Relationship between Pulmonary Oxygen Uptake Kinetics and High-Intensity Running Performance in Professional Soccer Players

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Abstract

The overall aim of this thesis was to identify whether oxygen uptake ($\dot{V}O_2$) kinetics are a determining factor in the performance of soccer-specific high-intensity exercise. To achieve this aim there were five objectives: 1) to design a protocol for the assessment of $\dot{V}O_2$ kinetics at the onset and cessation of moderate- and heavy-intensity treadmill running; 2) to assess the reproducibility of $\dot{V}O_2$ kinetics measured during such a protocol; 3) to quantify the characteristics of $\dot{V}O_2$ kinetics during the onset and cessation of moderate- and heavy-intensity running; 4) to identify if $\dot{V}O_2$ kinetics discriminate between elite and non-elite soccer players and 5) to identify the physiological processes ($\dot{V}O_2$ kinetics, $\dot{V}O_2$ max, GET, anaerobic capacity) that determine soccer-specific high-intensity running capacity.

To establish the day-to-day variability in aerobic markers of moderate- (80%GET) and heavy- (50%Δ) intensity exercise domains, the pulmonary gas exchange of nine participants was measured during an incremental treadmill test to exhaustion on two occasions. Narrow 95% limits of agreement (LOA) and low coefficients of variation (CV) indicated that such markers of intensity were reproducible. Based on these findings, eight participants performed a repeated exercise transition treadmill protocol (six moderate (80%GET) and two heavy (50%Δ) transitions) on two occasions. Two-way analysis of variance with repeated measures (ANOVA) revealed the phase II time constant (τ_1) to be invariant across intensity domains for both exercise transients (τ_{1on}, moderate 23.2 ± 2.9 s vs. heavy 23.7 ± 3.1 s; τ_{1off} moderate 27.4 ± 3.5 s vs. heavy 27.1 ± 2.4 s), while both phase II and III τ were quicker during the onset than cessation of exercise (phase III, τ_{2on} 177.5 ± 43.9 s vs. τ_{2off} 396.1 ± 52.3). The 95% LOA and CV for phase II parameters were small for both intensities and transients of exercise. Conversely, broad 95% LOA were identified for all the phase III parameters. To address this problem, the treadmill protocol was modified to include four very heavy-intensity exercise transients (80%Δ) to improve the signal-to-noise ratio of the phase III response. Analysis of test-retest data obtained from ten participants revealed that although the CV and 95% LOA for the phase III parameters were improved, they were still larger than for phase II parameters.

Using the very-heavy intensity treadmill protocol, a relationship (bivariate correlation) was found between τ_{1on} and soccer-specific high-intensity running capacity, both for professional (Pro, n = 18) (r = -0.71; P =0.013) and amateur (Am, n = 18) (r = -0.69; P = 0.014) soccer players. However, the role $\dot{V}O_2$ kinetics plays in such exercise appears to be limited, as a mixed design two-way ANOVA revealed that the Pro players ran further in a test of soccer-specific fitness (Pro 966 ± 153 m vs. Am 840 ± 156 m) despite the $\dot{V}O_2$ kinetic profiles of the two groups being indistinguishable (τ_{1on}, Pro 24.5 ± 3.2 s vs. Am 24.7 ± 1.8 s; τ_{1off}, Pro 28.7 ± 2.8 s vs. Am 29.3 ± 3.5 s). To identify which physiological processes did determine soccer-specific high-intensity running capacity among elite players, a longitudinal study was conducted with 16 Pro soccer players (8 = controls, 8 = training), whose soccer specific fitness, aerobic ($\dot{V}O_2$ max, $\dot{V}O_2$ kinetics) and anaerobic profiles (anaerobic capacity) were assessed before and after a six week high-intensity training intervention. A two-way ANOVA mixed design revealed soccer-specific fitness (P=0.015) and anaerobic capacity (P = 0.021) were the only measures that increased among the training group following the intervention. The change between the two measures was also correlated (r = 0.89; P = 0.012). It is plausible that due to the sporadic nature and high-intensity of the running performed in soccer, $\dot{V}O_2$ kinetics are not a determinant of performance, and above a certain threshold of aerobic fitness, it is the capacity for anaerobic energy production that is crucial for the performance of soccer-specific high-intensity running.

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Conference communications relevant to this thesis

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Contents

List of Symbols and Abbreviations

ACSM	American College of Sports Medicine
ADP	adenosine diphosphate
Am	amateur soccer players
ANT	adenine nucleotide translocase
A_{1on}	amplitude of phase II $\dot{V}O_2$: on-transient
A_{1off}	amplitude phase II $\dot{V}O_2$: off-transient
A_{2on}	amplitude phase III $\dot{V}O_2$: on-transient
A_{2off}	amplitude phase III $\dot{V}O_2$: off-transient
ANOVA	analysis of variance
AT	anaerobic threshold
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BASES	British Association of Sport and Exercise Sciences
Ca^{++}	calcium ion
$C(a\text{-}v)O_2$	arterial-venous O_2 content difference
Cn	control group
Cr	creatine
CS	citrate synthase
CK	creatine kinase
CV	coefficient of variation
CO_2	carbon dioxide
d	distance
ETC	electron transport chain
FAD	flavin-adenine dinucleotide (oxidised form)
$FADH_2$	flavin-adenine dinucleotide (reduced form)
FIO_2	fractional concentration of O_2 in total inspired gas
GET	gas exchange threshold
H^+	hydrogen ion
HCO_3	bicarbonate ion
H_2O	water
HLa	blood lactate
HR	heart rate
HR_{max}	maximum heart rate
IMP	inosine monophosphate
kg	kilogram
l	litre
LDH	lactate dehydrogenase
LOA	limits of agreement
LT	lactate threshold

MART	maximal anaerobic running test
MDH	β-hydroxyy-CoA-dehydrogenase
min	minute: unit of time
MiCK	mitochondria
ml	millilitre
MLa	muscle lactate
MMK	mechanical machinery
mmol	millimole
ms	millisecond: unit of time
NAD^+	nicotinamide-adenine dinucleotide (oxidised form)
NADH	nicotinamide-adenine dinucleotide (reduced form)
NMR	nuclear magnetic resonance
O_2	oxygen
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
$PETCO_2$	partial pressure of CO_2
$PETO_2$	partial pressure of O_2
Phos	glycogen phosphorylase
PFK	phosphofructokinase
Pi	inorganic phosphate
^{31}P-MRS	phosphorous nuclear magnetic resonance spectroscopy
PO_2	partial pressure of oxygen
Pro	professional soccer players
$\dot{Q}$	cardiac output
$\dot{Q}max$	maximum cardiac output
$\dot{Q}O_2$	rate of muscle oxygen consumption
RER	respiratory exchange ratio
RST	repeated sprint test
s	second: unit of time
SD	standard deviation
SDH	succinate dehydrogenase
S_o	SD of the breath-by-breath noise
STPD	standard temperature and pressure dry
t	time
TCA	tricarboxylic acid cycle
T_{D1on}	phase II time delay: on-transient
T_{d1off}	phase II time delay: off-transient
T_{D2on}	phase III time delay: on-transient
τ_{1on}	phase II time constant: on-transient
τ_{1off}	phase II time constant: off-transient

τ_{2on}	phase III time constant: on-transient
τ_{2off}	phase III time constant: off-transient
Tr	training group
$\dot{V}CO_2$	rate of carbon dioxide production
$\dot{V}E$	minute ventilation
$\dot{V}O_{2\,½}$	time to reach one half of the final oxygen uptake response
$\dot{V}O_2$	rate of oxygen uptake
$\dot{V}O_{2}$ max	maximal oxygen uptake
$\dot{V}O_2$ kinetics	oxygen uptake kinetics
YIRT1	Yo-Yo intermittent recovery test level 1
YIRT2	Yo-Yo intermittent recovery test level 2
80%GET	80% of GET (moderate intensity)
50%Δ	midpoint between GET and $\dot{V}O_2$ max
80%Δ	80% of the way between GET and $\dot{V}O_2$ max
Δ	the change in
[ ]	concentration

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CHAPTER 1

Introduction

1.1 Introduction

The physiology of exercise is the study of how the body responds and adapts to exercise. Although this study can be traced to the observations of Empedocles in 490 BC, it was not until the early 20th century through the work of Krogh and Lindhard (1913) and A.V. Hill and colleagues (1924) that concentrated and systematic efforts were made to measure human physiological responses to the onset of exercise. Since these pioneering studies, physiological assessments have advanced considerably, making it possible to identify physiological processes that influence the ability to perform exercise. In the context of sport, such information has been used to maximise the adaptation of relevant physiological systems through appropriate physical training.

The use of physiological assessments to enhance performance in repeated sprint-type sports such as soccer is challenging, as intermittent exercise involves a complex interaction of several physiological processes. Although successful soccer performance requires a high degree of technical ability, it has been reported that the most successful teams are also the fittest (Ekblom, 1986; Bangsbo, 1994; Reilly, 1996). Recent investigations have demonstrated that the strongest indicator of a player's soccer-specific fitness is their ability to perform high-intensity running during a game (Krustrup and Bangsbo, 2001; Krustrup *et al.*, 2003; Mohr *et al.*, 2003). Aerobic metabolism is widely accepted to play an important role during prolonged high-intensity intermittent exercise, as it contributes to energy production during both exercise and recovery. However, recent research (Krustrup and Bangsbo, 2001; Krustrup *et al.*, 2003) has shown that measures of aerobic fitness such as maximum oxygen uptake

($\dot{V}O_2$ max) and gas exchange threshold (GET) are not related to these indices of soccer performance. Perhaps such gross measures of aerobic function that are the product of integrating cardiovascular, pulmonary and muscular systems are insensitive measures of the oxidative processes involved in soccer performance. This raises a key question: what are the aerobic processes that help determine a player's capability to perform soccer-specific exercise?

An integral component of a performer's aerobic profile (Whipp *et al.*, 1982) that has not been considered in the context of soccer performance is pulmonary oxygen uptake kinetics ($\dot{V}O_2$ kinetics). Measures of these kinetics have been shown to mirror changes in muscle oxygen uptake ($\dot{Q}O_2$) at the onset and cessation of exercise (Barstow and Molé, 1987; Grassi *et al.*, 1996). Such information on the oxidative processes of muscle might provide valuable information about a soccer player's capability to perform the required bouts of repeated high-intensity exercise. Fast $\dot{V}O_2$ kinetics at the onset of such exercise have been shown to reduce the oxygen deficit (Demarle *et al.*, 2001) and hence the reliance on potentially fatigue-inducing anaerobic energy production. Similarly, quick $\dot{V}O_2$ kinetics during the rest periods of this exercise might indicate enhanced recovery capabilities within muscle, such as the resynthesis of phosphocreatine (PCr), restoration of oxygen stores and the metabolism of lactate. The net effect of quick $\dot{V}O_2$ kinetics during both exercise transients would be a minimal disruption of muscle homeostasis, which would benefit performance during subsequent bouts of exercise. In addition, $\dot{V}O_2$ kinetics appear to be more sensitive to training than both $\dot{V}O_2$ max and GET among endurance trained athletes (Phillips *et al.*, 1995; Norris and Peterson, 1998; Demarle *et al.*, 2001). Therefore $\dot{V}O_2$ kinetics might reflect

training-induced physiological adaptations that other measures of aerobic function fail to detect.

