{2max}$  within each condition was considered as the mean peak  $\dot{V}O_2$  attained in Ramp 1 and Ramp 2 ( $3.81 \pm 0.64$ ,  $4.24 \pm 0.65$ , and  $4.55 \pm 0.63$  L·min<sup>-1</sup> for hypoxia, normoxia, and hyperoxia, respectively).

There were no differences in the intensity of the submaximal step tests ( $P = 0.654$ ) between conditions expressed relative to each condition-specific  $\dot{V}O_{2max}$  ( $44 \pm 8\%$  to  $80 \pm 9\%$ ,  $42 \pm 5\%$  to  $76 \pm 10\%$ , and  $46\% \pm 3\%$  to  $80\% \pm 7\%$  for hypoxia, normoxia, and hyperoxia, respectively). Absolute intensities,

however, were higher as  $\text{FiO}_2$  increased ( $82 \pm 12$  W to  $186 \pm 28$  W,  $96 \pm 24$  W to  $220 \pm 55$  W and  $106 \pm 22$  W to  $243 \pm 51$  W for hypoxia, normoxia, and hyperoxia, respectively; all  $P < 0.001$ ). All participants showed strong linear relationships between  $\dot{V}\text{O}_2$  and power output across all three conditions ( $0.975 \leq r \leq 0.999$ ; all  $P < 0.001$ ). Furthermore, there were no differences in the slope (grand mean ( $n = 30$ )  $12.03 \pm 1.64$   $\text{mL}\cdot\text{kg}^{-1}\cdot\text{W}^{-1}$ ;  $P = 0.279$ ) and intercept (grand mean:  $729 \pm 291$  mL;  $P = 0.139$ ) of the lines of best fit between conditions.



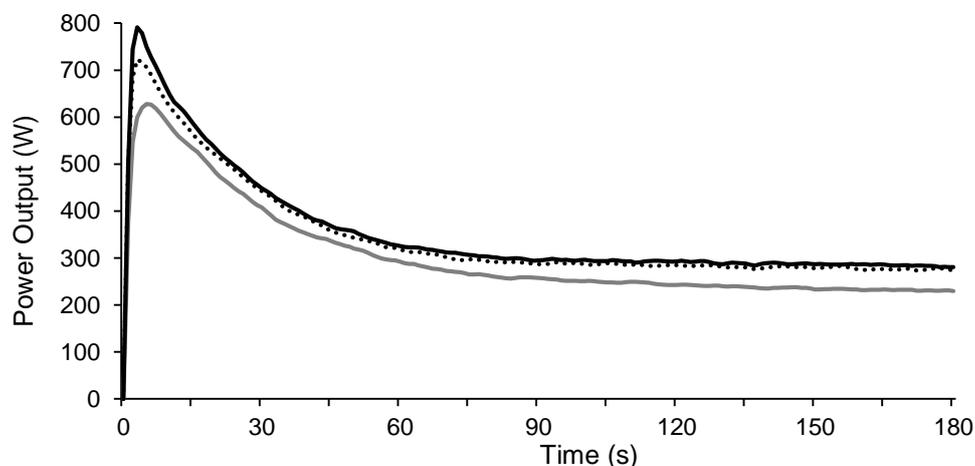
**Figure 7.2.** Effects of exercising under hypoxia, normoxia and hyperoxia on peak  $\dot{V}O_2$  (Ramp 1: empty bars, Ramp 2: filled bars; Panel A), critical power (Panel B), and the gas exchange threshold (GET; Panel C). # denotes a significant ( $P < 0.05$ ) difference from hypoxia. Bars and lines represent means and standard deviations, respectively. Mean values are also reported numerically within bars.

#### 7.4.2. Constant work-rate and 3-min all-out tests

The physiological responses to the CWR tests to exhaustion and the 3AO tests are presented in Table 7.1. All 3AO tests were deemed as valid (Figure 7.3). End-exercise  $\dot{V}O_2$  (pooled data,  $n = 30$ ) was  $94 \pm 15\% \dot{V}O_{2max}$ , power output attained its peak in  $4 \pm 2$  s and decreased thereafter so that, after 90 s,  $W'$  was depleted to  $4 \pm 3\%$  of its magnitude. At the end of the test, cadence was  $2 \pm 8$  rpm different from each participants preferred cadence. There were no differences in TTE between CWR and 3AO tests ( $P = 0.098$ ), or between conditions ( $P = 0.726$ ). However, there was a high ( $44 \pm 22\%$ ) within-individual

variation in TTE between CWR tests in hypoxia, normoxia and hyperoxia. The intensity of the CWR tests, expressed relative to CP, was not different between conditions ( $123 \pm 17\%$  CP in hypoxia,  $124 \pm 13\%$  CP in normoxia, and  $129 \pm 13\%$  CP in hyperoxia ( $P = 0.501$ ), with the within-individual variation being  $8 \pm 5\%$  across the three conditions. The accumulated oxygen demand was not affected by condition ( $P = 0.855$ ) or test ( $P = 0.152$ ). Similarly, the accumulated oxygen uptake was not affected by condition ( $P = 0.540$ ) or test ( $P = 0.500$ ). The AOD was not affected by the condition ( $P = 0.207$ ), though it was significantly affected by the type of test ( $P = 0.019$ ). Specifically, CWR tests produced greater AODs than 3AO tests. There was a large within-individual variation in the AOD between conditions during CWR ( $48 \pm 38\%$ ) and 3AO ( $54 \pm 26\%$ ) tests. With regards to  $W'$ , there was no condition ( $P = 0.535$ ) or test ( $P = 0.162$ ) effect. The within-individual variation in the  $W'$  was  $38 \pm 23\%$  and  $16 \pm 6\%$  for CWR and 3AO tests, respectively

Peak heart rate was unaffected by the test ( $P = 0.488$ ) or condition ( $0.429$ ), whilst peak BLa was greater in 3AO tests to exhaustion than CWR tests ( $P = 0.001$ ), but it was unaffected by condition ( $P = 0.210$ ). Peak  $\dot{V}O_2$  relative to the condition-specific  $\dot{V}O_{2max}$ , was not affected by the condition ( $P = 0.089$ ) or the test ( $P = 0.452$ ). In contrast, if expressed in absolute terms, peak  $\dot{V}O_2$  was affected by the condition ( $P < 0.001$ ), with no effect on test ( $P = 0.446$ ). *Post hoc* tests revealed that peak  $\dot{V}O_2$  was lower in hypoxia than normoxia ( $P = 0.014$ ) and hyperoxia ( $P = 0.004$ ), but there were no differences between normoxia and hyperoxia ( $P = 0.080$ ). In the three CWR tests to exhaustion, estimations of AnC derived from AOD tended to be greater than those derived from  $W'$  in all three environmental conditions ( $P \leq 0.066$ ). There were no differences in the AnC estimation derived from AOD and  $W'$  in all-out tests.



**Figure 7.3.** Power output during 3-min all-out tests in hyperoxia (black – continuous line), normoxia (black – dotted line) and hypoxia (grey line). Lines are means, standard deviations are not reported for clarity.

**Table 7.1.** Physiological responses to a constant work-rate (CWR) exercise to exhaustion and a 3-min all-out (3AO) test in hypoxia (15% oxygen), normoxia (21% oxygen) and hyperoxia (35%) oxygen.

	CWR tests			3AO tests		
	Hypoxia	Normoxia	Hyperoxia	Hypoxia	Normoxia	Hyperoxia
Duration (s)	239 ± 76	203 ± 107	200 ± 148	180 ± 0	180 ± 0	180 ± 0
Power output (W)	285 ± 30	336 ± 51	367 ± 67	307 <sup>#</sup> ± 35	346 <sup>#</sup> ± 48	356 <sup>#</sup> ± 41
Acc oxygen demand (L)	16.96 ± 5.70	14.85 ± 5.95	15.67 ± 8.48	13.38 ± 2.50	13.96 ± 2.31	14.85 ± 1.47
Acc oxygen uptake (L)	12.17 ± 4.45	11.28 ± 5.69	12.32 ± 5.69	9.75 ± 1.24	10.64 ± 1.99	12.74 ± 2.21
AOD (L)	4.79 ± 2.16	3.57 ± 1.22	3.35 ± 1.83	3.51 ± 1.83	3.26 ± 1.40	2.04 ± 2.11
AOD (% Acc oxygen demand)	28.3 ± 9.2	26.5 ± 11.2	26.0 ± 11.7	25.07 ± 10.1	23.2 ± 8.1	13.5 ± 13.9
Work (kJ)	67.53 ± 19.86	64.82 ± 25.38	68.61 ± 25.38	55.20 ± 6.36	62.23 ± 8.71	64.15 ± 7.45
W' (kJ)	12.18 ± 6.18	10.20 ± 8.11	12.10 ± 6.17	13.51 ± 3.78	12.67 ± 3.26	13.21 ± 4.73
W' (% Work)	18.6 ± 8.6	17.4 ± 10.7	21.5 ± 8.9	24.6 ± 7.2	20.6 ± 5.7	20.5 ± 6.6
End-exercise $\dot{V}O_2$ (L·min <sup>-1</sup> )	3.38 ± 0.75	3.85 ± 0.66	4.30 ± 0.73	3.33 ± 0.50	3.81 ± 0.77	4.68 ± 0.84
End-exercise $\dot{V}O_2$ (% $\dot{V}O_{2max}$ )	89 ± 12	91 ± 9	96 ± 16	88 ± 10	90 ± 11	104 ± 19
Peak HR (beats·min <sup>-1</sup> )	164 ± 11	165 ± 11	167 ± 12	163 ± 11	164 ± 8	166 ± 13
Peak BLa (mmol·L <sup>-1</sup> )	10.00 ± 2.19	9.82 ± 1.74	9.19 ± 1.90	12.56 ± 2.29	11.80 ± 2.62	11.38 ± 2.71

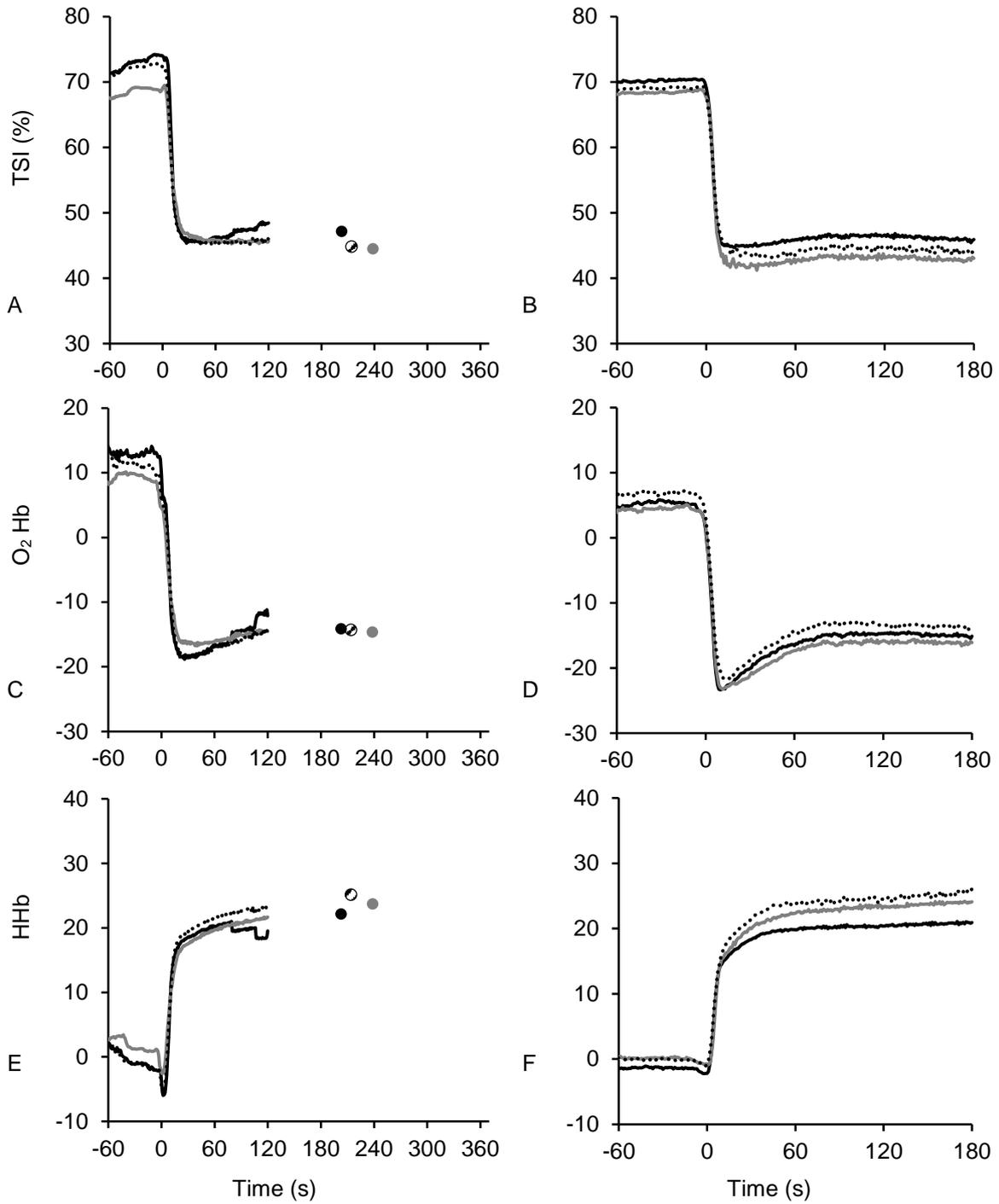
<sup>#</sup> indicates average power output during the entire test.