Much of the research that has investigated $\dot{V}O_2$ kinetic responses to the onset and cessation of exercise has involved moderate-intensity cycling. Such an exercise pattern is clearly unrelated to soccer-specific activities. As $\dot{V}O_2$ kinetic responses appear to be dependent on the form of exercise (Carter *et al.*, 2000a) and its intensity (Paterson and Whipp, 1991; Koga *et al.,* 1999; Carter *et al.*, 2002), it would be more appropriate to measure the $\dot{V}O_2$ kinetics of soccer players during the on- and off-transients of moderate- and heavy-intensity treadmill running, as this would replicate the type of exercise performed in a game. However, investigations using a treadmill protocol to measure on- and off-transients of $\dot{V}O_2$ kinetics are limited (Williams *et al.*, 2001; Carter *et al.*, 2002), and have in some cases provided conflicting results to those from cycle ergometry (Carter *et al.*, 2000a). Therefore, to investigate the relationship between $\dot{V}O_2$ kinetics and soccer performance, an appropriate and reliable square-wave exercise protocol must be established. Such a study would also provide an opportunity to assess the characteristics and day-to-day variability of $\dot{V}O_2$ kinetics. Reproducible measures of $\dot{V}O_2$ kinetics are essential if they are to be considered as a determinant of soccer performance and to be used as a tool to distinguish between players of differing ability.

If the speed of $\dot{Q}O_2$ is a determinant of soccer performance, then a player's $\dot{V}O_2$ kinetic profile should differ in relation to their level of soccer-specific fitness. Speeded phase II τ (Koppo *et al.*, 2004) and reduced slow component (Billat, 2002; Carter *et al.*, 2002) at the onset of exercise have been reported for trained compared to less trained individuals. Furthermore, Kilding *et al.* (2003) found that $\dot{V}O_2$ kinetics measured

during the on- and off-transients of moderate-intensity exercise were faster for highly trained long- than middle-distance runners. This implies that even for athletes who perform similar sporting events, $\dot{V}O_2$ kinetics are sensitive to the influence of differences in training on the oxidative capacity of muscle. The possibility that $\dot{V}O_2$ kinetics can distinguish between soccer players who perform different types and volumes of training due to standard of competition has not been addressed.

If $\dot{V}O_2$ kinetics play a determining role in soccer performance, training undertaken to improve soccer-specific high-intensity running capability should lead to a change in $\dot{V}O_2$ kinetic responses. Improvements in $\dot{V}O_2$ kinetic measures during the on-transients of exercise following endurance training have been shown to coincide with enhanced cycling (Norris and Peterson, 1998) and running performance (Demarle *et al.*, 2001) without changes to $\dot{V}O_2$ max or lactate threshold (LT). High-intensity interval training similar to that performed by some soccer players (Bangsbo, 1994) has been shown to speed $\dot{V}O_2$ kinetics to a greater extent than moderate-intensity continuous training (Berry and Moritani, 1985). This suggests that oxidative adaptations generated from intermittent exercise influence $\dot{V}O_2$ kinetics in a way that will be beneficial for performance. However, no studies have been undertaken directly to assess the relationship between soccer performance and training induced speeding of $\dot{V}O_2$ kinetics responses to the onset and cessation of exercise.

1.2 Aims and objectives

The overall aim of this thesis is to identify if $\dot{V}O_2$ kinetics are a determining factor in the ability to perform soccer-specific high-intensity running. To achieve this aim there are 6 specific objectives:

1. To assess the reproducibility of physiological markers used to set moderate- and heavy-intensity exercise.

2. To design a treadmill protocol for the measurement of $\dot{V}O_2$ kinetics at the onset and cessation of moderate- and heavy-intensity running in soccer players.

3. To assess the reproducibility of $\dot{V}O_2$ kinetic responses measured during moderate- and heavy-intensity treadmill running.

4. To assess the characteristics of $\dot{V}O_2$ kinetic responses during moderate- and heavy-intensity treadmill running.

5. To identify if $\dot{V}O_2$ kinetics discriminate between players who possess differing levels of soccer-specific fitness.

6. To identify the physiological processes ($\dot{V}O_2$ kinetics, $\dot{V}O_2$ max, GET, anaerobic capacity) that are associated with an increase in soccer-specific fitness following a period of high-intensity intermittent training.

CHAPTER 2

Review of Literature

2.1. Explanation of soccer

2.1.1 Historical background

The earliest evidence of participation in an activity resembling soccer dates back to around the 2nd and 3rd centuries BC, when Chinese military kicked a ball into a net as part of a game or skill building exercise (Hill, 2003). However, it is widely accepted that the modern game of soccer was developed in Britain, where it is more commonly known as football. Played by the masses from the 8th century onwards, football often involved hundreds of people from rival towns and villages attempting to move a ball to a predetermined spot. The game could last all day and was notoriously violent, with kicking, gouging, biting and punching allowed. It was not until 1815 that Eton College established a set of rules that started to resemble those of the modern game. In 1845 these rules were standardised and adopted by most of England's universities, becoming known as the Cambridge rules. Later in 1863, the Football Association was created, establishing the rule that banned any handling of the ball, marking the split between association and rugby football. Consequently, the name soccer is derived from the "soc" of association. The first Football Association challenge cup was contested in 1871 and a competitive league of 12 English clubs was formed in 1888. From these modest beginnings soccer has grown to become the most popular participation sport in the world, crossing cultural and economic barriers (Reilly, 1996).

2.1.2 Rules

The modern game of soccer is played on a rectangular pitch by two teams of 11 players, each consisting of one goalkeeper and 10 outfield players. According to the English

Football Association (2004), a pitch must be within 90 and 120 m in length and 45.5 and 90 m in width. Play is split into 45 min halves separated by a 15 min interval. The objective of soccer is to score a goal by placing a ball into the opponent's net/goal (7.32 m x 2.44 m). This is primarily achieved by kicking the ball, although a goal can be scored using any body part except the upper limbs, as handling of the ball by outfield players is prohibited. Only the goalkeeper, whose role is to prevent the opposition from scoring is permitted to handle the ball, although this is restricted to a designated area encompassing their team's goal. Outfield players can typically be separated into: 1) defenders, whose primary role is to defend their goal by limiting the opposition scoring opportunities, 2) midfielders, who link defence with attack and 3) forwards, whose major responsibility is to score in the opposition's goal. The team that has scored the most goals by the end of a game is the winner. If both teams score the same number of goals by the end of play the game is drawn. In certain cup competitions however, if a game is drawn at the end of the 90 min, extra time is played (two x 15 min) to allow a team to try to score the winning goal. If the score is level after extra time the outcome of the game is determined by penalty kicks.

2.2 The activity profile of an outfield soccer player

Over the course of a game, players are required to perform bouts of sub-maximal running interspersed by high-intensity runs and dynamic actions such as jumps, tackles, turns and kicks. Such an activity profile is highlighted by the observations of several studies that a player makes on average over 1000 changes in playing activity during a game, which equates to a change in movement every four to six seconds (Thomas and Reilly, 1976; Bangsbo, 1993; Drust *et al.*, 1998). The total distance covered during a game while performing such variable activities typically ranges from 10 to 12 km, with midfielders and central defenders covering the furthest and least distances respectively

(Saltin, 1973; Thomas and Reilly, 1976; Van Gool *et al.*, 1988; Bangsbo and Lindquist 1992; Mohr *et al.*, 2003).

It has been widely reported that 85 to 90% of the many activities a player performs during a game are at low- or sub-maximal intensities (Reilly and Thomas, 1976; Withers *et al.*, 1982; Yamanaka *et al.*, 1988; Drust *et al.*, 1998; Mohr *et al.*, 2003). Consequently, soccer has been reported to involve a large ratio of low- to high-intensity running, in terms of time on the pitch (7:1, Thomas and Reilly, 1976; 4:1, Bangsbo, 1993) and distance run (2.2: 1, Thomas and Reilly, 1976). An example of the running speeds used to classify game activities into low- or high-intensity categories is listed below.

Table 2.1 The intensity classification of match activities by Mohr *et al.* (2003).

Activity	**Speed ($km.h^{-1}$)**	**Intensity Classification**
Walking	6	Low-intensity
Jogging	≥8	
Low Speed Running	≥12	
Moderate Speed Running	≥15	High-intensity
High Speed Running	≥18	
Sprinting	≥30	

Although such ratios emphasise the large amount of low-intensity running a player is required to perform during a game, the distance run at a high-intensity is not insignificant. Mohr *et al.* (2003) reported that with the exception of central defenders, all outfield players ran in excess of 2 km at a high-intensity during a game. Furthermore, it has been observed that a soccer player performs a high-intensity activity

every 28 to 90 s (Withers *et al.*, 1982; Bangsbo, 1994). The capability to repeatedly perform high-intensity exercise would therefore seem to be a necessity of soccer performance.

2.2.1 High-intensity running as a marker of soccer-specific exercise capability

The total distance covered during a soccer game is a poor gauge of the physical demand placed on a player as it largely consists of walking and jogging, which are not physically demanding activities for a trained athlete. Instead, the distance covered performing high-intensity running has been suggested to be a more valid and reliable indicator of a player's performance capability, even though it constitutes a much smaller proportion of a player's activity profile. There are several observations to support this viewpoint: 1) during the second half, the volume of high-intensity running can be 35% to 45% less than in the first half without a reduction in low intensity running (Mohr *et al.*, 2003). Therefore, it is the reduced ability to exercise at high- not low-intensities that characterises fatigue in soccer (Krustrup and Bangso, 2001); 2) elite soccer players have been observed to run 28 ± 6% further at a high-intensity than moderate standard players during a game (Bangsbo, 1992; Mohr *et al.*, 2003); 3) it is during high-intensity periods of play that the outcome of games are often decided (Bangsbo, 1994) and 4) the total distance covered between games can vary considerably, whereas the distance covered performing high-intensity running is more stable (Reilly, 1996). Therefore the physiological assessment of soccer players should focus on the processes that influence the performance of high-intensity intermittent exercise.

2.3 Energy provision for soccer

It has been widely demonstrated that both anaerobic (Gerisch, 1988; Bangsbo *et al.*, 1991; Smith *et al.*, 1993; Florida-James and Reilly, 1995) and aerobic (Seliger, 1968;

Reilly and Holmes, 1983; Kawakami *et al.*, 1992; Florida James and Reilly, 1995) energy systems are heavily taxed during the course of a soccer game. Rather than acting separately, the energy systems interact in an attempt to maintain ATP provision for muscle contraction.

2.3.1 Anaerobic energy metabolism

At the start of intermittent exercise, oxygen (O_2) bound to myoglobin in the muscle and haemoglobin in the blood provide a direct source of O_2 than can be used for energy provision (Saltin *et al.*, 1976). However, this aerobic contribution is not sufficient to provide all the energy required at the onset of high-intensity exercise (Spriet, 1995). To ensure muscle force development can continue anaerobic energy systems must contribute. The immediate source of anaerobic energy provision is the hydrolysis of the high energy phosphate compound, adenosine tri-phosphate (ATP) which is stored in muscle:

$$ATP \xrightarrow{ATPase} ADP + P_i + H^+ + \text{energy} \quad (1)$$

where ADP is adenosine diphosphate, Pi is inorganic phosphate and H^+ is a hydrogen ion. Only a small amount of ATP is stored in cells, therefore it must be resynthesised at the rate it is used to allow muscular activity to continue. There are two main anaerobic energy producing pathways which interact to maintain the supply of ATP for muscular force development: ATP- PCr and anaerobic glycolysis.

In the ATP-PCr pathway, the resynthesis of ATP can be achieved by combining ADP and Pi via a creatine kinase (CK) reaction in the cytoplasm of the cell:

$$ADP + PCr + H^+ \xleftrightarrow{CK} ATP + Cr \quad (2)$$

This pathway provides immediate energy for muscular contraction at the onset of exercise and during short term high-intensity exercise, acting as an energy buffer to reduce the degradation of ATP stores within the muscle. However, there is only enough PCr stored in the muscle to aid ATP provision for approximately 10 s, therefore to maintain ATP provision contributions are required from simultaneously operating metabolic pathways.

The activation of anaerobic glycolysis occurs probably immediately at the onset of exercise and involves the resynthesis of ATP via degradation of glucose or glycogen to pyruvate in the cytoplasm of the cell. However, when glycolytic flux exceeds mitochondrial activity, as observed at the onset of exercise or during heavy-intensity exercise, pyruvate is subsequently converted to lactate, where:

$$Glucose + 2\ ADP + 2\ Pi \longrightarrow 2\ Lactate + 2\ H^+ + 2\ ATP \quad (3)$$

2.3.2 Anaerobic energy metabolism during intermittent high-intensity exercise

The role of anaerobic energy production is complex during high-intensity intermittent exercise as it is directly influenced by the intensity and duration of both the exercise and recovery periods (Åstrand *et al.*, 1960; Essen, 1978). During the initial seconds of a high-intensity exercise bout, large reductions in stores of PCr (Balsom *et al.*, 1992; Holymard *et al.*, 1998; Bogdanis *et al.*, 1996; Bangsbo, 2000) occur that cannot be resynthesised until the intensity of exercise is reduced (Tomlin and Wenger, 2001). The total restoration of PCr stores can take between 3 to 5 min (Hultman *et al.*, 1967), yet

the recovery periods in soccer are often much shorter. This could have implications for soccer performance, as the inhibition of PCr resynthesis during intermittent exercise by insufficient recovery periods (Bogdanis *et al.*, 1995; Bogdanis *et al.*, 1996) has been shown to considerably reduce power output and hence performance during subsequent bouts of exercise.

However, the capability to perform high-intensity intermittent exercise is not entirely dependent on the resynthesis of PCr, as decrements in the repeated performance of both abrupt (Gaitanos *et al.*, 1993) and prolonged sprints (McCartney *et al.*, 1986; Greenhaff *et al.*, 1994; Bogdanis *et al.*, 1996) have been linked to reductions in anaerobic energy turnover from glycolysis. This was clearly demonstrated by Spriet *et al.* (1989) who reported that a 25% reduction in power output during repeated sprints was related to a 20% reduction in glycogenolysis, with no change being detected in energy contribution from the ATP-PCr system. However, a reduction in anaerobic glycolysis during repeated high-intensity exercise is not always associated with a reduction in performance (Bangsbo, 2000). An increased contribution from aerobic metabolism can help maintain ATP supply for muscle contraction as anaerobic glycolysis becomes down-regulated, highlighting the complex interaction that occurs between the different energy systems during intermittent exercise

2.3.3 Aerobic energy metabolism

The regeneration of ATP from aerobic glycolysis involves the conversion of glucose or glycogen to pyruvate. However, if glycolytic flux does not exceed mitochondrial activity, lactate is not formed and oxidative phosphorylation occurs in the mitochondria. The beta-oxidation of fatty acids inside the mitochondria will also take place. The final reaction of oxidative phosphorylation is:

$$NADH + \frac{1}{2}O_2 + H^+ + 3\ ADP + 3\ Pi \longrightarrow 3\ ATP + NAD^+ + H_2O \quad (4)$$

There are two major metabolic pathways involved in oxidative phosphorylation: the tricarboxylic acid cycle (TCA), which breaks down acetyl units derived from fuel molecules and generates the reduced coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) as well as carbon dioxide (CO_2) and the electron transport chain (ETC) where free energy, released when electrons are transferred from NADH and $FADH_2$ to O_2, gets channelled into phosphorylation of ADP to make ATP, that is, it drives the reaction:

$$ADP + Pi \longrightarrow ATP + H_2O \quad (5)$$

During electron transfer from NADH and $FADH_2$ to O_2, the free energy released pumps protons (H^+) from the matrix side of the inner membrane of the mitochondria to the outer side thus creating an electrochemical gradient. When protons return down the gradient, the free energy released is used to resynthesise ATP from ADP and Pi.

2.3.4 Aerobic energy metabolism during high-intensity intermittent exercise

Aerobic metabolism is integral to the performance of high-intensity intermittent exercise as not only does it contribute to energy provision during abrupt (Seresse, 1988; Balsom *et al.*, 1992) and prolonged sprints (McCartney *et al.*, 1986; Bangsbo *et al.*, 1992; Bogdanis *et al.*, 1996), but is fundamental to the restoration of muscle homeostasis during recovery periods (Bogdanis *et al.*, 1996; Tomlin and Wenger, 2001).

Substantial aerobic contributions during high-intensity intermittent exercise are necessary to maintain performance when rates of anaerobic glycolysis are reduced. Bogdanis *et al.* (1996) reported a 45% reduction in anaerobic ATP production during a second 30 s sprint performed 5 min after the first sprint, yet only recorded an 18% drop in power output. This mismatch between anaerobic energy release and power output during sprint two was partly compensated for by an 18% increase in aerobic energy provision. Such elevation in aerobic metabolism during repeated bouts of exercise has been reported previously for high-intensity cycling (McCartney *et al.*, 1986; Green *et al.*, 1987), and maximal leg extensor exercise (Bangsbo *et al.*, 1992). It has also been demonstrated that $\dot{Q}O_2$ is speeded during repeated bouts of exercise (Bangsbo, 2000), further demonstrating the increasing importance of aerobic metabolism during repetitive high-intensity exercise. Small increases in O_2 uptake are beneficial as they will translate into large amounts of extra ATP due to carbohydrate (CHO) being oxidised rather than metabolised to lactate (Spriet, 1995).

During the recovery periods of intermittent exercise, it has been shown that aerobic metabolism not only provides the energy for muscle activity but also plays a fundamental role in the restoration of muscle homeostasis (Lee *et al.*, 1987; Balsom *et al.*, 1992). Lee *et al.* (1987) reported that if the oxygen supply to muscle was restricted following a high-intensity exercise bout, PCr resynthesis was inhibited. It has also been demonstrated by Yoshida *et al.* (1993) that PCr resynthesis is faster in muscles that have a high oxidative capacity, and furthermore PCr reysnthesis is speeded through the intra-muscular oxidative adaptations that occur as a consequence of endurance training.

The second major contribution aerobic metabolism plays in the recovery of muscle homeostasis is the oxidation of lactate and hence removal of H^+. Although lactate is an

important precursor for gluconeogenisis in muscle and the liver via the TCA cycle (Gaesser and Brooks, 1984; Brooks, 2000), the primary fate of lactate is its oxidation by the heart, liver, resting muscles and muscles that are exercising at a low- to moderate-intensity (Mazzeo *et al.*, 1986; Brooks, 2000; Gladden, 2000). This process has been commonly termed the lactate shuttle (Brooks, 1986). Recently, Brooks (2000) has suggested that intracellular uptake and oxidation of lactate might also occur, although this theory has been disputed (Sahlin *et al.*, 2002). The complexities of the argument are too in-depth to cover in this review.

2.3.5 Adaptation of energy systems to high-intensity intermittent training

If recovery time between bouts of prolonged (20 to 30 s) high-intensity exercise is limited, anaerobic glycolysis will be heavily taxed as ATP provision from high energy phosphagens will be reduced due to limited PCr resynthesis (Margaria *et al.*, 1969; Balsom, 1992; Bogdanis *et al.*, 1996). However, as glycolysis becomes inhibited during repeated bouts of high-intensity exercise (Spriet, 1995) there is a considerable contribution from aerobic metabolism in an attempt to maintain force development (Bogdanis *et al.*, 1996). The reliance on aerobic energy production increases in relation to the length of the exercise bout. Another important consequence of short recovery periods is that they will cause $\dot{V}O_2$ to remain elevated through out the exercise, further increasing the demand on aerobic metabolism. This simultaneous stimulation of energy systems has been shown to improve anaerobic and to a lesser extent aerobic fitness. In a 6 week training study by Tabata *et al.* (1996), recreationally active students performed 7, 20 s exercise bouts at ~ 170% $\dot{V}O_2$ max, with 10 s of recovery between each bout. Increases of 13% and 28% were observed for $\dot{V}O_2$ max and MAOD respectively. MacDougall *et al.* (1998) demonstrated in a 7 week training study, where 10, 30 s bouts of maximal exercise were separated by 2.5 min of recovery, peak power output (+ 49%)

and $\dot{V}O_2$ max (+ 7%) both increased. There were also marked increases in the activity of anaerobic and aerobic metabolic enzymes.

When short exercise bouts (5 - 10 s) are interspersed by long recoveries (~ 4 min) the demand on anaerobic glycolysis and aerobic metabolism is decreased, placing greater importance on the rapid breakdown and resynthesis of PCr stores. This will result in lower levels of lactate accumulation and muscle acidosis, which might to some extent protect against reductions in PCr resynthesis (Billat, 2001). Long recoveries will also result in $\dot{V}O_2$ dropping before the next exercise bout, reducing the demand on aerobic metabolism. Therefore, this intermittent training model would appear useful for improving ATP production from high energy phosphagens. Such training has been shown to benefit sprint performers, where the quickest athletes are the ones who are capable of breaking down their stores of PCr the fastest (Hirnoven *et al.*, 1987). Although a number of studies have shown no increase in PCr degradation or CK levels following periods of sprint training (Jacobs *et al.*, 1987; Nevill *et al.*, 1989). Cadefau *et al.* (1990) have suggested that a relatively large activity of CK is evident in the muscle of sedentary individuals and the stress induced by periods of short sprint training is not sufficient to stimulate an increase in CK activity.

If exercise bouts are repeatedly performed at an intensity that will enable an individual to exercise for several minutes or more, aerobic metabolism will be the major source of ATP provision. Research by Billat *et al.* (1999) has shown that in well trained runners, interval training at $\geq$ 90 % velocity $\dot{V}O_2$ max can lead to a substantial increase in $\dot{V}O_2$ max as it places a maximal demand on aerobic metabolism. This would indicate that for larger improvements in aerobic fitness, the intensity of exercise should be lower than

for sprint training so that exercise bouts can be performed for longer to allow greater stimulus of the aerobic energy system.