### 7.4.3. Near-infrared spectroscopy

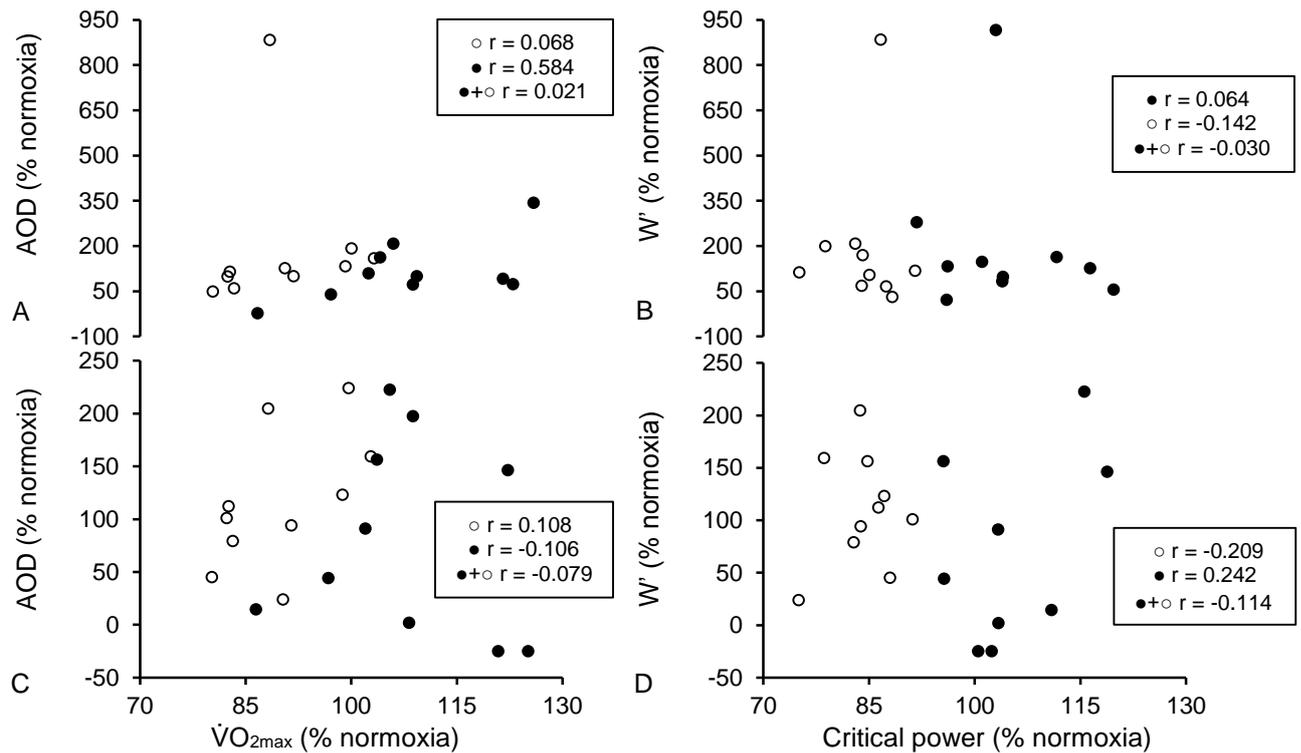
The acquisition of NIRS data was consistently faulty (4 out of 6 tests) in one participant, and his data were removed from the analysis. The overall response of O<sub>2</sub>Hb, HHb, and TSI of the remaining nine participants is presented in Figure 7.4. None of the variables were affected by the test at rest and at the point of exhaustion ( $P = 0.346$ ,  $P = 0.119$ , and  $P = 0.380$  for TSI, O<sub>2</sub>Hb, and HHb, respectively). However, whilst O<sub>2</sub>Hb and HHb were not affected by environmental conditions ( $P = 0.504$  and  $P = 0.106$ , respectively), there was a significant effect on TSI ( $P = 0.021$ ). *Post hoc* tests, however, were unable to locate these differences. The delta values for TSI and HHb were not affected by condition ( $P = 0.943$  and  $P = 0.344$ , respectively) or test ( $P = 0.508$  and  $P = 0.595$ , respectively). Moreover, environmental conditions had no effect on  $\Delta\text{O}_2\text{Hb}$  ( $P = 0.367$ ). However,  $\Delta\text{O}_2\text{Hb}$  was further decreased in CWR tests than in the 3AO tests ( $P = 0.001$ ).

### 7.4.4. Correlation between relevant variables

The correlation between the changes during hypoxic and hyperoxic conditions, relative to normoxia, in AOD and  $\dot{V}\text{O}_{2\text{max}}$ , and between  $W'$  and CP, are presented in Figure 7.5. None of the variables were significantly correlated, irrespective of the condition (hypoxia, hyperoxia, or both), test (CWR or 3AO) or variables (AOD and  $\dot{V}\text{O}_{2\text{max}}$ , or  $W'$  and CP).



**Figure 7.4.** Muscle oxygenation during (tissue saturation index (TSI) and oxy- and deoxy-haemoglobin concentration (O<sub>2</sub> Hb and HHb, respectively) during constant work-rate (Panels A, C and D) and 3-min all-out tests (Panel B, D and F). Black - continuous lines represent hyperoxia, black – dotted lines lines represent normoxia, and grey lines represent hypoxia. In constant work-rate tests, only the initial 120 s are shown, and dots indicate mean values at the point of exhaustion. Lines indicate means, standard deviations are not shown for clarity.



**Figure 7.5.** Change, relative to normoxia, in accumulated oxygen deficit (AOD) and maximal oxygen uptake ( $\dot{V}O_{2max}$ ) during constant work-rate (Panels A) and 3-min all-out (Panel C) tests during hypoxia ( $\circ$ ) and hyperoxia ( $\bullet$ ). Panel B and Panel C represent the change in the curvature constant  $W'$  and critical power, relative to normoxia, in constant work-rate (Panel B) and 3-min all-out (Panel C) tests during hypoxia ( $\circ$ ) and hyperoxia ( $\bullet$ ).

## 7.5. Discussion

The aim of the present study was to investigate the physiological effects of acute hypoxia and hyperoxia exposure on physiological variables, with particular attention to AOD and  $W'$ . The main findings from this investigation were that i) AOD was not different between conditions, though AOD was higher during CWR tests to exhaustion than in the 3AO tests; ii)  $W'$  remains constant, irrespective of condition (hypoxia, normoxia, or hyperoxia) and pacing (CWR and 3AO tests); iii) there was no significant correlation between changes in CP and  $W'$ , or between changes in AOD and  $\dot{V}O_{2max}$ , during the hypoxic and hyperoxic trials; iv) hypoxia and hyperoxia had no effect on muscle oxygenation during either CWR or 3AO tests; and v) hypoxia reduces GET, CP, and  $\dot{V}O_{2max}$ ; but none of these variables were increased by hyperoxia.

The data from the present study challenges the hypothesis that AOD, as a test aiming to estimate AnC, remains consistent irrespective of power output profile and oxygen availability. First, it has been hypothesised that CWR tests to exhaustion lasting > 2 min and all-out test of at least 90 s result in a

consistent AOD (Gastin et al., 1995; Medbø et al., 1988). In contrast, but consistent with the results from Chapter 8, the AOD in the present study was greater during CWR tests than 3AO tests. Given that BLa was lower during CWR tests to exhaustion, it is possible that the 3AO efforts caused a greater metabolic perturbation than the CWR tests, as discussed previously (see Section 6.5). Secondly, as hypothesised in the present study and previously reported (Feriche et al., 2007; Friedmann et al., 2007; Medbø et al., 1988; Morales-Alamo et al., 2012; Ogura et al., 2006), there were no differences in AOD between conditions. Nonetheless, compared to normoxia, the mean AOD was increased by ~25% and ~7% in hypoxia (CWR and 3AO tests, respectively) and decreased by ~7% and ~37% in hyperoxia (CWR and 3AO tests, respectively). In turn these data suggest that the large intrasubject variability in the results might have prevented hypoxia and hyperoxia from affecting AOD. Indeed, in the hyperoxic trials, the AOD was negative in one participant during the CWR tests and in two participants during the 3AO tests. It is not possible to reconcile a negative AOD with the hypothesis that AOD provides a measure of AnC. Negative AODs have been already reported in the literature (Jones et al., 2008b; Özyener et al., 2003). For instance, Jones et al. (2008b) reported that a 'fast start' pacing strategy, where power output progressively decreases, resulted in increased performance and reduced AOD, so that two out of the seven subjects in that study attained a negative AOD. Moreover, these two participants exhibited the largest TTE (see Figure 6 in Jones et al., 2008b). Jones et al. (2008b) speculated that, although power output (and, therefore, oxygen demand) was decreasing, the slow component of  $\dot{V}O_2$  prevented  $\dot{V}O_2$  from decreasing. Özyener et al. (2003) also suggested that the slow component of  $\dot{V}O_2$  increased  $\dot{V}O_2$  above the projected oxygen demand, and therefore AOD becomes negative. Indeed, the slow component of  $\dot{V}O_2$  can be manifested either as a decrease in power output, despite  $\dot{V}O_2$  remaining elevated; or as an increase in  $\dot{V}O_2$ , despite power output being constant (Grassi et al., 2015; Jones et al., 2011; Vanhatalo et al., 2011b; Zoladz et al., 2008). Then again, hyperoxia seems to have no effect on the  $\tau$  of the primary phase of  $\dot{V}O_2$  on-kinetics, but reduces the amplitude of the slow component of  $\dot{V}O_2$  (Wilkerson et al., 2006). Therefore, although the slow component of  $\dot{V}O_2$  seems to be the strongest candidate to explain the negative AOD observed in some hyperoxic trials of the present study, other factors need to be considered.

The other variable hypothesised to represent anaerobic energy production in the present study,  $W'$ , remained constant irrespective of power output profile (CWR vs. 3AO tests) and environmental conditions (hypoxia, normoxia and hyperoxia). This finding is consistent with previous research, and

adds to the body of evidence suggesting that  $W'$  is a robust measure (Chidnok et al., 2013a, b; Dekerle et al., 2012; Simpson et al., 2015; Vanhatalo et al., 2008b). Valli et al. (2011) reported an increase in  $W'$  at 5050 m ( $FiO_2 \sim 0.11$ ) compared to sea level. Importantly, severe levels of hypoxia ( $FiO_2 < 0.13$ ) have been shown to alter the relative contribution of central and peripheral components of fatigue (Amann, Romer, Subudhi, Pegelow, & Dempsey, 2007). Thus,  $W'$  appears to remain constant irrespective of pacing during moderate hypoxia (providing  $FiO_2 > 0.13$ ) and hyperoxia, suggesting that anaerobic energy production underpins  $W'$ . It is interesting to note that, whilst  $W'$  was decreased during CWR tests in Chapter 8, this was not the case in the present study. Most likely, the longer TTE during the CWR of the present study (164 vs. 214 s) allowed  $W'$  to be fully depleted. In turn, these data support previous research (Chidnok et al., 2013a) and suggests that  $W'$  remains consistent during exercise  $\geq 3$  min.

It has been recently suggested that, although mean  $W'$  is not affected by oxygen availability, there is a negative relationship between changes in CP and  $W'$  in hypoxia and hyperoxia, compared to normoxia (Dekerle et al., 2012; Simpson et al., 2015; Vanhatalo et al., 2010). In contrast, such a relationship was not observed in the present study (Figure 7.5). First, it is plausible to explain these differences by the exercise-induced hypoxemia that athletes might or might not experience during maximal tests to exhaustion in normoxia, as denoted by a normoxic end-exercise  $SaO_2 < 90\%$  or  $> 92\%$ , respectively (Chapman, Emery, & Stager, 1999; Nielsen, 2003). Those athletes who did not experience exercise-induced hypoxemia had their  $\dot{V}O_{2max}$  unaffected by mild hypoxia (Chapman et al., 2003). Therefore, given the strong relationship between CP and  $\dot{V}O_{2max}$  (Murgatroyd et al., 2011), it is plausible that some of the participants in the current study exhibited no exercise-induced hypoxemia, so their CP was not affected in hypoxia. Then again, although Dekerle et al. (2012) only included participants exhibiting exercise-induced hypoxemia, Simpson et al. (2015) reported an inverse relationship between changes in CP and  $W'$  in hypoxia without verifying whether the participants in that study exhibited exercise-induced hypoxemia. Secondly,  $W'$  seems to be determined not only by AnC, but by other factors such as the slow component of  $\dot{V}O_2$ ,  $\dot{V}O_{2max}$  and/or the ability to acquire and tolerate fatigue (Broxterman et al., 2015a, b; Grassi et al., 2015; Johnson et al., 2013; Murgatroyd et al., 2011; Nicolò et al., 2015; Skiba et al., 2015).

According to the hypothesis of the present study, there was a decrease in aerobic metabolism during the hypoxic trials, as denoted by decreases in the GET, CP and  $\dot{V}O_{2max}$  (Figure 7.2). Furthermore, the reduction in  $\dot{V}O_{2max}$  in the present study (~10%), was similar to that reported by Dekerle et al. (2012) using a similar hypoxic exposure (~12%). In contrast to the hypothesis, however, hyperoxic trials did not increase GET, CP, or  $\dot{V}O_{2max}$ . Peltonen et al. (2001) reported a ~13% increase in  $\dot{V}O_{2max}$  with a similar hyperoxic exposure ( $FiO_2 = 0.32$ ). In the current study, mean  $\dot{V}O_{2max}$  increased by ~8%, with 8 out of 10 participants achieving a larger  $\dot{V}O_{2max}$  hyperoxia compared to normoxia. Similarly, 8 and 7 participants exhibited a larger GET and CP during under hyperoxic conditions, respectively, whereas 9 out of 10 participants attained their highest end-exercise  $\dot{V}O_2$  during both CWR and 3AO tests in hyperoxia. It is plausible that the low number of participants in the current study prevented GET, CP, and  $\dot{V}O_{2max}$  from being increased in hyperoxia compared to normoxia.

There are a couple of limitations to this study that need to be addressed. First, to the best of the researchers knowledge, this is the first study to conduct 3AO tests under hyperoxic conditions. Indeed, with the exception of Simpson et al. (2015), 3AO tests have been conducted exclusively in normoxia. These authors (Simpson et al., 2015) validated the 3AO tests in hypoxia by determination of CP and  $W'$  using both the traditional approach of modelling the TTE from several CWR exercise bouts to exhaustion and the 3AO test. However, using the traditional approach to validate the measurement of CP and  $W'$  from the 3AO test would have increased an already demanding experimental design by 3-4 trials. Instead, following current recommendations (Dekerle et al., 2008; Simpson et al., 2015; Vanhatalo et al., 2008b), all 3AO tests were performed using condition-specific alpha factors. Indeed end-test cadence was within 10 rpm of the preferred cadence, peak power was attained early in the test; therefore,  $W'$  was largely depleted halfway through the test, and end-exercise  $\dot{V}O_2$  approached  $\dot{V}O_{2max}$  (Figure 7.3). Whilst further studies need to validate the 3AO test in hyperoxia, the present study suggests that the 3AO test can be used to determine CP and  $W'$  during hyperoxia. Secondly, measuring  $\dot{V}O_2$  under hyperoxic conditions is technically difficult, and might result in an overestimation of  $\dot{V}O_2$  (Linossier et al., 2000; Prieur et al., 1998, Wilkerson et al. 2006). Nonetheless,  $\dot{V}O_2$  has been accurately determined using an online gas analyser under conditions of mild hyperoxia ( $FiO_2 = 0.30$ ) (Prieur et al., 1998). In addition, the test-retest variability in peak  $\dot{V}O_2$  during Ramp 1 and Ramp 2 in hypoxia was 5%, which approximates values reported in the literature (e.g. Barbosa et al., 2014; Chapter 5), and

was similar to the corresponding values in normoxia, suggesting that  $\dot{V}O_2$  measurements were not overestimated in hyperoxia.

### **7.5.1. Conclusion**

In conclusion, this study demonstrated that AOD is not affected by oxygen availability during acute hypoxic and hyperoxic exposure, though it was greater in CWR than 3AO tests. In contrast, the magnitude of  $W'$  remains constant, irrespective of the distribution of power output and oxygen availability. Compared to normoxia, changes in AOD and  $W'$  were not correlated with  $\dot{V}O_{2max}$  and CP, suggesting that both represent, to some extent, anaerobic energy production. Nonetheless, large individual variability between and within subjects supports the need for caution when using either approach to estimate AnC.

# **Chapter 8: General discussion and conclusions**

The overarching aim of this PhD thesis was to investigate the assumptions and limitations surrounding AOD and  $W'$  as indirect tests to estimate AnC. The main findings of the PhD were i) the use of 10 × 3-min bouts of exercise at increasing exercise intensities results in a linear  $\dot{V}O_2$ -power output relationship, which can be projected to predict the oxygen demand at supramaximal intensities. ii) The magnitude of AOD is not consistent. Instead, it reaches its maximum value during a CWR exercise bout to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ . At intensities below 112.5%  $\dot{V}O_{2max}$  or above 120%  $\dot{V}O_{2max}$ , AOD underestimates AnC. Similarly, the AOD determined from a 3AO test underestimates AnC. iii) In a CWR exercise bout to exhaustion at 112.5%  $\dot{V}O_{2max}$ , the reliability of AOD is 8.72%. iv) The AOD determined from a supramaximal CWR exercise bout to exhaustion should not be determined from a single-day trial, but from a separate trial on a different day. v) There is a strong correlation between AOD and  $W'$ . The strength of this correlation remains constant, irrespective of pacing, and suggests ~43% of AOD and  $W'$  is determined by a shared factor. vi) AOD and  $W'$  remain unaffected by hypoxia and hyperoxia. vii) There was a high within-subject variation in the response of  $W'$  and, in particular, AOD to hypoxia and hyperoxia. Changes due to acute hypoxia and hyperoxia exposure in  $\dot{V}O_{2max}$  and CP, compared to a control condition in normoxia, did not correlate with those of AOD and  $W'$ , respectively. Overall, the current PhD thesis suggests that AOD should be determined from a CWR test to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ . In addition, the positive correlation between AOD and  $W'$ , and the fact that hypoxia and hyperoxia had no effect on AOD and  $W'$ , suggest that both measures represent AnC. However, there was a high variability within and between subjects in  $W'$  and, in particular, AOD. In conclusion, although  $W'$  is likely determined by other factors besides AnC, it appears to be a favourable option to estimate anaerobic energy production.

## **8.1. Anaerobic capacity estimated from the accumulated oxygen deficit**

The AOD has been a constant approach to estimate AnC throughout the present PhD thesis, as it is considered the best non-invasive approach to estimate AnC (Noordhod et al., 2010). For instance, all direct and indirect methods described in the literature review have been compared to the AOD as a means to assess their validity. In addition, AOD is sensitive enough to be able to differentiate between populations that are expected to have different AnC (e.g. endurance vs. sprinter athletes) and to identify

changes after a high-intensity training programme. Furthermore, the AOD has been used to determine the energy demands in a variety of sporting events. Nonetheless, the AOD relies on a number of assumptions, some of which have been addressed in the present PhD thesis.

The determination of AOD necessitates the estimation of the oxygen demand, typically from a linear extrapolation of the  $\dot{V}O_2$ -power output relationship. In turn, the estimation of the oxygen demand above the LT (or GET) is complex due to the effect that the slow component of  $\dot{V}O_2$  might have on the  $\dot{V}O_2$ -power output relationship. Nonetheless, using 3 min stages, it has been demonstrated throughout the current thesis that the  $\dot{V}O_2$ -power output relationship remains linear below and above the LT up to ~95%  $\dot{V}O_{2max}$  (Chapter 5) with exceptionally high correlation coefficients (Chapters 5-9). These results suggest that the slow component of  $\dot{V}O_2$  was minimised due to the relatively short duration of the stages. However, although the slow component of  $\dot{V}O_2$  is not readily visible during exercise above CP because the increase in  $\dot{V}O_2$  is truncated at  $\dot{V}O_{2max}$  (Figure 1.12), the mechanisms underpinning the slow component of  $\dot{V}O_2$  were most likely present at submaximal intensities above the LT and throughout the CWR and 3AO tests (Jones et al., 2011; Vanhatalo et al., 2011b). Given that oxygen demand is a theoretical construct that cannot be directly measured, it is difficult to determine whether linear or non-linear models to describe the energy demand-exercise intensity relationship should be used to calculate AOD. However, Li et al. (2015) recently reviewed the relative contribution of the aerobic and anaerobic energy systems during high-intensity exercise lasting up to 10 min. The authors reported a lower estimation of anaerobic energy production using AOD compared to estimations derived from [PCr] and BLa measurements, suggesting that AOD underestimates AnC. In summary, throughout this PhD thesis consecutive 3 min stages at increasing intensities have been shown to result in strong  $\dot{V}O_2$ -power output linear relationships, which can be used to predict supramaximal oxygen demands, as required to determine AOD. However, future research is warranted to investigate whether the oxygen demand predicted from this linear relationship best reflects the estimated energy cost at supramaximal intensities.

An alternative approach to calculating AOD may be derived from the difference between energy demand and oxygen uptake, as opposed to the difference between oxygen demand and oxygen uptake. Indeed, running economy has been traditionally determined using the oxygen cost, defined as the oxygen required for covering a given distance (e.g. 1 km) (Fletcher, Esau, & MacIntosh, 2009).