2.4 Measures of physiological function and soccer performance

Extensive physiological assessments of soccer players have been undertaken in an attempt to quantify the importance of aerobic and anaerobic energy provision for the performance of high-intensity soccer-specific exercise.

2.4.1 Maximal oxygen uptake

Maximal oxygen uptake is defined as the maximum rate at which an individual can extract, transport and utilise O_2 at sea level (Åstrand and Rodahl, 1986). The $\dot{V}O_2$ max of an individual is achieved when both cardiac output ($\dot{Q}$) and the arterial-venous O_2 content difference ($C(a\text{-}v)O_2$) are maximal, which is expressed in a rearrangement of the Fick equation:

$$\dot{V}O_2 \text{ max} = \dot{Q} \text{ max} \cdot C(a\text{-}v)O_2 \text{ max} \qquad (6)$$

During activites such as soccer, where an individual is required to support their body, $\dot{V}O_2$ max is expressed relative to body mass ($ml.kg^{-1}.min^{-1}$).

2.4.1.1 Limitations of $\dot{V}O_2$ max

Although a person's $\dot{V}O_2$ max can be increased through appropriate training (Thoden, 1996), there appears to be a ceiling to its development. Both central and peripheral physiological mechanisms have been suggested as factors that limit $\dot{V}O_2$ max (Rowell, 1986). Central mechansims involve cardiac and pulmonary function and hence the transport of O_2 to exercising muscle. Peripheral mechanisms relate to the utilisation of

O_2 in the muscle and include factors such as capillary density, fibre type composition, oxidative enzyme activity and mitochondrial content and function (Wilmore and Costill, 1994).

Support for the central limitation argument is provided by observations that perturbation to any step of the O_2 transport pathway affects $\dot{V}O_2$ max. Stray-Gundersen *et al.* (1986) noted that peri-myocardial patients who underwent surgery to increase cardiac output and hence oxygen delivery capabilities experienced an 8% improvement in $\dot{V}O_2$ max. It has also been demonstrated that $\dot{V}O_2$ max can be altered through the manipulation of the blood's oxygen carrying capacity. Richardson *et al.* (1999) reported that the leg $\dot{V}O_2$ max of cyclists increased under hyperoxic breathing conditions. Comparable reductions in leg $\dot{V}O_2$ max were achieved through hypoxic breathing. Similarly, the infusion and removal of red blood cells has been shown to increase and decrease $\dot{V}O_2$ max respectively (Ekblom *et al.*, 1972).

Support for the peripheral argument is based on the observation that not all the O_2 delivered to the muscle is extracted (Taylor, 1987). In fact, during exhaustive exercise the delivery of O_2 to exercising muscle is sufficient to keep muscle PO_2 above the critical threshold required for normal mitochondrial function (Weibel, 1987). Support for the peripheral argument is provided by studies that have found improvements in $\dot{V}O_2$ max to be associated with increases in mitochondrial enzyme activity (Saltin *et al.*,1976b) and muscle capillary density (Weibel, 1987). A study by Henriksson and Reitman (1977) however questions the argument that increases in $\dot{V}O_2$ max can be solely attributed to peripheral adaptations. They reported that at the end of an 8 week training programme, $\dot{V}O_2$ max had increased by 19% and succinate-dehydrogenase (SDH), which is an indicator of a muscles aerobic potential by 32%. However, after 6

weeks of detraining, $\dot{V}O_2$ max remained unchanged but SDH had returned to pre-training levels. It is conceivable that it is an interaction of both central and peripheral mechanisms that determines $\dot{V}O_2$ max, and so an integrated model of oxygen delivery and utilisation might provide the best explanation (Di Prampero and Cerretelli, 1987).

2.4.1.2 Measurement of $\dot{V}O_2$ max

Due to its practicality, a field-based maximal shuttle run test (Leger and Lambert, 1982) is often used in soccer to provide an indirect estimate of a player's $\dot{V}O_2$ max. Although the test is convenient to use with squads of players, Leger *et al.* (1988) reported the error of the estimate for $\dot{V}O_2$ max to be 5.9 $ml.kg^{-1}.min^{-1}$ or ~12%, which could mask small changes in a player's $\dot{V}O_2$ max. A more accurate measure of a soccer player's aerobic capacity can be obtained through direct determination of $\dot{V}O_2$ max (Armstrong and Costill, 1985), where pulmonary gas exchange is typically measured during an incremental (step or ramp) treadmill test to volitional exhaustion. The intensity of exercise is progressively increased by manipulating the speed or gradient of the treadmill. Importantly, $\dot{V}O_2$ max has been reported to be ~ 4% lower during a speed compared to a gradient protocol (Draper *et al.*, 1998). This might be explained by the fact that if individuals are not accustomed to running at high speeds, it is their inability to run quickly that stops them prematurely, rather than the attainment of volitional exhaustion. A second explanation is that to run uphill, increased contributions of the upper extremities and less efficient type II fibres are required, which would result in a greater consumption of O_2.

2.4.1.3 Relationship between $\dot{V}O_2$ max and soccer performance

The $\dot{V}O_2$ max of soccer players has been observed to range from 56 to 69 $ml.kg^{-1}.min^{-1}$ (Puga *et al.*, 1990; Rahkila and Luhtanen, 1991; Davis and Brewer, 1992; Bangsbo,

1993; Reilly, 1996), which is higher than for sedentary individuals and similar to that reported for athletes from other team sports (Bangsbo, 1998). Such a range in $\dot{V}O_2$ max values appears to be the result of positional differences in aerobic fitness, with midfielders and fullbacks typically possessing the greatest aerobic capacity, followed by forwards and then central defenders (Puga *et al.*, 1990; Rahkila and Luhtanen, 1991). Such differences in aerobic fitness among players might be attributable to position-specific physiological loads, as $\dot{V}O_2$ max has been found to be associated with the total distance run during a game (r = 0.98, Smaros, 1980; r = 0.68, Reilly, 1996).

A high $\dot{V}O_2$ max is advantageous for soccer performance as it enables aerobic metabolism to substantially contribute to energy provision during high-intensity exercise, reducing reliance on anaerobic energy sourses above the AT. A large $\dot{V}O_2$ max might also be beneficial for soccer performance as a high level of aerobic fitness has been associated with enhanced recovery capabilitites following high-intensity exercise (Tomlin and Wenger, 2001).

Direct support for the importance of $\dot{V}O_2$ max for soccer performance was provided by Helgerud *et al.* (2001), who reported that an increase in the $\dot{V}O_2$ max of well-trained soccer players from 58.1 ± 4.5 $ml.kg^{-1}.min^{-1}$ to 64.3 ± 3.9 $ml.kg^{-1}.min^{-1}$ was associated with an increase in the number of sprints performed during a game. Furthermore, the distance run in a test of soccer-specific high-intensity running capacity has been found to be positively correlated with $\dot{V}O_2$ max (r = 0.79, Krustrup *et al.*, 2003). However several studies have noted that $\dot{V}O_2$ max is not associated with the amount of high-intensity running performed during a match-play (Bangsbo and Mizuno, 1988; Krustrup and Bangsbo, 2001; Krustrup *e al.*, 2003; Mohr *et al.*, 2003), which as discussed in section 2.2 appears to be the most important aspect of soccer performance. Bangsbo and

Mizuno (1988) also reported $\dot{V}O_2$ max to be an insensitive indicator of training status, as they observed a player's performance in a soccer-specific fitness test to decrease markedly after 3 weeks of inactivity while their $\dot{V}O_2$ max remained unchanged. Furthermore, Krustrup and Bangsbo (2001) observed that elite referees who undertook a 12 week intermittent training programme that comprised a range of exercise to rest patterns (4 x 4 min runs, 8 x 2 min runs, 16 x 1 min runs and 24 x 30 s runs at heart rates above 90% of an individual's maximum) were found to perform more high-intensity running during a game with no change being detected in $\dot{V}O_2$ max. Krustrup *et al.* (2003) also noted performance in a test of soccer-specific fitness to increase considerably (25 ± 6%) following an intense period of pre-season training with only a small improvement in $\dot{V}O_2$ max (+ 7% ± 1%).

2.4.2 Anaerobic threshold

The anaerobic threshold refers to the $\dot{V}O_2$ at which the onset of metabolic (lactate) acidosis occurs within muscle. (Wasserman and McIlroy, 1964). However, there is a lack of consensus as to the existence, definition and identification of the AT (Yeh *et al.*, 1983; Brooks, 1985; Hughson *et al.*, 1987). Consequently, several approaches have been developed for its measurement, which can make cross study comparisons problematic. However, Wasserman *et al.* (1994) stated that the use of contrasting methods of AT measurement does not dispute the existence of the underlying mechanism.

2.4.2.1 Mechanisms of AT

During exercise intensities performed below the AT, most of the hydrogen ions stripped from fuel substrates and carried by NADH are oxidised within the mitochondria and passed to oxygen via the ETC to form water. In these conditions, there is minimal

lactate accumulation as the rate of lactate appearance is equal to the rate of lactate removal, creating a biochemical steady-state. However, as the intensity of exercise exceeds the AT, an increased contribution from anaerobic glycolysis and recruitment of less oxygen efficient type II fibres causes an increase in the lactate-pyruvate ratio. Consequently, pyruvate reacts with $NADH + H^+$ and is reduced to lactate, via lactate dehydrogenase (LDH), while regenerating NAD^+ and allowing anaerobic glycolysis to continue. The increased H^+ is buffered intra-cellularly by HCO_3, generating additional CO_2.

The traditional explanation of the AT devised by Wasserman (1983) identified the following sequence of processes as forming the basis of AT: 1) The O_2 required by exercising muscle can exceed the O_2 supply to the mitochondria as exercise intensity progresses, 2) The imbalance between the O_2 supply and O_2 requirement causes the mitochondrial membrane shuttle to lose pace with the rate of $[NADH + H^+]$ production, resulting in a reduced redox state. The condition of O_2 limited oxidative phosphorylation is termed dysoxia. Recently, evidence has mounted that suggests dysoxia is not necessarily the primary cause of increased lactate production. During progressive exercise Richardson *et al.* (1998) reported increases in lactate even though intramuscular PO_2 remained above the critical mitochondria PO_2. It is also widely accepted that during rest and low-intensity exercise in fully aerobic conditions, lactate is continuously being produced and removed (Brooks, 1986). As cell acidosis is dependent on the rates of production and removal of lactate, it is conceivable that it is the ability to remove lactate that determines where AT occurs.

2.4.2.2 Measurement of AT

One method for identifying AT is to determine the point at which lactate production exceeds its removal, leading to increases in muscle and blood lactate concentrations, referred to as the lactate threshold (LT). Typically, LT is identified during a protocol which consists of several small incremental stages that tend to be 3 to 4 min long. Lactate measurements are taken at the end of each stage. When represented graphically, the lactate response to the increasing exercise intensity typically consists of a two component curve, the first being shallow, often not passing 2 $mmol.l^{-1}$ or exceeding 1 $mmol.l^{-1}$ above resting. The second component then tends to lead into a steep rise, often resulting in values above 4 $mmol.l^{-1}$ (Karlson and Jacobs, 1982). Beaver *et al.* (1985) suggested that the point at which the two components intersect is LT. Although it must be stressed that there are several methods of LT identification and each can provide an alternative $\dot{V}O_2$ value for LT.