Recently, it has been suggested that energy cost, as opposed to oxygen cost, provides a more physiologically sound approach to assess running economy (Fletcher et al., 2009; Shaw et al., 2014; Shaw, Ingham, Atkinson, & Folland, 2015). The oxygen cost is determined as the product of  $\dot{V}O_2$  and its corresponding energy equivalent, derived from the determination of the respiratory exchange ratio. In brief, the  $\dot{V}O_2$ -running speed relationship is linear when determined using 3 min stages, minimising the slow component of  $\dot{V}O_2$  (Fletcher et al., 2009; Shaw et al., 2014; see discussion above), and as a result the oxygen cost is independent of changes in speed. However, as running speed increases, so does the respiratory exchange ratio and, therefore, the energy cost of running (Fletcher et al., 2009; Jeukendrup & Wallis, 2005; Shaw et al., 2014). There have been some attempts to determine AOD using the energy cost-intensity relationship, as opposed to  $\dot{V}O_2$ -intensity relationship (Keir, Zory, Boudreau-Larivière, & Serresse, 2012). Nonetheless, the overwhelming majority of the research has used the  $\dot{V}O_2$ -work rate relationship (Table 2.1 and 2.2), including the studies of the current PhD thesis. Future research should investigate whether the energy cost-work rate relationship overcomes some of the limitations highlighted in the present PhD thesis with regards to using AOD as a measure of AnC.

Medbø et al. (1988) originally reported that the AOD peaks during CWR exercise that lasts 2-5 min, which corresponded to intensities of ~110-120%  $\dot{V}O_{2max}$ . In Chapter 6, this assumption was challenged by calculating the AOD during CWR exercise at 105, 112.5, 120 and 127.5%  $\dot{V}O_{2max}$ . The results showed that the AOD follows an inverted-U shape, with AOD from trials at 105 and 127.5%  $\dot{V}O_{2max}$  being reduced. At 127.5%  $\dot{V}O_{2max}$ , exercise was sustained for only ~1.5 min, suggesting that exercise was terminated before AnC was fully depleted. In contrast, at 105%  $\dot{V}O_{2max}$ , exercise lasted ~4.4 min, but AOD was reduced compared to 112.5 and 120%  $\dot{V}O_{2max}$ . Moreover, all-out tests with a duration > 60 s have been shown to produce a AOD similar to CWR test to exhaustion (Gastin et al., 1995; Withers et al., 1991, 1993). However, in Chapters 8 and 9, the AOD was increased during CWR tests versus 3AO. If, as discussed above, the mechanisms of the slow component of  $\dot{V}O_2$  were present during supramaximal exercise, it would be possible to explain the above results by a decrease in efficiency during supramaximal exercise, which in turn challenges the assumption that the predicted oxygen demand from the  $\dot{V}O_2$ -power output relationship remains constant. Indeed, there is evidence of an increase in the rate of ATP resynthesis during constant-load exercise (Bangsbo et al., 2001; Cannon et al., 2014; Krusturup et al., 2003). Accounting for this decrease in efficiency during exercise, Mulder et al. (2015) demonstrated that the AOD remained constant for durations from ~0.75 to ~5.5 min, but

assuming a constant efficiency resulted in a decrease in AOD in the longer trials. In summary, data from the current PhD thesis suggest that AOD reaches its peak during a CWR test to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ . In contrast, CWR tests to exhaustion at intensities below 112.5%  $\dot{V}O_{2max}$  or above 120%  $\dot{V}O_{2max}$  and 3AO tests underestimate AnC by means of a reduced AOD. In summary, the results of the current PhD thesis suggest that a supramaximal CWR exercise to exhaustion at 112.5-120%  $\dot{V}O_{2max}$  elicits the highest AOD, and therefore should be used to estimate AnC. Future research should address whether accounting for the decrease in efficiency during supramaximal exercise improves the validity of the test.

Further to methodological considerations regarding the protocol that should be used to produce the highest AOD, and therefore represent AnC, the current thesis addressed whether hypoxia and hyperoxia have any effect on AOD. In Chapter 9, the AOD was determined from CWR tests to exhaustion at 112.5%  $\dot{V}O_{2max}$  and 3AO tests. As discussed above, CWR tests produced greater AOD than 3AO tests. The results of that study showed that there was no effect of hypoxia and hyperoxia on AOD. Close inspection of the data, however, revealed a high within-subject variability during hypoxic and hyperoxic trials. Indeed, in some hyperoxic trials, the AOD determined from CWR and 3AO tests, became negative, which is not physiologically possible if AOD is supposed to represent AnC. In effect, the results from that study challenge the assumption that AOD is a valid measure of AnC.

In addition to the physiological assumptions discussed above, this PhD aimed to investigate whether the AOD could be determined in a more time-efficient single-day trial. Chapter 7 investigated whether performing 10 × 3 min consecutive exercise bouts at increasing intensities (to construct the  $\dot{V}O_2$ -power output relationship) followed by an incremental test to exhaustion (to determine  $\dot{V}O_{2max}$ ) affected the AOD determined from a subsequent CWR exercise bout to exhaustion at 112.5%. Both TTE and AOD were reduced by ~16% in the single-trial approach, suggesting that 25 min of rest between the end of the incremental test to exhaustion and the CWR exercise bout to exhaustion did not allow full recovery of AnC. From a practical perspective, the results from Chapter 7 challenge the applicability of this method, and two trials seem to be required to assess AnC by means of AOD.

In summary, using 3 min stages, a linear  $\dot{V}O_2$ -power output relationship can be established to predict supramaximal oxygen demands. With this protocol, a CWR test to exhaustion at 112.5-120%  $\dot{V}O_{2max}$  seems to elicit the highest AOD. In contrast, using CWR tests at intensities below 112.5%  $\dot{V}O_{2max}$ , above

120%  $\dot{V}O_{2\max}$ , or 3AO tests appear to reduce AOD, and therefore underestimate AnC. Although AOD was not affected by hypoxia or hyperoxia, the fact that i) there was a large within-subject variation observed in the AOD, with some subjects displaying a negative AOD during tests under hyperoxic conditions; ii) AOD was greater during CWR than 3AO tests; and iii) two trials were required to determine AOD, suggest that AOD might not be the best non-invasive approach to estimate AnC, as previously suggested.

## **8.2. Anaerobic capacity estimated as $W'$**

The other main approach used in the current PhD thesis to estimate AnC was  $W'$ , derived from the parameters of the  $P-d$  relationship. The  $P-d$  relationship is non-linear, and traditionally fit with a hyperbolic function (Hill, 1995; Jones et al., 2010). The asymptote of the hyperbola denotes the CP, whereas the curvature constant  $W'$  represents a finite amount of work that can be completed above CP. At intensities above CP, physiological systems do not attain homeostasis. Thus, for instance,  $\dot{V}O_2$  does not attain a steady state, but increases inexorably towards  $\dot{V}O_{2\max}$  (Poole et al., 1988). In order to sustain exercise above CP, ATP is resynthesised continuously from anaerobic pathways until exercise is terminated (Jones et al., 2008c; Vanhatalo et al., 2010).

Under the assumption that AOD represents the best non-invasive test to estimate AnC (Noordhof et al., 2010), a positive linear correlation between  $W'$  and AOD has been reported previously (Chatagnon et al., 2005; Miura et al., 2002; Leclair et al., 2010). These studies, however, determined  $W'$  from a time-consuming protocol that requires each participant to complete 3-5 exhaustive tests with durations between 2 and 15 min (Chatagnon et al., 2005; Miura et al., 2002; Leclair et al., 2010). Chapter 8 demonstrated that  $W'$ , determined from a time-efficient 3AO test, also correlates with AOD. Moreover, the relationship between AOD and  $W'$  remains constant for CWR tests to exhaustion and 3AO tests. Altogether, these results suggest that a shared factor, most likely related to anaerobic energy production, underpins both AOD and  $W'$ . In turn, these data support the idea that  $W'$  can be used as a means to estimate AnC.

Previous research has suggested that  $W'$  remains constant during exhaustive exercise within the severe exercise domain and during 3AO tests (Chidnok et al. 2013a; Fukuba et al. 2003; Jones et al. 2008b; Souza et al., 2015; Vanhatalo et al. 2008; see Figure 1.8). In Chapter 8, however,  $W'$  was reduced in

the CWR tests compared to 3AO tests, but no such difference was observed in Chapter 9. The shorter TTE during the CWR tests to exhaustion in Chapter 8 compared to Chapter 9 (~164 vs. ~214 s) most likely prevented  $W'$  from being completely depleted before exhaustion. In effect, the results from the current PhD thesis suggest that, during a CWR exercise to exhaustion lasting < 3 min,  $W'$  might not be fully depleted.

Chapter 9 investigated the effects of hypoxia and hyperoxia on  $W'$ . As hypothesised mean  $W'$  was not affected by either condition, but there was a large within-subject variability. Some authors have suggested a 'compensation' mechanism whereby participants whose CP is more severely reduced by hypoxia (or augmented by hyperoxia), have their  $W'$  increased (or decreased, for hyperoxia) (Dekerle et al., 2012; Simpson et al., 2015; Vanhatalo et al., 2010). This was not the case in Chapter 9 (Figure 7.5), suggesting that the magnitude of  $W'$  does not depend on CP, and therefore, that it may be underpinned by anaerobic energy production (i.e. AnC).

In recent years, the traditional assumption that  $W'$  exclusively represents AnC has been challenged (Broxterman et al., 2015a, b; Copp et al., 2010; Grassi et al., 2015; Johnson et al., 2013; Murgatroyd et al., 2011; Nicolò et al., 2015; Skiba et al., 2015). Specifically, novel evidence has linked  $W'$  to a myriad of interlinked factors, which ultimately accelerates the development of fatigue until exercise becomes unsustainable and exhaustion occurs. In brief, at intensities above CP, there appears to be an increase in the recruitment of type II fibres (Copp et al., 2010). In turn, additional recruitment of type II fibres results in a reduction in efficiency, manifested as the slow component of  $\dot{V}O_2$  (Jones et al., 2011; Murgatroyd et al., 2011; Vanhatalo et al., 2011b). Moreover, recruitment of type II fibres results in the accumulation of fatiguing metabolites, such as  $[P_i]$  and  $[H^+]$  and/or the depletion of  $[PCr]$  (Fitts, 2008; Kent-Braun et al., 2012; Nelson, Debold, & Fitts, 2014). The accumulation (or depletion) of these metabolites continues until a critical threshold is reached,  $W'$  is depleted, and ultimately exercise is terminated (Chidnock et al., 2013a, b; Grassi et al., 2015; Vanhatalo et al., 2010). Alternatively, before  $W'$  has been completely depleted, the intensity of the exercise can be reduced below CP, so  $W'$  is recharged, and exercise can be continued (Chidnok et al., 2013c; Coats et al., 2003; Skiba et al., 2012, 2014, 2015). Because all the above processes are physiologically linked (Grassi et al., 2015; James & Green, 2012), it seems reasonable to assume that AnC does not exclusively explain the magnitude of  $W'$ . Nonetheless, irrespective of the precise mechanisms underpinning the  $W'$ , the parameters of the

*P-d* relationship offer athletes a unique framework to monitor fatigue during exercise and prevent exercise intolerance during training and competition (Skiba et al., 2014). Future research is warranted to determine the exact mechanisms underpinning  $W'$ , and how AnC, the slow component of  $\dot{V}O_2$  and fatigue all integrate to determine exercise intolerance (Poole & Barstow, 2015).

In summary, the present thesis demonstrated that  $W'$ , determined from a 3AO test, correlates with AOD. Moreover, providing the duration of the exercise is  $> 3$  min, the magnitude of  $W'$  is constant, irrespective of pacing or oxygen availability. However, recent evidence suggests that in addition to providing a measure of AnC,  $W'$  represents the magnitude of fatigue, and is influenced by the slow component of  $\dot{V}O_2$ , muscle fibre recruitment and/or  $\dot{V}O_{2max}$ . These results, in turn, suggest that the CP- $W'$  framework should be used to determine AnC, as well as to promote novel insights into the mechanisms of  $W'$  as a measure of fatigue.

### **8.3. Limitations**

The specific limitations surrounding AOD and  $W'$  have been addressed throughout this thesis and, therefore, will not be repeated here. However, further methodological considerations and potential limitations of the overall thesis are discussed below. All experimental studies (Chapters 5-9) have been conducted on endurance-trained cyclists and triathletes. However, AnC is likely a more interesting parameter for athletes performing sprint events. Unfortunately, the access to a sufficient number of track and sprint cyclists would have been difficult, with the risk of diminishing the statistical power of these studies. Moreover, all experimental studies were conducted using men. The female population is underrepresented in Sport and Exercise Science studies: only 39% of the participants in Sport and Exercise Science studies are women (Costello, Bieuzen, & Bleakley, 2014). The current PhD thesis has failed to address the under-representation of women in Sport and Exercise Science studies. Women could have been included by either using a single experimental group with both men and women, or using a group with only women (irrespective of using another group of men). The former option has been used by some studies (see Table 2.1 and 2.2). However, as already discussed, AnC is typically higher in men than women, and the causes underpinning this difference are not yet completely understood. Using a group of men and women, therefore, might have added a confounding variable and was therefore disregarded. The alternative option was to include a group of women, either

as the whole sample of study or together with a group of men. Unfortunately, the population of female athletes is lower than that of male athletes. It was anticipated that recruiting a large number of female participants would have been difficult. It is interesting to note that, despite the differences in absolute AnC between men and women, acute responses (Weber & Schneider, 2001) and chronic adaptations (Eckerson et al., 2004, 2005; Weber & Schneider, 2002; cf. Fukuda, Smith, Kendall, & Stout, 2010b) are similar. It seems reasonable to assume, therefore, that the results reported herein would not be different to those obtained from women.

## 8.4. Conclusions

The aims, and corresponding conclusions, of this PhD thesis were:

1. To establish the validity and reliability of the  $\dot{V}O_2$ -power output relationship to predict supramaximal oxygen demands.

Using 3 min consecutive stages in trained cyclists, the  $\dot{V}O_2$ -power output relationship is linear for intensities up to 95%  $\dot{V}O_{2max}$ . Projection of the line predicts supramaximal intensities with a test-retest reliability of 6.7%

2. To determine the test-retest reliability of AOD.

The test-retest reliability of AOD is 8.7%.

3. To test the hypothesis that AOD remains constant during a constant-work rate cycling to exhaustion at different supramaximal intensities.

The AOD does not remain constant during constant-load cycling at supramaximal intensities. Instead, AOD peaks at intensities corresponding to 112.5-120%  $\dot{V}O_{2max}$ .

4. To determine whether AOD can be determined in a single-day trial.

A single-day trial underestimates AOD by 17%. Therefore, AOD should be determined using a separate trial on a different day.

5. To establish the correlation between AOD and  $W'$ , and whether the strength of the correlation is affected by the type of exercise.

There is a positive correlation between AOD and  $W'$ . Moreover, the strength of the correlation is similar during CWR tests to exhaustion ( $r = 0.654$ ) and 3AO tests ( $r = 0.664$ ).

6. To test the hypothesis that AOD and  $W'$  remain unchanged during a CWR test to exhaustion and an all-out test.

AOD is greater during CWR tests to exhaustion than 3AO tests. The magnitude of  $W'$  may or may not remain constant during CWR and 3AO tests. If the duration of CWR tests is  $> 3$  min, the magnitude of  $W'$  does not appear to be affected. However, in shorter CWR tests,  $W'$  might be underestimated.

7. To determine the effects of hypoxia and hyperoxia on AOD and  $W'$ .

There was no effect of hyperoxia or hypoxia on AOD or  $W'$ . However, large within-subject variation warrants caution in assuming that either test provides a valid measure of AnC.

Altogether, this PhD thesis suggests that when using 3 min stages to construct the  $\dot{V}O_2$ -power output relationship, the AOD reaches its maximum during a CWR test to exhaustion at 112.5-120%  $\dot{V}O_{2max}$ . The test-retest coefficient of variation of this method to estimate AnC is 8.7%. Unfortunately, however, this protocol requires two trials in order to determine AOD. Moreover, the AOD determined from 3AO tests underestimates AnC. Although the AOD was not affected by hypoxia and hyperoxia, close inspection of the data revealed large within-subject variability, with some participants displaying a negative AOD under hyperoxic conditions, suggesting that AOD might not be the best non-invasive approach to estimate AnC. The strong correlation between AOD and  $W'$  suggests that  $W'$  provides an indication of AnC. Furthermore,  $W'$  remains constant irrespective of pacing and oxygen availability during CWR and 3AO tests, providing the duration of the CWR test lasts longer than 3 min. Although  $W'$  might be determined by several factors, it appears to be the favourable option for estimating AnC.

## 8.5. Further Research

Throughout the current PhD thesis, two approaches to estimate AnC, AOD and  $W'$ , have been scrutinized. In brief, the take-home message of this thesis is that the AOD might not be the best approach to estimate AnC, as it was considered (e.g. Noordhof et al., 2010). Instead, it appears that  $W'$  might offer a better alternative to estimate AnC, even though factors other than AnC appear to account for the magnitude of  $W'$ .

Further research on AOD as a potential tool to estimate AnC should, primarily, investigate the assumption that oxygen demand represents a proxy measure of energy demand. First, recent evidence

has suggested that energy cost (rather than oxygen cost) should be used to determine running economy (e.g. Shaw et al. 2014). Using a similar approach, further research should address whether using the energy cost-power output relationship (instead of the relationship between  $\dot{V}O_2$  and power output) offers a better alternative to estimate AnC by means of AOD determination. The energy cost-power output relationship appears to be non-linear (Shaw et al., 2014), and implies that efficiency decreases as the intensity of the exercise increases. Secondly, it is assumed that the parameters of the  $\dot{V}O_2$ -power output relationship remain constant during exercise, so that during a CWR bout of exercise within the severe exercise domain, the oxygen demand remains constant. This assumption has also been challenged, and evidence suggests that efficiency might also decrease during exercise, despite the absolute work rate being constant (Cannon et al., 2014; Mulder et al., 2015). Whilst there have been some attempts to account for this reduction in efficiency during AOD calculations (Mulder et al., 2015), this is seminal work, which needs to be further investigated.

The second approach used in the present thesis as a measure of AnC was  $W'$ . There is growing evidence suggesting that the magnitude of  $W'$  is not exclusively explained by AnC (see Section 1.6.3). On the one hand, knowing CP and  $W'$  allow accurate prediction of TTE during exercise within the severe exercise domain, irrespective of pacing, for exercise lasting ~3 to ~30 min (e.g. Chidnok et al., 2013a; Morton, 2011; Chapter 6; Chapter 7). Moreover, there are mathematical models (Skiba et al., 2012, 2014, 2015) that allow online, real-time estimation of the remaining  $W'$  ( $W'$  balance) during exercise that alternates exercise above CP ( $W'$  is depleted) and below CP ( $W'$  is restored) at any given time (Figure 1.9). In practical terms, the athlete can benefit from knowing  $W'$  balance during exercise, given exhaustion (or fatigue that forces the athlete to slow down) is likely to occur as  $W'$  approaches zero (Skiba et al., 2014). Skiba and co-workers (2012, 2014, 2015) developed this model for cycling, so further research should address whether similar tools can be applied for other forms of motion, such as running, rowing, or swimming. Moreover, Skiba et al. (2015) recently suggested that the speed of  $W'$  recovery during exercise below CP might be determined by intramuscular carnosine concentration, which can be increased by  $\beta$ -alanine supplementation (Sale, Saunders, & Harris, 2010). It remains unknown whether an increased intramuscular carnosine concentration derived by  $\beta$ -alanine supplementation can accelerate the rate of  $W'$  recovery. On the other hand, further research should explore the physiological mechanism underpinning  $W'$ . For instance, it has been suggested that the recruitment of type II fibres underpins the slow component of  $\dot{V}O_2$  (for a review, Jones et al., 2011), and the

amplitude of the slow component correlates with  $W'$  (Murgatroyd et al., 2011). Moreover, type II fibers primarily rely on anaerobic glycolysis, and are broadly defined as 'anaerobic' (e.g. Karatzaferi et al., 2001). It could be hypothesised, therefore, that the magnitude of  $W'$  could be correlated with the percentage of type II fibers. However, Vanhatalo et al. (2016) recently reported no correlation between  $W'$  and muscle fiber composition. Further research, therefore, needs to address the physiological mechanisms underpinning  $W'$ , and whether a link exists between type II fibre recruitment, the slow component of  $\dot{V}O_2$ , and fatigue, as it has been hypothesised (Grassi et al., 2015).

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# Appendices

## Appendix 1. Example of ethics forms (Chapter 5).

St Mary's  
University College  
Twickenham  
London

### St Mary's University College

#### ETHICS SUB-COMMITTEE

#### APPLICATION FOR ETHICAL APPROVAL

All individuals who are intending to carry out research relating to human participants are expected to complete this form. For students, forms should be submitted to your supervisor for signature in the first instance. If consideration is required at Level 2 the form should be submitted to your School Ethics Sub-Committee representative. If consideration is required at Level 3 the form should be signed and submitted in hard copy to the Secretary to the University College Ethics Sub-Committee, at least 10 working days prior to the meeting at which it is being considered. All forms and guidance notes are available on the intranet: <http://portal.smuc.ac.uk/ethics-committee.html>

<b>1. Title of project: The effects of training status, exercise modality and intensity in the power output-oxygen consumption relationship</b>	
The relationship between power output and oxygen uptake and its implications on maximal accumulated oxygen deficit determination.	
<b>2. School</b>	School of Health, Sport and Applied Sciences.
<b>3. Programme</b> ( <i>if undergraduate research or taught Masters</i> )	
<b>4. Type of activity/research</b> ( <i>Staff/undergraduate student research/postgraduate student/ new module, programme or revalidation</i> )	Postgraduate student: PhD student
<b>5. Name of Proposer(s) Regnum</b>	
<b>6. Name of Supervisor</b> ( <i>if applicable</i> )	Mark Glaister and Charles Pedlar
<b>7. Proposed start and completion date</b> Please indicate when the study is due to commence, timetable for data collection and expected date of completion	
Data collection is likely start in February 2012. It is expected, based on the final number of subjects recruited, to be completed by Easter Holydays (April – May 2012).	
<b>8. Sponsors/Collaborators</b> This project has no external sponsors / collaborators.	

Ethics Sub-Committee  
Updated September 2010

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**9. Other Research Ethics Committee Approval**

Please indicate whether other approval is required or has been obtained (e.g. NHS, LEA etc) and whether approval has previously been given for any element of this research by the University College Ethics Sub-Committee

No approval from other Research Ethics Committee is required.

**10. Purpose of the study**

Please give the aims of the research and provide a **brief** rationale for the study including any existing knowledge and benefits of the proposed research.

The maximal amount of ATP re-synthesised anaerobically is termed anaerobic capacity, which is an indicator of performance and exercise tolerance. The maximal accumulated oxygen deficit (MAOD) in a supramaximal bout is currently the reference method to estimate anaerobic capacity during whole body exercise, despite this physiological and practical limitations remain debated and compromise its use as valid and reliable tool to estimate anaerobic capacity. First, energy demand during a supramaximal exercise bout is calculated from a projection of the relationship between power output and  $\dot{V}O_2$ . As a consequence of the delayed attainment of a  $\dot{V}O_2$ -steady state at high submaximal intensities, due to the slow component of  $\dot{V}O_2$ , this linear relationship appear to be eventually broken and show an upward drift, although this might be affected by training status and exercise mode. Second, the power output- $\dot{V}O_2$  relationship has been traditionally constructed in a time-consuming protocol since maximal  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ) and several 4 to 10 minutes-bouts of constant load exercise are required and performed in different days.

A single-day protocol in which the relationship between power output and  $\dot{V}O_2$  and  $\dot{V}O_{2max}$  are determined in a step-incremental test and then used to calculate supramaximal intensity for MAOD will address this limitation. On the other hand, this new approach might be constrained as recent evidence suggests that prior exercise affects  $\dot{V}O_2$  kinetics and time to exhaustion. Of note is that priming exercise requires certain characteristics in terms of intensity, duration and recovery to lead to a positive effect on the primed bout. The aim of the project, therefore, is to investigate the power output and  $\dot{V}O_2$  relationship during different exercises among cyclists and determine whether supramaximal intensity calculation and prior exercise affect MAOD values.