The gas exchange threshold (GET) is a non-invasive method of determining AT. Pulmonary gas exchange is recorded during a continuous incremental exercise test, where at the low and moderate exercise intensities; $\dot{V}CO_2$ and $\dot{V}E$ rise in a linear pattern until GET. Above GET, CO_2 production from the buffering of lactate results in an increase in $\dot{V}CO_2$ relative to $\dot{V}E$. As $\dot{V}E$ and $\dot{V}CO_2$ initially accelerate linearly above GET, there is a short period where $\dot{V}E/\dot{V}CO_2$ and $PETCO_2$ do not change while $\dot{V}E/\dot{V}O_2$ and $PETO_2$ increase, which is referred to as the isocapnic buffering stage. This point can be used to identify GET. However, this approach can be inaccurate because of its reliance on the ventilatory response to metabolic acidosis. In some people with insensitive ventilatory chemoreceptors, the expected ventilatory response might be absent. To overcome this problem Beaver *et al.* (1986) developed the V-Slope method to determine the occurrence of GET. It is based upon the fact that CO_2 is released when

lactic acid is buffered by bicarbonate in the cells, and that this CO_2 is quickly transported to the lungs. This additional CO_2 can be detected by an increase in CO_2 output over and above the CO_2 produced from aerobic metabolism. When breath-by-breath $\dot{V}CO_2$ is plotted against $\dot{V}O_2$, the point at which $\dot{V}CO_2$ increases disproportionally to the aerobic energy production is GET (Wasserman *et al.*, 1988).

2.4.2.3 Relationship between AT and soccer performance

The LT and GET of soccer players has been reported to occur between 72 to 86% of $\dot{V}O_2$ max (Whitė *et al.*, 1988; Chin *et al.*, 1992; Edwards *et al.*, 2003), which is similar to that reported for both team players and endurance athletes (Kilding *et al.*, 2003). The range in values is probably due to positional differences in endurance fitness as well as the use of different criteria to assess where LT and GET occur.

Recently, Edwards *et al.* (2003) observed that after a period of preseason training, $\dot{V}O_2$ at both LT and GET increased considerably, although $\dot{V}O_2$ max remained unchanged. This suggests that the ability to delay lactate accumulation as exercise intensity increases is more important for soccer performance than a large aerobic capacity. Alternatively, it might be that AT is a more sensitive marker of soccer-specific fitness than $\dot{V}O_2$ max. Bangsbo and Lindquist (1992), using a modified measure of LT reported that players whose lactate levels did not reach 3 $mmol.l^{-1}$ until a high percentage of their $\dot{V}O_2$ max ran further during a game. Although, as stated in section 2.2, the total distance covered during a game provides limited information concerning a player's physical performance. Furthermore, Balsom, (1991) noted little correspondence between an individual's $\dot{V}O_2$ at LT and decrement in high-intensity running performance over the course of a game. This observation is logical as the high-intensity runs involved in soccer are often at a speed that is well above a player's AT. Although

this does not account for the beneficial role a high AT might have in recovery capabilities and the removal of lactate.

2.4.3 Anaerobic Capacity

The concept of anaerobic capacity refers to the total amount of ATP that can be derived from the high-energy phosphagen and glycolytic energy systems (Green *et al.*, 1987).

2.4.3.1 Mechanisms of anaerobic capacity

The capacity to produce energy via the high energy phosphagen and glycolytic energy systems is strongly influenced by the architecture of exercising muscle. A high distribution of type IIa and IIx fibres predisposes a muscle for anaerobic energy production as they possess high concentrations of the metabolic enzymes (Table 2.2) that drive and regulate PCr degradation and glycolysis. An association has been demonstrated between type II fibre distribution and the glycolytic capacity of muscle (Simoneau *et al.*, 1985). Also strong links have been observed between the amount of energy that can be derived anaerobically and concentrations of these key enzymes before and after a period of high-intensity training (Hirnoven *et al.*, 1987; Neville *et al.*, 1989; Linnossier *et al.*, 1993; MacDougall *et al.*, 1998).

Table 2.2 Enzymes and their role in energy production.

Enzyme	Role
Creatine phosphokinase (CPK)	Breakdown of PCr
Phosphofructokinase (PFK)	Regulation of glycolysis
Glycogen Phosphorylase (PHOS)	Breakdown of glycogen
Lactate dehydrogenase (LDH)	Conversion of pyruvate to lactate
ATPase	Splitting of ATP

It is also feasible that a high distribution of type II muscle fibres will enhance muscle's anaerobic energy producing capacity as they contain high concentrations of PCr and glycogen; the substrates required by the high energy phosphagen and glycolytic energy systems (Ross and Leveritt, 2001).

Associated with the capacity for anaerobic energy production is the acidic buffering capacity of muscle. During prolonged high-intensity exercise, the accumulation of H^+ dissociating from lactic acid has been linked with fatigue by lowering muscle pH to such an extent that energy metabolism is reduced or prohibited. Human skeletal muscle has the ability to offset this change in pH through the use of various buffering mechanisms, including chemical buffers HCO_3^-, phosphate, proteins and haemoglobin in red blood cells (Ross and Leveritt, 2001). Recent evidence (Jeul, 1998) has shown that enhanced buffering capacity is associated with lower blood pH levels, indicating it is the ability of skeletal muscle to transport H^+ into the blood rather than tolerate them that is key to maintaining exercise. Several studies have demonstrated that the buffering capacity of muscle can be increased following a period of high-intensity training (Parkhouse and McKenzie, 1984; Allen *et al.*, 1995; Jeul, 1998). Such an adaptation might contribute to a large anaerobic capacity as it would enable anaerobic energy metabolism to continue during high-intensity exercise.

2.4.3.2 Measurement of anaerobic capacity

A direct estimation of anaerobic capacity is obtained by measuring changes in ATP, PCr and lactate in muscle following high-intensity exercise. Such measures have only been made possible by the introduction of the muscle biopsy technique. A small sample of tissue is taken from the exercising muscle and immediately frozen in liquid nitrogen. It is important that the procedure is performed quickly as resynthesis of ATP and PCr

could otherwise occur. To obtain valid estimates of anaerobic capacity from a muscle biopsy, the muscle mass involved in the exercise must be accurately determined. During cycling or treadmill running working muscle mass is estimated to be approximately 25% of body mass (Medbo and Tabata, 1993), however this is only an assumption and does not take into account the possibility that different muscles or muscle fibres are utilised as exercise continues (Bangsbo, 1993). A further limitation of the muscle biopsy technique is that it only accounts for lactate in the muscle, and does not provide a measure of lactate that has been released into the blood. This could lead to an underestimation of anaerobic energy production from glycolysis.

A commonly used indirect measure of anaerobic capacity is the maximal accumulated oxygen deficit (MAOD). To calculate MAOD, a linear relationship between $\dot{V}O_2$ and a range of sub-maximal exercise intensities is established. On a later occasion, this relationship is used to exercise a participant at 120% $\dot{V}O_2$ max until exhaustion (Medbo, 1988). The MAOD is calculated as the difference between the accumulated O_2 demand and the accumulated O_2 uptake. Medbo *et al.* (1988) demonstrated that MAOD would reach a maximum value and plateau off for exhaustive bouts of running which lasted 2 minutes or more, indicating the attainment of maximum anaerobic capacity. In support of MAOD as a measure of anaerobic capacity, Medbo *et al.* (1988) found a strong correlation between MAOD and measures of maximal anaerobic energy production determined from muscle biopsies. The authors also noted MAOD to be a valid measure of anaerobic energy production as it was unaffected by reductions in PO_2 during hypoxic breathing conditions.

However there is controversy surrounding the use of MAOD as a measure of anaerobic capacity, as the physiological processes involved in its assumptions are complex and

poorly understood in large exercising muscle groups (Bangsbo, 1993; Bangsbo, 1998). It might be more appropriate to use the MAOD as a measure of anaerobic energy release or a performance measure of prolonged high-intensity running capability, rather than saying it provides a measure of anaerobic capacity *per se*.

Several indirect estimates of maximal anaerobic capacity have since been developed that are less time consuming and hence more practical than the MAOD (Rusko *et al.*, 1993; Ramsbottom *et al.,* 1997; Hill *et al.*, 1998), however it is beyond the scope of this review to describe them all. One particular test that appears to be applicable to soccer is the Maximal Anaerobic Running Test (MART) devised by Rusko *et al.* (1993) and later modified by Maxwell and Nimmo (1996). The test involves 20 s running bouts on a motorised treadmill separated by 100 s of passive recovery. The starting speed is 14.3 $km.h^{-1}$ and increases by 1.2 $km.h^{-1}$ for each subsequent running bout. Treadmill gradient is kept constant at 10.5%. It is the aim of the participant to complete as many 20 s bouts as possible until exhaustion. The time to fatigue and the running speed achieved is then used to calculate maximal anaerobic power. Maxwell and Nimmo (1996) observed a strong correlation ($r = 0.83$) between the maximal anaerobic power values obtained in the MART and MAOD for a group of 18 recreationally active students. Earlier research by Rusko *et al.* (1993) showed the MART to be reliable on a test-retest basis, reporting correlation coefficients of $r = 0.93$ for maximal anaerobic power. It has also been suggested that the MART is sensitive to differences in training status as sprinters have been observed to achieve significantly greater max anaerobic power scores (119.2 ± 5.4 $ml.kg^{-1}.min^{-1}$) than endurance runners (97.8 $ml.kg^{-1}.min^{-1}$) (Nummela *et al.* 1996; Vuorima *et al.* 1996). So it would appear that the MART is an appropriate indicator of an individual's ability for anaerobic energy provision, and its intermittent nature might

make it a more appropriate indirect measure of anaerobic capacity for soccer players than MAOD.

2.4.3.3 Relationship between anaerobic capacity and soccer performance

It is conceivable that the ability to derive large amounts of energy anaerobically would benefit soccer performance as it would enable a player to exercise for longer at supra $\dot{V}O_2$ max intensities. Odetoyinbo and Ramsbottom (1998) observed the MAOD of University standard soccer players to increase from 74.3 ml.O_2-Eq.kg to 80 ml.O_2-Eq.kg after a period of high-intensity training, while studies by Ramsbottom *et al.* (1997) and Ramsbottom *et al.* (2001) reported that an increase in anaerobic capacity was positively correlated with improvements in continuous high-intensity shuttle running capacity.