### 11. Study Design/Methodology

Please provide details of the design of the study (qualitative/quantitative etc) and the proposed methods of data collection (exactly what you will do and how; nature of tests, questionnaires, type of interview, ethnographic observation etc) including what will be done to participants, the extent of their commitment and the length of time they will be required to attend for testing. Please also include details of where the testing will take place.

**Copies of questionnaires to be used and/or interview schedules should be attached to this application.**

In this quantitative study, voluntary participants will be required to attend the laboratory on five occasions, lasting approximately 1 h each. Data will be collected during maximal exercise in each session, which will be conducted at a similar time of the day and separated by a minimum of 48 h to ensure recovery. The first testing day will be used to familiarise participants with the equipment (i.e. cycle-ergometer and treadmill) and to perform an incremental ramp cycling exercise for maximum  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ) determination. On the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> sessions, two step-incremental cycling tests and one step-incremental running test will be performed in random order to establish the relationship between power output and  $\dot{V}O_2$  on different exercises (cycling and running). After each test, following 20 min at rest, participants will complete a square-wave (i.e. constant load) exercise at an intensity equivalent to 120% of  $\dot{V}O_{2max}$  determined as a projection of the power output- $\dot{V}O_2$  relationship to exhaustion to determine MAOD. In the final session, cycling exercise at the same supramaximal intensity used in previous tests will be used to determine MAOD.

Body composition will be assessed on the first session prior to any exercise. Physiological variables such as heart rate, gas exchange (i.e.  $\dot{V}O_2$ , minute ventilation and carbon dioxide production) and blood lactate will be monitored prior, during and after exercise. In addition, subjects will be asked to follow a similar diet and abstain from alcohol ingestion and heavy exercise the day before each trial. All tests and data collection will be conducted in the physiology laboratory of the School of Health, Sport and Applied Science at St Mary's University College.

## 12. Participants

Please describe how many participants will be required to complete the study, their age, sex, how they will be chosen/recruited and inclusion/exclusion criteria.

I expect to recruit about 20 trained male cyclists. For a participant to be included in the study certain fitness status and training experience (determined as a minimum  $\dot{V}O_{2max}$  and/or power output/kg values and minimum years of training and competing experience, respectively) will be required.

Participants' age must be within the range of 18 – 35 years and be regularly involved in cycling training only. Participants suffering of medical conditions or disease, or in which their health could be compromised during the study will be excluded.

## 13. Consent

**Please provide copies of the consent form, information sheet, debriefing sheets (if relevant) for participants and any other documentation in relation to consent, e.g. letters to parents, Heads of Schools etc.**

13a) Are there any incentives/pressures which may make it difficult for participants to refuse to take part (i.e. will coercion be used in the recruitment of participants)?

Participants will volunteer to participate in the study, and, therefore, will not receive any financial (or otherwise) incentive to finish the study. They will be completely free to withdraw the study at any moment, without any reason given and without any consequence.

13b) Will any of the participants be from any of the following groups?

- Children under 18
- Participants with learning disabilities
- Participants suffering from dementia
- Other vulnerable groups

No vulnerable participant will be involved in this project.

If children under 18 years of age are participating has the researcher/investigator a current CRB disclosure?

No children under 18.

13c) How will consent be obtained?

The participants will be given a brief oral explanation and information sheet in which the procedures and aims are carefully explained. Participants will be then encouraged to ask any question they may have about the study and finally will be asked to sign a written consent form. A copy of the written consent form is attached.

#### **14. Risks and benefits of research/ activity**

14a) Are there any potential risks or adverse effects (e.g injury, pain, discomfort, distress, changes to lifestyle) associated with this study? If so please provide details including information on how they will be minimised.

As the nature of the study includes exercising at high intensities and maximal effort, some of the subjects might experience tiredness or discomfort. The protocol, however, is carefully designed to minimize fatigue or distress providing resting periods interspersed between efforts and between testing sessions. In order to minimize injury risk, a physical activity test questionnaire (PAR-Q) will be filled by participants prior to any data collection. A warm up will be completed by each participant prior to any test. Based on the protocol and exercises involved in this project, it is unlikely that pain (muscular soreness) or changes in lifestyle will be associated with the project.

14b) Does the study involve any invasive procedures? If so, please list the researchers' or collaborators' experience in the use of these procedures.

The study includes capillary blood samples collection from either the earlobe or fingertip. The researchers have demonstrated experience in this procedure.

14c) Will individual/group interviews/questionnaires include anything that may be sensitive or upsetting?

No sensitive or upsetting question/test/measurement is included in the project.

14d) Please describe how you would deal with any adverse reactions participants might experience.

In the event of an adverse reaction by any participant test, the will be automatically stopped. The laboratory manager will be immediately contacted to provide first aid. In the event of a serious adverse reaction, after calling emergencies (999), laboratory manager, security and first aid personnel will be called immediately.

14e) Are there any potential benefits of participating in the research to the participants (e.g gaining knowledge of their fitness, finding out personality type, improving performance etc)?

The participants will gain knowledge on their fitness status through physiological tests and evaluations. This will help them on the design of training plans.

#### **15. Confidentiality, privacy and data protection**

15a) What steps will be taken to ensure participant's confidentiality?

At the beginning of the test all the participants will be allocated to a code for the data analysis, so their data will be treated anonymously.

15b) Will data be stored securely?

Data will be stored by duplicate in a PC and external hard disk, both permanently kept in the University in a locked room.

15c) Who will have access to the data?

Only the principle investigator will have access to the raw data.

15d) Will the results of analysis include information which may identify people or places?

Data will be presented as group mean. In any case where information might identify people or places, this will be omitted.

**16. Feedback to participants**

Please give details of how, if appropriate, feedback will be given to participants.

Once data collection is completed, an information sheet will be given to participants with their personal results on each test and a comparison with the group average.

The proposer recognises their responsibility in carrying out the project in accordance with the University College's ethical guidelines and procedures and will ensure that any person(s) assisting in the research/ teaching is also bound by these. The Ethics Sub-Committee must be notified of and approve any deviation from the information provided on this form.

17. Signature of Proposer(s)	Date:
18. Signature of Supervisor (for student research projects)	Date:
19. Signature of Head of School	Date:

## Appendix 2. Physical activity readiness questionnaire (PAR-Q).

St Mary's University College, School of Human Sciences

### CONFIDENTIAL CONSENT FORM & PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

All sections of this form must be completed

#### Confidential Consent Form

I have been made aware of the exercise protocol by the \*Test Coordinator and I have had the opportunity to ask questions. Consequently, I agree to participate in the proposed protocol(s) which I am voluntarily undertaking. I fully understand the risks and benefits involved and that I am free to withdraw from the test/study at any point.

I agree that the \*Test Coordinator will retain this form in accordance with the Data Protection Act 1998 throughout the testing period and will retain the form after all tests have been completed.

I release St Mary's University College and the \*Test Coordinator from all liability for injury and/or illness during or after testing, unless this has arisen due to negligence on the part of the test coordinator/university.

<b>Print name:</b>		<b>Signature:</b>		<b>Date:</b>	
<b>Witness name:</b>		<b>Witness signature:</b>		<b>Date:</b>	

#### Confidential Physical Activity Readiness Questionnaire

Full Name:  Date of Birth:   
 Height (cm):  Weight (kg):

Have you ever suffered from any of the following medical conditions? If yes please give details:

	Yes	No	Details
Heart Disease or attack	<input type="checkbox"/>	<input type="checkbox"/>	_____
High or low blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	_____
Stroke	<input type="checkbox"/>	<input type="checkbox"/>	_____
Cancer	<input type="checkbox"/>	<input type="checkbox"/>	_____
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	_____
Asthma	<input type="checkbox"/>	<input type="checkbox"/>	_____
High cholesterol	<input type="checkbox"/>	<input type="checkbox"/>	_____
Epilepsy	<input type="checkbox"/>	<input type="checkbox"/>	_____
Other, please give details	<input type="checkbox"/>	<input type="checkbox"/>	_____

Do you suffer from any blood borne diseases? If yes please give details; \_\_\_\_\_

Please give details of any medication you are currently taking or have taken regularly within the last year:

Please give details of any musculoskeletal injuries you have had in the past 6 months which have affected your capacity to exercise or caused you to take time off work or seek medical advice:

#### Other Important Information

During a typical week approximately how many hours would you spend exercising?

If you smoke please indicate how many per day:

If you drink please indicate how many units per week:

Are you currently taking any supplements or medication? Please give details:

**Signature (Participant):**  **Date:**

**Signature (\*Test Coordinator):**  **Date:**

*\*Test coordinator: The individual responsible for administering the test(s) and subsequent data collection.*

### Appendix 3. Example of consent forms (Chapter 9).



St Mary's  
University College  
Twickenham  
London

NAME OF PARTICIPANT:

Title of the project: The effect of hyperoxia, normoxia and hyperoxia on anaerobic capacity.

Main investigator and contact details: Daniel Muniz ([daniel.muniz@smuc.ac.uk](mailto:daniel.muniz@smuc.ac.uk))

Members of the research team:

1. I agree to take part in the above research. I have read the Participant Information Sheet which is attached to this form. I understand what my role will be in this research, and all my questions have been answered to my satisfaction.
2. I understand that I am free to withdraw from the research at any time, for any reason and without prejudice.
3. I have been informed that the confidentiality of the information I provide will be safeguarded.
4. I am free to ask any questions at any time before and during the study.
5. I have been provided with a copy of this form and the Participant Information Sheet.

Data Protection: I agree to the University College processing personal data which I have supplied. I agree to the processing of such data for any purposes connected with the Research Project as outlined to me.

Name of participant (print).....Signed.....Date.....

Name of witness (print).....Signed.....Date.....

-----  
If you wish to withdraw from the research, please complete the form below and return to the main investigator named above.

Title of Project: Effects of training status, exercise modality and intensity in the power output-oxygen consumption relationship

I WISH TO WITHDRAW FROM THIS STUDY

Name: \_\_\_\_\_

Signed: \_\_\_\_\_ Date: \_\_\_\_\_

St Mary's University College  
Waldegrave Road, Strawberry Hill, Twickenham TW14SX  
Switchboard 020 8240 4000 Fax 020 8240 4255  
[www.smuc.ac.uk](http://www.smuc.ac.uk)



# Appendix 5. Poster presented at the International Sport Science and Sport Medicine Conference. Newcastle, August 2012.

## THE RELATIONSHIP BETWEEN OXYGEN UPTAKE AND WORK RATE BELOW AND ABOVE LACTATE THRESHOLD

Muniz-Pumares, D<sup>1</sup>; Godfrey, R<sup>2</sup>; Pedlar, C<sup>1</sup>; Glaister, M<sup>1</sup>

<sup>1</sup> School of Sport, Health and Applied Science, St Mary's University College, Twickenham, UK, TW1 4SX

<sup>2</sup> The Centre for Sports Medicine and Human Performance, Brunel University, Uxbridge, UK, UB8 3PH



St Mary's  
University College  
Twickenham  
London

School of Sport, Health  
and Applied Science

### Introduction

The assessment of finite capacity of anaerobic pathways to release energy during whole-body exercise represents a challenge.<sup>1</sup> The maximal accumulated O<sub>2</sub> deficit is widely used estimate anaerobic capacity by subtracting accumulated oxygen uptake (VO<sub>2</sub>) from energy demand in a supramaximal bout to exhaustion.<sup>1,2</sup>

Supramaximal energy demand is typically estimated as a projection of submaximal measures of VO<sub>2</sub> and work rate (WR) assuming a linear relationship. However, the slow component of VO<sub>2</sub> might upward-drift this relationship at intensities above lactate threshold (LT).<sup>3</sup> Because the slow component emerges 2-3 minutes into constant WR exercise, short bouts (3-4 min) are now recommended to estimate WRs predicted to elicit supramaximal energy demands.<sup>1</sup>

### Aims

- To determine whether submaximal measures of VO<sub>2</sub> and WR display a linear relationship at intensities below and above lactate threshold, during a familiar (trained) and unfamiliar (untrained) 3-min incremental exercise.

- To establish the reliability of predicted supramaximal cycling WRs.

### Methods

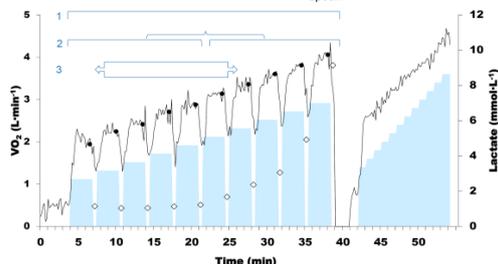
- Eight male cyclists, with no otherwise training, were recruited. Mean ± SD age: 40.8 ± 7.8 years, mass: 75.6 ± 7.6 kg, height: 1.77 ± 0.05 m.

- Two identical cycling trials and a running trial were completed; each comprising a 10 x 3-min submaximal bouts test, followed by five minutes rest and a ramp test to exhaustion (Figure 1).

- The slope, intercept and Pearson's coefficient of line for best fit for the VO<sub>2</sub>-WR relationship were studied by three approaches:

- Entire 10 x 3-min test (1 in figure 1).
- Measures below and above lactate threshold (2 in figure 1).
- Rolling (moving) cluster of five measures (3 in figure 1).

- Characteristics of the line (slope, intercept and correlation coefficient) and predicted WRs at 110% and 130% of VO<sub>2peak</sub> were compared with repeated measures analysis of variance. Significance was accepted at p<0.05. Coefficient of variation (CV) and intraclass correlation coefficient (ICC) were calculated for the predicted WRs at 110% and 130% of VO<sub>2peak</sub>.



**Figure 1.** Schematic representation of the 10 x 3-min and ramp tests employed to determine the relationship between WR (○) and VO<sub>2</sub> (solid line, steady state denoted by ●) and VO<sub>2max</sub> (solid line, dashed state denoted by ○) respectively. Lines for best fit were determined for: 1) the entire test, 2) values below and above LT (lactate values denoted by ◇); and 3) a moving cluster of 5 data-points throughout the test.

### Results

- The characteristic of the lines for best fit WR and VO<sub>2</sub> and supramaximal WRs were not significantly different in the two cycling trials for the entire 10 x 3-min test and below and above LT (Table 1), and exhibited strong linear relationships (all r > 0.95).

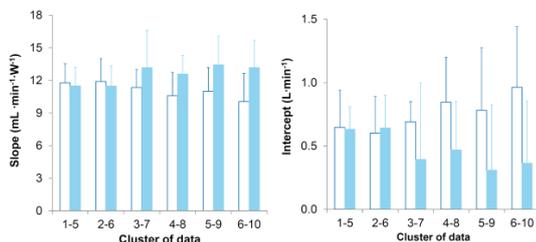
- Similarly, there were no differences in slope, intercept and correlation factors of the rolling five-measures cluster throughout the entire cycling test (Figure 2) or running test (260 ± 62 mL O<sub>2</sub>·min<sup>-1</sup>·km<sup>-1</sup>, 276 ± 461 mL O<sub>2</sub> and 0.98 ± 0.01; respectively).

- The CV of the predicted WR in the two cycling trials at 110% (325 ± 40 W and 322 ± 43 W) and 130% (398 ± 51 W and 389 ± 54 W) VO<sub>2peak</sub> were 7.0% and 7.2%; being ICC 0.80 and 0.83, respectively.

**Table 1.** Characteristics of the line for best fit VO<sub>2</sub> and WR; and power output estimated to elicit supramaximal energy demands.

	Cycling trial 1			Cycling trial 2		
	Entire test	< Lactate Threshold	> Lactate Threshold	Entire test	< Lactate Threshold	> Lactate Threshold
Slope	10.9 ± 1.66	12.1 ± 2.1	9.72 ± 2.65	12.56 ± 1.90	11.07 ± 1.57	13.15 ± 2.81
Intercept	765 ± 230	601 ± 348	1045 ± 498	494 ± 258	682 ± 164	373 ± 575
Correlation	0.98 ± 0.04	0.97 ± 0.04	0.95 ± 0.11	0.99 ± 0.02	0.97 ± 0.07	0.97 ± 0.04
PO@110%	325 ± 40	311 ± 60	338 ± 43	322 ± 43	349 ± 45	319 ± 44
PO@130%	398 ± 51	378 ± 77	423 ± 61	389 ± 54	424 ± 56	384 ± 56

Slope (mL O<sub>2</sub>·min<sup>-1</sup>·km<sup>-1</sup>), intercept (mL O<sub>2</sub>) and correlation coefficient of the line of best fit the relationship between WR and O<sub>2</sub> at different intensities determined from a 10 x 3-min test.



**Figure 2.** Slope (left panel) and intercept (right panel) of the lines for best fit for five submaximal measures of WR and VO<sub>2</sub> in two identical trials (trial one: □; trial two: ■). No significant differences were observed throughout the tests.

### Discussion

- The WR – VO<sub>2</sub> relationship presented a strong linear relationship, which was not affected by the exercise intensity or familiarity of the exercise. The use of relatively short (3-min) submaximal bouts likely prevent the slow component to affect this relationship.<sup>1,4</sup>

- This protocol can be used to predict supramaximal WRs. However, moderate reliability figures on predicted supramaximal WRs, warrants further research on the reliability of the accumulated oxygen deficit test.

### Key Points

- From a 3-min incremental test, WR and VO<sub>2</sub> displayed a strong linear relationship, regardless exercise intensity or familiarity.

- This linear relationship can be projected to estimate supramaximal intensities, as required for MAOD determination.

### References

- Noordhof, D. A., et al. (2010). *Sports Medicine*, 40, 285–302.
- Medbo, J.I., et al. (1988). *Journal of Applied Physiology*, 64, 50–60.
- Zoladz, J.A., et al. (1995). *Journal of Physiology*, 488, 211–217.
- Jones, A.M., et al. (2011). *Medicine & Science in Sports & Exercise*, 43, 2046–62.

**Appendix 5.** Poster presented at the British Association of Sport Sciences conference. St George's Park, November 2014.

**DETERMINATION OF THE ACCUMULATED OXYGEN DEFICIT IN A SINGLE TRIAL**

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**Introduction**

Anaerobic capacity represents the finite amount of energy released from anaerobic energy sources<sup>(1)</sup>. Due to invasive and/or expensive procedures, direct quantify anaerobic capacity remains challenging and two indirect approaches have been developed<sup>(2)</sup>: the accumulated oxygen deficit (AOD)<sup>(3)</sup> and the curvature constant of the power-duration relationship ( $W'$ )<sup>(4)</sup>. Beyond physiological assumptions discussed elsewhere<sup>(2)</sup>, traditional protocols to determine both AOD and  $W'$  are limited by a time- and effort-consuming protocols, since both require at least two laboratory visits. Recently<sup>(5)</sup>, it was observed that, if interspersed with 20 min of rest, the ramp test to exhaustion and 3-min all-out tests required to determine  $W'$  could be determined in a single trial. The aim of this study, therefore, was to determine whether AOD can be determined in a single trial.

**Aim**

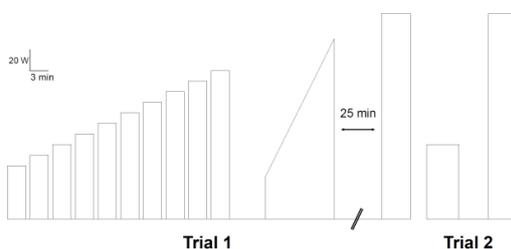
To determine whether anaerobic capacity, estimated as the accumulated oxygen deficit, can be determined in a single trial.

**Methods**

- Twenty male competitive cyclists and triathletes volunteered to participate in this study, which was approved by the institutional ethics committee. Their means  $\pm$  standard deviations for age, mass, height and  $\dot{V}O_{2max}$  were  $41 \pm 7$  years,  $80 \pm 7$  kg,  $1.82 \pm 0.07$  m and  $57 \pm 6$  ml·kg<sup>-1</sup>·min<sup>-1</sup>, respectively.

- Participants completed two trials (Figure 1). Trial 1 consisted of a 10x3 min step test to determine the linear  $\dot{V}O_2$  – power output relationship followed by a ramp test to exhaustion to determine  $\dot{V}O_{2max}$ . After 10 min of unloaded cycling and a further 15 min of passive resting, participants completed a supramaximal, constant-load exercise bout to exhaustion at 112.5%  $\dot{V}O_{2max}$  linearly predicted from the  $\dot{V}O_2$  – power output relationship. Subsequently, in Trial 2, participants repeated the supramaximal exercise to exhaustion at 112.5%  $\dot{V}O_{2max}$  after a 5-min warm-up and 5 min of passive resting.

- Breath-by-breath  $\dot{V}O_2$  were filtered and linearly interpolated to produce second-by-second values. The AOD was then determined as the difference between accumulated oxygen demand and accumulated oxygen uptake for the single trial and the control trial. During supramaximal constant-load exercise bouts, peak values for heart rate (HR),  $\dot{V}O_2$  and blood lactate concentration (BLA) were recorded, with the latter also measured immediately before supramaximal exercise (resting BLA). Physiological variables from the single and control trials were compared using a paired samples t-test, with significance accepted at  $P < 0.05$ .



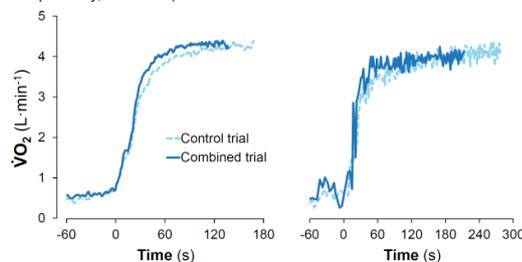
**Figure 1.** Outline of the protocol. AOD is determined from a supramaximal, constant-load, cycle to exhaustion form a single trial (Trial 1) and a control trial (Trial 2).

**Reference List**

1. Green & Dawson (1993). *Sports Medicine*, 15, 312-327.
2. Heck et al. (2003). *European Journal of Sport Sciences*, 3, 1-23.
3. Medbo et al. (1988). *Journal of Applied Physiology*, 64, 50-60.
4. Morton (2006). *European Journal of Applied Physiology*, 96, 339-54.

**Results**

The supramaximal constant-load exercise bouts at 112.5%  $\dot{V}O_{2max}$  were performed at  $373 \pm 56$  W. AOD determined in a single trial ( $3.40 \pm 0.70$  L) was 17% lower than AOD determined in the control trial ( $4.20 \pm 1.00$  L;  $P < 0.01$ ; mean difference: 0.80 L, 95% likely range: [0.51 – 1.09]). Similarly, time to exhaustion was reduced by 16% in the supramaximal test of the single trial ( $137 \pm 41$  s) compared to the control trial ( $168 \pm 53$  s;  $P < 0.01$ ; mean difference: 0.31 s, [17, 45]), whilst resting BLA was greater in the single trial ( $0.81 \pm 0.22$  mmol·L<sup>-1</sup>) vs. the control trial ( $4.97 \pm 1.98$  mmol·L<sup>-1</sup>  $P < 0.01$ ). However, there were no differences between the single and control trials for peak values in  $\dot{V}O_2$  ( $93 \pm 6$  vs.  $93 \pm 7\%$   $\dot{V}O_{2max}$  respectively;  $P = 0.865$ ), heart rate ( $167 \pm 11$  vs.  $167 \pm 11$  beats·min<sup>-1</sup> respectively;  $P = 0.932$ ) or BLA ( $11.09 \pm 3.56$  vs.  $10.69 \pm 2.61$  mmol·L<sup>-1</sup> respectively,  $P = 0.573$ ).