In contrast, Bangsbo and Michalsik, (1993) reported that a high anaerobic capacity might not be crucial in order to succeed in soccer as the MAOD of elite Danish soccer players (49.5 ± 3.0 ml.O_2-Eq.min^{-1}.kg^{-1}) did not differ to that of distance runners (51.9 ± 3.8 ml.O_2-Eq.min^{-1}.kg^{-1}) and oarsmen (47.3 ± 6.3 ml.O_2-Eq.min^{-1}.kg^{-1}). Bangsbo and Michalsik (1993) also noted a large variation in MAOD to exist within the group of soccer players. Unfortunately, there does not appear to be any research that has investigated whether anaerobic capacity is a determining factor in the ability to perform high-intensity soccer-specific exercise. If it is the case that aerobic processes do not limit soccer performance, then the link between anaerobic capacity and soccer performance warrants investigation.

2.5 Soccer-specific performance measures

Soccer-specific performance tests can provide a direct means of investigating the physiological processes involved in the performance of high-intensity soccer-specific exercise. Due to the variable physical demands of soccer, a broad range of performance tests have been devised. However, for the purpose of this thesis, attention shall be focused on measures of intermittent high-intensity exercise capability.

2.5.1 Intermittent high-intensity exercise capacity

Tests of capacity provide information about the total amount of exercise that an individual can perform. Several soccer-specific exercise capacity tests have been devised (Ekblom, 1986; Balsom, 1992; Bangsbo and Lindquist, 1992; Nicholas *et al.*, 2000), all of which are field based and involve an intermittent exercise pattern. Recently, the Yo-Yo intermittent recovery test (YIRT) devised by Bangsbo (1996) has received considerable attention. The test requires players to perform repeated 20 m shuttle runs interspersed by 10 s recovery periods. During the recovery periods, players are required to jog around a marker set 5 m back from the 20 m shuttle. The time allowed for each 20 m shuttle is progressively decreased and is dictated by audible signals generated from a cassette tape. Players must try to keep in time with the signals until they reach volitional exhaustion. The test result is the total distance run. The reproducibility of the YIRT test has been reported to be high, with a coefficient of variation of 4.9% for test-retest data (Krustrup *et al.*, 2003). Performance in the YIRT is strongly related with the amount of high-intensity running a player is capable of performing during a game ($r = 0.71$) (Krustrup *et al.*, 2003). The YIRT has also been observed to distinguish between players of different standards (Mohr *et al.*, 2003), and improvements in YIRT performance following a period of training are mirrored by an increase in the amount of game related high-intensity running (Krustrup and Bangsbo,

2001). Such findings would indicate that the YIRT provides a valid and reliable measure of the capacity to perform high-intensity soccer-specific exercise.

2.5.2 Repeated sprint performance

Repeated sprint tests provide information about the capability to maintain performance over a series of maximal exercise bouts. Although there are a variety of repeated sprint tests, very few have actually been based on match analysis data. Bangsbo (1994) developed a repeated sprint protocol where players are required to sprint along a 30 m course that involves a 5 m deviation to the left. On completing the course players have 25 s to jog back to the start before performing the next trial. Seven trials are performed in total. Performance measures from the test include best sprint time, mean time for the 7 sprints and fatigue index (fastest minus slowest sprint). Wragg *et al.* (2000) reported that the ratio of high- to low-intensity running involved in the test is 1:3.3 and the mean time taken to perform the 7 repetitions was 203.6 s These values correspond very closely with match analysis data published by Withers *et al.* (1982) who found that the most physiologically demanding situation experienced by a player involved a high to low-intensity ratio of 1:3.1 that lasted for a duration of 178.2 s. Wragg *et al.* (2000) also reported a coefficient of variation of 1.8% for within subject variability. Unfortunately the test has not been used to gain information regarding the physiological mechanisms that influence high-intensity soccer-specific exercise capability. Research is therefore warranted to address this paucity in knowledge.

2.6 Pulmonary $\dot{V}O_2$ kinetics and soccer performance

The performance of high-intensity soccer-specific running has been shown to have a limited association with traditional measures of aerobic fitness. Yet, it is clear that aerobic energy provision plays an important role during the performance of high-

intensity intermittent exercise (Bogdanis *et al.*, 1996; Tomlin and Wenger, 2001). A potential reason for this apparent contradiction is that tests of aerobic fitness such as $\dot{V}O_2$ max and AT do not measure the aerobic processes that might benefit soccer performance. It is plausible that it is the speed at which aerobic metabolism can meet the energy demands of a change in exercise intensity that is fundamental for the performance of intermittent exercise. A rapid onset of aerobic metabolism would decrease the oxygen deficit and result in less reliance on anaerobic energy pathways. Also quick recovery kinetics might indicate enhanced PCr resynthesis and fast removal of lactate, leading to a quicker restoration of muscle homeostasis which would benefit the performance of subsequent exercise bouts. Furthermore, as the slow component has been associated with fatigue processes (Casaburi *et al.*, 1987; Barstow *et al.*, 1994), a small slow component might suggest an enhanced capacity to tolerate high-intensity exercise which could be beneficial for soccer performance. Previous research has demonstrated a link between quick $\dot{V}O_2$ kinetics at the onset of exercise and superior performance of continuous high intensity exercise (Norris and Peterson, 1998; Demarle *et al.*, 2001). It is conceivable therefore that $\dot{V}O_2$ kinetics might be a determining factor in the capability to perform soccer-specific high-intensity intermittent exercise.

2.7 Pulmonary $\dot{V}O_2$ kinetics

Pulmonary $\dot{V}O_2$ kinetics is a measure of the rate at which $\dot{V}O_2$ adjusts to alterations in exercise intensity. As the dynamics of $\dot{V}O_2$ measured at the mouth during exercise are closely coupled to metabolic cellular events (Cooper *et al.*, 1985), the measurement of $\dot{V}O_2$ kinetics can provide a useful insight into the mechanisms and effectiveness of cellular energy production. It is important to consider the exercise intensity domain in which $\dot{V}O_2$ kinetics are measured, as the kinetic response both for on- and off-transients become more complex as exercise intensity exceeds AT. Since soccer

involves a range of running speeds, the dynamic $\dot{V}O_2$ kinetic responses both to moderate- (sub AT) and heavy- (supra AT) intensity exercise will be discussed.

2.7.1 The $\dot{V}O_2$ response to moderate- and heavy-intensity square-wave exercise

2.7.1.1 Onset of exercise

A square-wave exercise transition in the moderate-intensity domain typically results in a three phased $\dot{V}O_2$ response (Linnarsson, 1974; Whipp *et al.*, 1982) which is illustrated below in Figure 2.1.

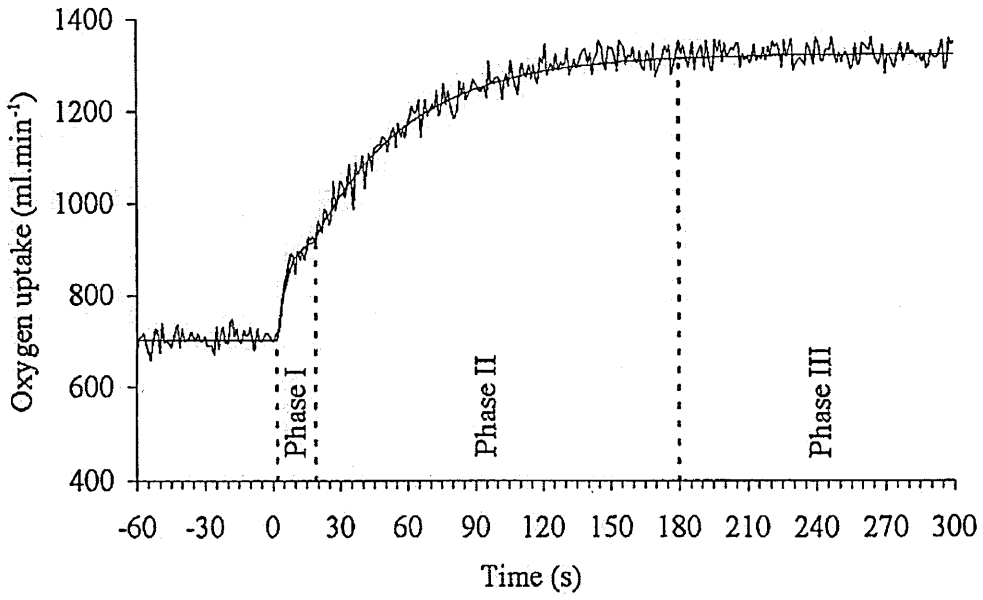


Figure 2.1 The $\dot{V}O_2$ response to the onset of exercise in the moderate-intensity domain. The three phases are described in the text (adapted from Sietsema *et al.*, 1989)

A sudden rise in exercise intensity causes an immediate increase in cardiac output ($\dot{Q}$) and pulmonary blood flow, the consequence of which is the abrupt increase in $\dot{V}O_2$ identified as phase I in Figure 2.1. This cardio-dynamic increase in $\dot{V}O_2$ lasts approximately 15 to 20 s and does not reflect increased muscle O_2 utilisation as it is not

the result of deoxygenated blood returning to the lungs from active muscle. Once venous blood arrives at the lungs there is a mono-exponential rise in $\dot{V}O_2$ termed phase II, which reflects the increased O_2 demand of the active muscles to sustain muscle contraction at the imposed intensity. This rise in $\dot{V}O_2$ continues until the O_2 demand of the exercising muscles is met, or the oxidative processes are at full capacity. If exercise is performed in the moderate-intensity domain, the energy demands of the active muscles can be met aerobically, which will result in a plateau of the $\dot{V}O_2$ response termed phase III. The time taken for $\dot{V}O_2$ to reach phase III typically takes two to three min, although variations have been observed in diseased (Hepple *et al.*, 1999) and highly trained individuals (Kilding *et al.*, 2003).

If exercise is performed in the heavy-intensity domain, aerobic metabolism alone is insufficient to meet the energy demands of exercising muscle and must be supplemented by anaerobic processes. This inability to meet the demands of the exercise aerobically prevents the attainment of a steady-state, instead phase III is extended, and takes the form of a gradual increase in the rate of oxygen utilisation. This development of excess $\dot{V}O_2$ has been termed the slow component and will continue to rise either to a delayed steady-state or $\dot{V}O_2$ max and exhaustion (Poole *et al.*, 1994).