**Figure 2.**  $\dot{V}O_2$  during supramaximal, constant load, cycling bouts to exhaustion at 112.5%  $\dot{V}O_{2max}$  for AOD determination in a single (solid lines) and control trial (broken lines). Left panel (A) denotes mean values and right panel (B) a denotes a representative participant (B).

**Discussion**

The present study demonstrates that, in contrast to  $W'$ <sup>(6)</sup>, AOD cannot be determined in a single trial. Fatigue from prior submaximal and maximal tests may have affected the single trial. Nevertheless, participants in the present study were given 25-min of active and passive recovery to prevent this from happening. There is a paucity of studies looking at the recovery kinetics of AOD, but it is known that the recovery of both phosphocreatine and  $W'$  follow exponential kinetics, and both would be expected to be largely (90-100%) restored after 25 min of recovery<sup>(5,6,7)</sup>. Another potential factor explaining the reduced AOD in the single trial is the effect that prior exercise had on  $\dot{V}O_2$  kinetics, and thus AOD. Prior heavy exercise speeds  $\dot{V}O_2$  kinetics in a subsequent exercise bout, resulting in a sparing of anaerobic energy release, potentially delaying exhaustion<sup>(8)</sup>. Indeed, visual inspection of  $\dot{V}O_2$  data supports faster  $\dot{V}O_2$  kinetics in the single trial. Surprisingly, the apparently faster  $\dot{V}O_2$  kinetics led to a reduction in the time to exhaustion. Unfortunately,  $\dot{V}O_2$  kinetics were not determined in the present study due to the completion of one transition only. As per the design, the order of the single and control trials were not randomised, and consequently a learning effect may have been present.

**Key findings**

- The accumulated oxygen deficit determined in a single trial after submaximal and maximal tests is reduced by 17% compared to a control trial.
- Determination of anaerobic capacity via the accumulated oxygen deficit requires two laboratory visits.

5. Constanini et al. (2014). *European Journal of Applied Physiology*, 114, 153-61.
6. Ferguson et al. (2010). *Journal of Applied Physiology*, 108, 866-874.
7. McMahon & Jenkins (2002). *Sports Medicine*, 32, 761-84.
8. Carter et al. (2005). *Medicine & Science in Sport & Exercise*, 37, 775-781.

**ABSTRACT**

**PURPOSE:** The main aim of the study was to investigate whether anaerobic capacity, estimated as the accumulated oxygen deficit (AOD) and the curvature constant of the power-duration relationship ( $W'$ ), remain constant during supra-maximal exercise to exhaustion at constant-load and all-out exercise. The secondary aim was to determine the relationship between AOD and  $W'$  during supra-maximal exercise to exhaustion at constant-load and all-out exercise.

**METHODS:** Twenty-one male cyclists completed 21 constant-load and 21 all-out tests. Standard deviations for age, height and mass were: 40 ± 6 years, 1.81 ± 0.08 m and 79.8 ± 7.5 kg, respectively. Each participant completed four trials on an individually-adjusted electromagnetically braked cycle-ergometer (Lode Excalibur Sport, Groningen, Netherlands): two trials to determine AOD and two trials to determine  $W'$ .

The first trial to determine  $W'$  consisted of an incremental exercise test whereby the resistance increased at a constant rate of 30 W·min<sup>-1</sup> until exhaustion. From this incremental test, 50% of the difference between the gas exchange threshold (GET) and peak  $\dot{V}O_2$  was determined (50%Δ).  $W'$  was subsequently determined from a 3AO test as described by Vanhatalo et al.<sup>1</sup> In brief, after 3 min of unloaded cycling, the ergometer switched to linear mode and participants were requested to turn the pedals as fast as they could for three minutes. The resistance was cadence-dependent, so that each participant's preferred cadence the power output corresponded to that at 50%Δ. In order to determine AOD, participants first completed 10 × 3 min bouts at increasing submaximal intensities to construct the linear  $\dot{V}O_2$ -power output relationship. After first minutes of rest, participants completed an incremental test to exhaustion to determine maximum  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ). In a subsequent trial, participants completed a CL test performed at 112.5%  $\dot{V}O_{2max}$ .

Both measures of AnC (AOD and  $W'$ ) were calculated during the CL and the 3AO tests (Figure 1). AOD was determined as the difference between the accumulated oxygen demand and the accumulated oxygen uptake,<sup>2</sup> where oxygen demand was calculated from a projection of the  $\dot{V}O_2$ -power output relationship and oxygen uptake was calculated via breath-by-breath  $\dot{V}O_2$  during the tests.  $W'$  was determined, for both tests, as the work completed above the mean power output during the last 30 s of the 3AO (Figure 1).<sup>7</sup>

**Statistical analyses**  
Differences in AOD and  $W'$  between CL and 3AO tests were evaluated using paired-samples *t*-tests. The correlation between AOD and  $W'$  was determined using Pearson product-moment correlation coefficient for both CL and 3AO tests. Significance was accepted at  $P < 0.05$ .

**INTRODUCTION**

During high-intensity exercise, energy demands are met by the interaction of aerobic and anaerobic energy systems. Whilst aerobic metabolism provides a virtually unlimited source of energy at a relatively low rate, anaerobic metabolism supplies energy to the exercising muscles at higher rates, but it has a limited and finite capacity. Aerobic metabolism can be easily assessed as the rate of oxygen uptake ( $\dot{V}O_2$ ). However, anaerobic capacity (AnC), defined as the finite capacity to produce energy or work from anaerobic energy systems,<sup>1</sup> remains challenging to quantify. Direct methods to measure AnC require invasive and/or expensive procedures, and it is commonly estimated through indirect approaches<sup>2</sup> such as the accumulated oxygen deficit (AOD)<sup>3</sup> and the curvature constant of the power-duration relationship ( $W'$ ).<sup>4</sup> Previous studies have demonstrated that AOD and  $W'$  are strongly correlated.<sup>5</sup>  $W'$ , however, was calculated using a time-consuming procedure that requires each participant to perform repeated exercise bouts at constant-load (CL) to exhaustion.<sup>6</sup> Whether the correlation between AOD and  $W'$  holds true when the latter is determined from the time-efficient 3-min all-out (3AO) test<sup>7</sup> remains unknown. Moreover, both methods to estimate AnC are assumed to remain constant regardless of the mode of exercise (i.e. CL vs. all-out effort).<sup>5,7</sup>

**AIM**

The aims of this investigation were a) to determine whether AOD and  $W'$  remain constant during a CL to exhaustion and a 3AO, and b) to quantify whether AnC estimated via AOD and  $W'$  are correlated during CL and 3AO tests.

**METHODS**

**Participants**  
Twenty-one male cyclists and triathletes signed a consent form to participate in this study, which was approved by St Mary's University Ethics Committee. Means ± standard deviation for age, height and mass were: 40 ± 6 years, 1.81 ± 0.08 m and 79.8 ± 7.5 kg, respectively.

**Procedures**  
Each participant completed four trials on an individually-adjusted electromagnetically braked cycle-ergometer (Lode Excalibur Sport, Groningen, Netherlands): two trials to determine AOD and two trials to determine  $W'$ .

The first trial to determine  $W'$  consisted of an incremental exercise test whereby the resistance increased at a constant rate of 30 W·min<sup>-1</sup> until exhaustion. From this incremental test, 50% of the difference between the gas exchange threshold (GET) and peak  $\dot{V}O_2$  was determined (50%Δ).  $W'$  was subsequently determined from a 3AO test as described by Vanhatalo et al.<sup>1</sup> In brief, after 3 min of unloaded cycling, the ergometer switched to linear mode and participants were requested to turn the pedals as fast as they could for three minutes. The resistance was cadence-dependent, so that each participant's preferred cadence the power output corresponded to that at 50%Δ. In order to determine AOD, participants first completed 10 × 3 min bouts at increasing submaximal intensities to construct the linear  $\dot{V}O_2$ -power output relationship. After first minutes of rest, participants completed an incremental test to exhaustion to determine maximum  $\dot{V}O_2$  ( $\dot{V}O_{2max}$ ). In a subsequent trial, participants completed a CL test performed at 112.5%  $\dot{V}O_{2max}$ .

Both measures of AnC (AOD and  $W'$ ) were calculated during the CL and the 3AO tests (Figure 1). AOD was determined as the difference between the accumulated oxygen demand and the accumulated oxygen uptake,<sup>2</sup> where oxygen demand was calculated from a projection of the  $\dot{V}O_2$ -power output relationship and oxygen uptake was calculated via breath-by-breath  $\dot{V}O_2$  during the tests.  $W'$  was determined, for both tests, as the work completed above the mean power output during the last 30 s of the 3AO (Figure 1).<sup>7</sup>

**Statistical analyses**  
Differences in AOD and  $W'$  between CL and 3AO tests were evaluated using paired-samples *t*-tests. The correlation between AOD and  $W'$  was determined using Pearson product-moment correlation coefficient for both CL and 3AO tests. Significance was accepted at  $P < 0.05$ .

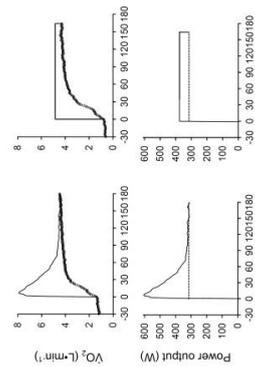


Figure 1. Schematic of the determination of AnC using AOD (top panels) and  $W'$  (bottom panels) from a 3AO (left panels) and CL exercise to exhaustion (right panels).

**RESULTS**

The GET and  $\dot{V}O_{2max}$  were 2.59 ± 0.33 L·min<sup>-1</sup> and 4.60 ± 0.61 L·min<sup>-1</sup>, respectively. All participants exhibited a linear  $\dot{V}O_2$ -power output relationship ( $P < 0.001$ ;  $r = 0.995 \pm 0.004$ ), which resulted in a predicted intensity at 112.5%  $\dot{V}O_{2max}$  of 376 ± 55 W. The end-test power output during the 3AO was 316 ± 50 W. Physiological responses to CL and 3AO are presented in Table 1. Despite no differences between CL and 3AO tests in duration, mean power and work completed (Table 1), both estimations of AnC (AOD and  $W'$ ) were different between CL and 3AO (Figure 2). There was a significant correlation between AOD and  $W'$  during the CL test to exhaustion ( $P < 0.001$ ,  $r = 0.654$ ), and during the 3AO test ( $P < 0.001$ ,  $r = 0.664$ ).

Table 1. Physiological responses to a constant load-test (CL) to exhaustion at 112.5% of  $\dot{V}O_{2max}$  and a 3-min all-out (3AO) test in 21 male cyclists.

	CL	3AO	P value
Duration (s)	164 ± 46	180 ± 0	0.127
Power output (W)	376 ± 55	376 ± 55*	0.882
Work completed (kJ)	60.8 ± 17.3	67.7 ± 9.8	0.057
Anaerobic contribution (%)	30 ± 6	23 ± 5	<0.001
AOD	20 ± 12	17 ± 6	<0.001
$W'$	20 ± 12	17 ± 6	<0.001

\* Indicates mean power during the 3AO test.

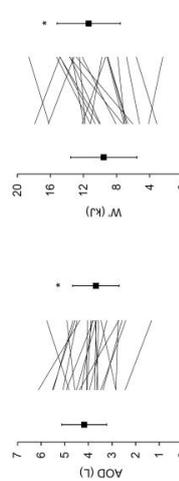


Figure 2. Estimation of AnC during CL and 3AO tests via the AOD (left panel) and  $W'$  (right panel). \* denotes  $P < 0.05$ .

**KEY POINTS**

- This investigation demonstrates that, in cycling, the mode of exercise (CL to exhaustion at 112.5%  $\dot{V}O_{2max}$  vs. a 3AO test), affects the magnitude of the AnC, estimated either as AOD or  $W'$ .
- Both approaches to estimate AnC, however, are positively and strongly correlated irrespective of the mode of exercise.

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