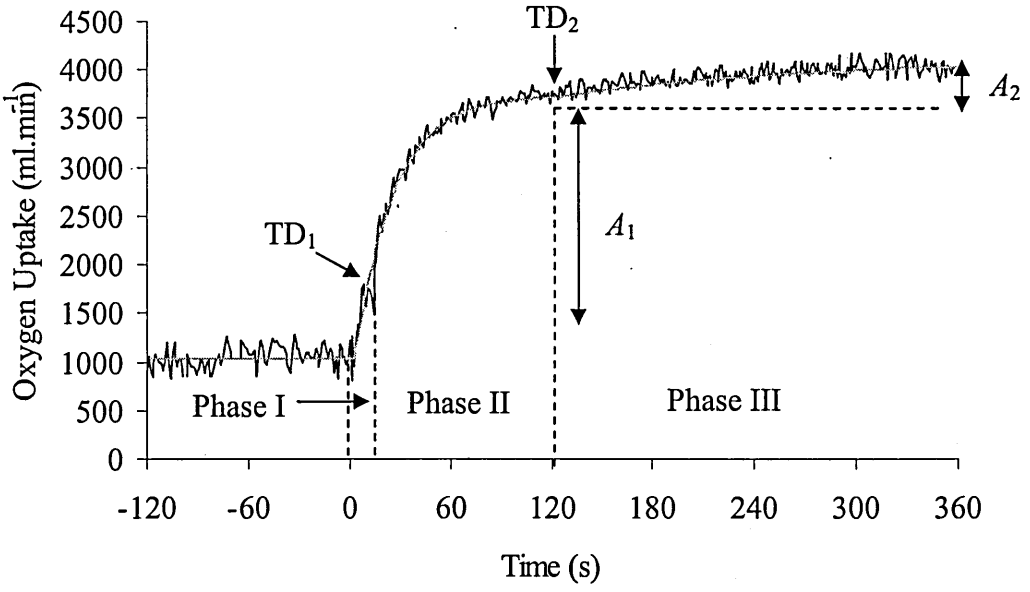


Figure 2.2 The three phased $\dot{V}O_2$ response to square-wave exercise in the heavy-intensity domain. The amplitude of phases II and III (slow component) are expressed as A_1 and A_2 respectively. The time delay between the onset of exercise and the beginning of phases II and III is represented by TD_1 and TD_2 respectively. A full explanation of terms is provided in chapter 2.7.5.

2.7.1.2 Cessation of exercise

At the cessation of exercise in the moderate-intensity domain, the $\dot{V}O_2$ response consists of three distinct phases that mirror those of the on-transient. To summarise, Phase I is characterised by a sudden drop in $\dot{V}O_2$ due to a decrease in $\dot{Q}$ reducing blood flow. Phase II immediately follows and is identified as the exponential fall in $\dot{V}O_2$ which is associated with the restoration of muscle oxygen stores and the resynthesis of PCr. Phase III (steady-state) represents the return of $\dot{V}O_2$ to pre-exercise values (Cunningham *et al.*, 2000; Özyener *et al.*, 2001). If exercise is performed in the heavy-intensity domain a slow component has been observed to develop (Fig 2.4), which keeps $\dot{V}O_2$ elevated above pre-exercise values.

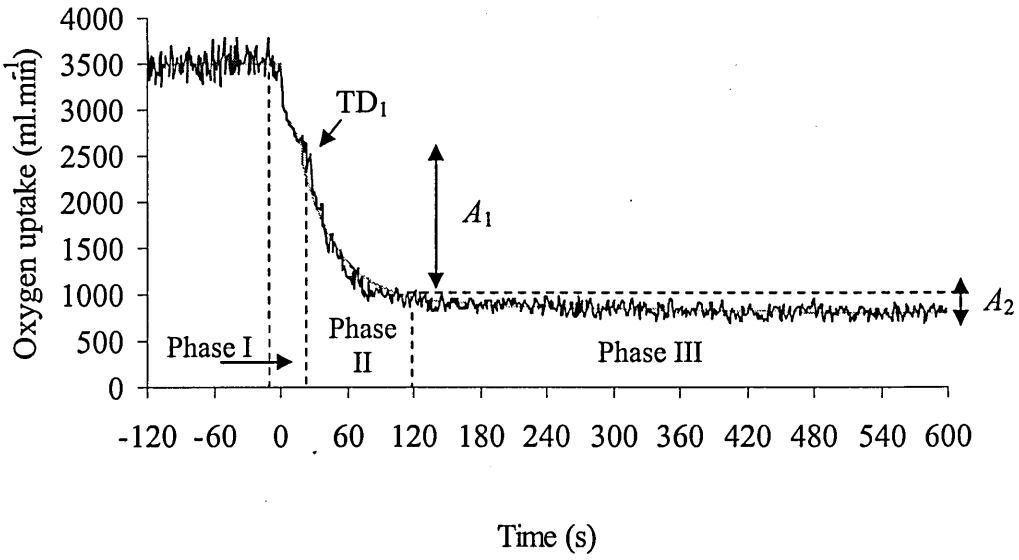


Figure 2.3 The $\dot{V}O_2$ response during recovery from square-wave exercise in the heavy-intensity domain. The amplitude of phases II and III (slow component) are expressed as A_1 and A_2 respectively. The time delay between the onset of exercise and the beginning of phases II and III is represented by TD_1 and TD_2 respectively. A full explanation of terms is provided in chapter 2.7.5.

2.7.2 Characterisation of $\dot{V}O_2$ kinetics as a function of exercise intensity

Several researchers have observed phase II τ to be invariant for cycle ergometry across a range of intensity domains in both the on- (Barstow and Mole, 1991; Barstow *et al.*, 1993) and off-transients (Cunningham *et al.*, 2000). This was demonstrated by Özyener *et al.* (2001) who observed the phase II τ for exercise onset and cessation did not differ between moderate- (33 ± 16 vs. 29 ± 6 s), very heavy- (34 ± 11 vs. 33 ± 5 s) and severe-intensity exercise (34 ± 7 vs. 35 ± 11 s). An earlier study by Barstow *et al.* (1993) reported similar findings where phase II τ was invariant across intensities ranging from 30 to 100% $\dot{V}O_2$ max.

There are however several studies that have observed τ to lengthen as exercise intensity increases (Paterson and Whipp, 1991; Koga *et al.,* 1999; Carter *et al.*, 2002), suggesting the control of $\dot{V}O_2$ kinetic responses are not independent of exercise intensity. Paterson and Whipp (1991) reported a slower phase II τ for heavy- (40.2 ± 2.7s) than moderate- (31.3 ± 3.3 s) intensity cycle ergometry. Barstow *et al.* (1994) questioned the validity of these results as a mono-exponential model was used to characterise the heavy exercise transition. This would not have allowed a distinction to be made between the dynamically different $\dot{V}O_2$ responses of phase II and the slow component. It is conceivable that the inclusion of the slow component would artificially slow the true phase II τ, leading to incorrect result interpretation. The use of a two-component model to take into account the slow component might have improved accuracy. More recent research by Carter *et al.* (2002) reported that phase II τ was shorter for moderate- than heavy-, very heavy- and severe-intensity treadmill running. The τ was found to be invariant across all the supra AT intensities.

The suggestion that τ is invariant across intensity domains has implications for the development of a testing protocol for soccer players. It is plausible that only heavy-intensity exercise bouts would have to be performed to gain valid information about the dynamics of the $\dot{V}O_2$ response both to low- and high-intensity running. Yet, the contradictory findings of the above studies make it difficult to draw firm conclusions about the characterisation of $\dot{V}O_2$ kinetics as a function of exercise intensity, prompting further research in the area.

2.7.3 Characterisation of $\dot{V}O_2$ kinetics as a function of the exercise transient

Studies into the characteristics of $\dot{V}O_2$ kinetics during both the on- and off-transients of exercise have reported conflicting results. Several investigations have reported

symmetry between $\dot{V}O_2$ kinetic responses during the onset and cessation of moderate-intensity square-wave cycling (Paterson and Whipp 1991; Barstow *et al.*, 1996; Özyener *et al.*, 2001). Conversely other studies have reported asymmetry between on- and off-$\dot{V}O_2$ kinetic parameters during such exercise (Hughson *et al.*, 1988; Rossiter *et al.*, 2002). A study which involved both cycling and running was conducted by Carter *et al.* (2000a) and reported the phase II τ to be asymmetrical between the on- and off-transients of moderate-intensity cycling (On: 18.0 ± 4.0 s vs. Off: 35.9 ± 4.2 s) and treadmill running (On: 15.0 ± 2.0 s vs. Off: 39.3 ± 3.0 s). The observation that $\dot{V}O_2$ kinetics are asymmetrical irrespective of exercise modality strongly supports the argument that $\dot{V}O_2$ kinetics are influenced by the exercise transient in which they are measured.

With regards to exercise performed in the heavy-intensity domain, several investigations have reported the phase II τ to be invariant across exercise transients for cycling (Engelen *et al.*, 1996; Scheurmann *et al.*, 2001). This is in disagreement however with more recent research that has reported phase II τ to be longer during the off- than the on-transient of heavy-intensity cycling (Cleuziou *et al.*, 2004) and knee extensor exercise (Rossitter *et al.*, 2002).

There is also uncertainty about the symmetry of $\dot{V}O_2$ slow component responses between exercise and recovery. Recently, Cleuziou *et al.* (2004) reported τ to be similar between the on- (113.7 ± 39.4 s) and off- (118.9 ± 41.4 s) transient slow component responses. However, in most cases τ for the slow component has been found to be much longer during recovery than exercise (Cunningham *et al.*, 2000; Özyener *et al.*, 2001). In a study by Özyener *et al.* (2001), τ of the slow component during recovery from very heavy exercise was 460 ± 123 s compared to 163 ± 46 s recorded during exercise onset.

Differences in τ of the slow component could be attributable to the modelling technique used to characterise the delayed $\dot{V}O_2$ response during recovery (Cleuziou *et al.*, 2004).

The amplitude of the slow component during recovery has been reported to be similar to that measured during exercise (Barstow *et al.*, 1996; Engelen *et al.*, 1996; Scheurman *et al.*, 2001; Cleuziou *et al.*, 2004). Barstow *et al.* (1996) concluded that the symmetry between slow components represents a clear exercise induced metabolic process that retains its distinction in recovery. However other studies have noted the amplitude of the slow component to be substantially smaller during recovery than exercise (Carter *et al.*, 2000a; Cunningham *et al.*, 2000; Özyener *et al.*, 2001). Cunningham *et al.* (2000) reported that the slow component during recovery was independent of the intensity and hence slow component contribution of the previous exercise, so refuting the earlier claims of Barstow *et al.* (1996).

2.7.4 Measurement of $\dot{V}O_2$ kinetics

The investigation of $\dot{V}O_2$ kinetics has resulted in several exercise protocols being devised to force a measurable change in cellular metabolism. The most common involves a square-wave (or step) transition in exercise intensity, where breath-by-breath pulmonary gas exchange is measured during an abrupt change from rest or low-intensity exercise to moderate- or high-intensity exercise. This exercise pattern can be reversed to measure dynamic $\dot{V}O_2$ responses during recovery. The intensity of the exercise transition is determined relative to an individual's AT and $\dot{V}O_2$max, ensuring that $\dot{V}O_2$ kinetic responses to the appropriate intensity domain are obtained.

To minimise the variability or noise inherent in breath-by-breath pulmonary gas exchange (Lamarra *et al.*, 1987), several square-wave transitions are performed, either

consecutively with appropriate rest periods or on separate occasions. The data from each transition are often interpolated and then ensemble averaged to produce a single representative measure of the $\dot{V}O_2$ response. Exponential modelling techniques can then be used to accurately determine a series of kinetic parameters.

2.7.5 Modelling $\dot{V}O_2$ kinetics at the onset and cessation of exercise

Exponential modelling techniques incorporating least squares regression identify the kinetic parameters that describe $\dot{V}O_2$ responses to the onset or cessation of square-wave exercise. In the moderate-intensity domain, a first-order exponential model is typically used to characterise $\dot{V}O_2$ responses during both the on- and off-transients (Chilibeck *et al.*, 1998; Özyener *et al.*, 2001). In an attempt to gain a true reflection of the exponential change in $\dot{V}O_2$ during phase II, interference from the initial cardio-dynamic phase (phase I) is reduced by either removing the first 20 s of $\dot{V}O_2$ data or constraining the model to start at the beginning of the phase II response (Whipp *et al.*, 1982). In the on- and off-transients of heavy-intensity exercise, a double-exponential model is commonly used to separately characterise the exponential rise in $\dot{V}O_2$ and subsequent slow component (Barstow *et al.*, 1996; Cunningham *et al.*, 2000). A number of researchers have included phase I in the modelling procedure, which will require two and three component exponential models for moderate- and heavy-intensity exercise respectively.

Kinetic parameters that are generated from this analysis of both the on- and off-transients are: 1) time delay (TD), which refers to the point(s) after the onset or cessation of exercise when phase II and/or the slow component begin; 2) τ, reflects the time taken to reach 63% of the exponential change in $\dot{V}O_2$ during phase II and/or the slow component following the onset or cessation of exercise; 3) amplitude term (*A*),

representing the magnitude of the $\dot{V}O_2$ change for each of the three phases. The above parameters are depicted in Figures 2.2 and 2.3 for the on- and off-transients of heavy-intensity exercise. In place of τ, early investigations into $\dot{V}O_2$ kinetics used the rate constant [k] or $\dot{V}O_2$ half-time [$\dot{V}O_{2\ t½}$] to characterise a $\dot{V}O_2$ response (Whipp and Casaburi, 1982; Powers *et al.*, 1985).

2.7.6 The site of $\dot{V}O_2$ kinetic limitation

The study of $\dot{V}O_2$ kinetics has indicated that at the onset of exercise, oxidative phosphorylation is limited either by: 1) the transport of oxygen to the exercising muscle (central) or 2) the inability of the muscle to utilise the O_2 delivered (peripheral). An O_2 transport limitation infers that mitochondria PO_2 is not saturating in all active muscle fibres during the exercise transition and O_2 utilisation could be increased if more O_2 was made available (Tschakovsky and Hughson, 1999). Alternatively, O_2 utilisation limitation suggests that the rate of oxidative phosphorylation during the exercise transition is determined by metabolic controllers. To investigate these opposing theories researchers have disrupted either one or more steps in the O_2 delivery chain, or attempted to identify physiological processes that follow the same response pattern as $\dot{V}O_2$ to the onset of exercise.

2.7.6.1 Disruption of the oxygen delivery chain

Several investigations have shown that when oxygen availability was lowered by the use of β-adrenergic receptor blockade drugs to reduce $\dot{Q}$, the $\dot{V}O_2$ response was slowed (Hughson and Smyth, 1983; Hughson, 1984). The use of such data to support an O_2 delivery limitation has however been questioned, as the use of β-adrenergic blocking drugs could cause participants to exercise in a heavier intensity domain (Hoffman *et al.*,

1994), which has been suggested to result in slower $\dot{V}O_2$ kinetic responses (Paterson and Whipp, 1991).

Studies that have reduced the amount of O_2 that can be delivered to exercising muscle through hypoxic breathing have reported a substantial slowing of $\dot{V}O_2$ kinetic responses (Murphy *et al.*, 1989) compared with normoxic conditions. Such an observation suggests that the site of $\dot{V}O_2$ kinetic limitation is central rather than peripheral. However, the inhalation of hyperoxic gas concentrations (70% O_2) does not affect the phase II $\dot{V}O_2$ kinetic responses (Hughson and Kowalchuck, 1995). Such findings suggest that the oxygen delivery only limits $\dot{V}O_2$ kinetics if inspired oxygen is reduced, under normal arterial O_2 content, oxygen transport is not limiting.

A less invasive approach to increasing oxygen delivery is the elevation and circulatory occlusion of both legs as this will increase central blood volume and so cardiac output. Under such conditions $\dot{V}O_2$ kinetic parameters have been observed to speed up during arm exercise (Hughson and Inman, 1986). A limitation of this study is that the information generated only relates to arm exercise, making assumptions about whole body exercise difficult. Such methodological problems can be overcome if circulatory changes are imposed by the manipulation of body position. When supine, venous return and so cardiac output are augmented as the effect of gravity is reduced compared to upright exercise. Consequently, supine exercise has been shown to slow the $\dot{V}O_2$ kinetic response (Convertino *et al.*, 1984; Hughson *et al.*, 1990) as the perfusion pressure across muscle capillary beds will decrease, therefore reducing muscle blood flow. This is supported by the observation that both blood flow and $\dot{V}O_2$ kinetics are slowed during knee extension exercise in the supine position (MacDonald *et al.*, 1998).

An alternative method of increasing blood flow and hence O_2 delivery is the use of lower body negative pressure. The increase in blood flow instigated by this technique has been associated with a speeding of $\dot{V}O_2$ kinetic responses in the supine position to a level similar to that recorded for upright exercise (Eiken, 1988; Hughson *et al.*, 1993). This would suggest that the slowing in $\dot{V}O_2$ kinetics observed by Hughson *et al.* (1990) during supine exercise is mediated by a reduction in oxygen delivery as it can be reversed by a technique that increases O_2 delivery. Following the same principles, lower body positive pressure would be expected to slow $\dot{V}O_2$ kinetic responses by restricting oxygen delivery. However Williamson *et al.* (1996) reported that lower body positive pressure had no effect on $\dot{V}O_2$ kinetic responses to moderate-intensity exercise, and so refuted findings that $\dot{V}O_2$ kinetics are limited by steps in the oxygen delivery process.

The potential influence a prior exercise bout might have on dynamic $\dot{V}O_2$ responses to the onset of exercise is particularly relevant to the study of soccer performance, as a soccer player's activity profile involves the execution of a series of exercise bouts that sporadically change in intensity. It is hypothesised that prior exercise could speed $\dot{V}O_2$ kinetics as oxygen delivery will be enhanced due to increases in the transport and perfusion of oxygenated blood to exercising muscle (Hughson and Morrissey, 1982). Recent investigations have reported that the $\dot{V}O_2$ kinetic responses to the onset of moderate-intensity exercise are unaltered by preceding bouts of moderate- and heavy-intensity exercise (Gerbino *et al.*, 1996; Burnley *et al.*, 2000). In contrast, the $\dot{V}O_2$ kinetic responses to heavy-intensity exercise have been found to be speeded by preceding heavy- but not moderate-intensity exercise (Gerbino *et al.*, 1996; Koppo and Bouckaert, 2000; Burnley *et al.*, 2000). Research by Gerbino *et al.* (1996) reported that τ for the entire $\dot{V}O_2$ response during a heavy-intensity exercise bout was 56.2 ± 37.8 s

and 36.9 ± 24.8 s when preceded by moderate and heavy-intensity exercise warm-ups respectively. It was also noted that the $\dot{V}O_2$ slow component response was substantially lower during heavy-intensity exercise that had been preceded by a warm-up in the heavy- (100 ± 60 ml.min^{-1}) compared with the moderate-intensity domain (250 ± 105 ml.min^{-1}). The authors took these kinetic responses as evidence that oxygen delivery is the rate-limiting step in $\dot{V}O_2$ kinetics, suggesting that the residual muscle acidema from prior heavy-intensity exercise improves muscle perfusion and hence speeded $\dot{V}O_2$ kinetics in the heavy-intensity domain.

This conclusion has since been questioned as the $\dot{V}O_2$ response to the onset of heavy-intensity exercise was characterised by a mono-exponential model. This does not allow a distinction to be made between the phase II and slow component $\dot{V}O_2$ responses. As the slow component was smaller during the second exercise bout a lower exercise end $\dot{V}O_2$ would be attained which would naturally lead to a reduced τ (Burnley *et al.*, 2000). When more appropriate double-exponential models have been used to describe $\dot{V}O_2$ responses to a second heavy-intensity exercise bout, phase II τ was found to be unaffected (Burnley *et al.*, 2000; Koppo and Bouckaert, 2000). It was also confirmed that a quicker τ for the entire $\dot{V}O_2$ response was a consequence of a reduced slow component. Such evidence refutes the claims that oxygen supply is the limiting factor in $\dot{V}O_2$ kinetic responses to heavy-intensity exercise.

An explanation for the reduced $\dot{V}O_2$ slow component during a second heavy-intensity exercise bout could be attributable to the recruitment of fewer type II muscle fibres. It has been hypothesised that type II fibres take longer to recover after intense exercise and more type I fibres might therefore be involved when exercise is repeated (Koppo

and Bouckaert, 2000). It has also been proposed that an increase in muscle temperature as a result of prior exercise increases mechanical efficiency of muscle contraction (Koga *et al.*, 1997; Burnley *et al.*, 2000), which contradicts the theory that the slow component response is caused by an increase in temperature via the Q_{10} effect. So the cause of a reduced $\dot{V}O_2$ slow component during repeated exercise still needs to be firmly established.

2.7.6.2 Physiological responses that mirror $\dot{V}O_2$

At the onset of exercise the rate at which cardiac output and heart rate increase has been found to be quicker than that of $\dot{V}O_2$. Such an observation indicates that $\dot{V}O_2$ kinetics are not limited by central factors as the mechanisms responsible for O_2 delivery appear to exceed its utilisation (Cerretelli *et al.*, 1966; Linnarsson, 1974; Yoshida and Whipp, 1994). However, such measures of cardiac function cannot be used totally to disregard central limitation theories of $\dot{V}O_2$ kinetics as they do not provide information about the redistribution of oxygenated blood delivery in the muscle. Furthermore, Hughson and Morrisey (1983) found the changes in heart rate and $\dot{V}O_2$ kinetics were comparable for a range of square-wave exercise intensities. The similar changes in the two physiological processes were taken as evidence that $\dot{V}O_2$ kinetics are limited by O_2 delivery assuming heart rate kinetics reflect muscle blood flow.

When $\dot{V}O_2$ kinetics have been measured simultaneously with muscle blood flow at the onset of exercise, the increase in muscle $\dot{V}O_2$ was much slower than the increase in muscle blood flow (Grassi *et al.*, 1996). This suggests that muscle is incapable of utilising all the O_2 delivered, providing strong evidence that the limitation of $\dot{V}O_2$ kinetics is peripheral. Yet when blood flow has been restricted during leg exercise in the

supine position or arm exercise above the heart, the reduction in muscle blood flow has been associated with slowed $\dot{V}O_2$ kinetics.

Studies that have employed phosphorous nuclear magnetic resonance spectroscopy (P-NMR) measurement techniques during the onset and cessation of exercise have identified a close association between the kinetics of PCr degradation in the muscle and $\dot{V}O_2$ at the lung (Barstow *et al.*, 1990; Grassi *et al.*, 1996; McCreary *et al.*, 1996; Rossiter *et al.*, 1999; 2002). Graphical representation of this association is depicted in Figure 2.4. Such observations provide strong evidence that the degradation of PCr through the PCr shuttle is involved in the control of cellular respiration and hence $\dot{Q}O_2$ (Whipp and Mahler, 1980). This implies that the rate limiting step of $\dot{V}O_2$ kinetics is the peripheral regulation of oxidative phosphorylation.

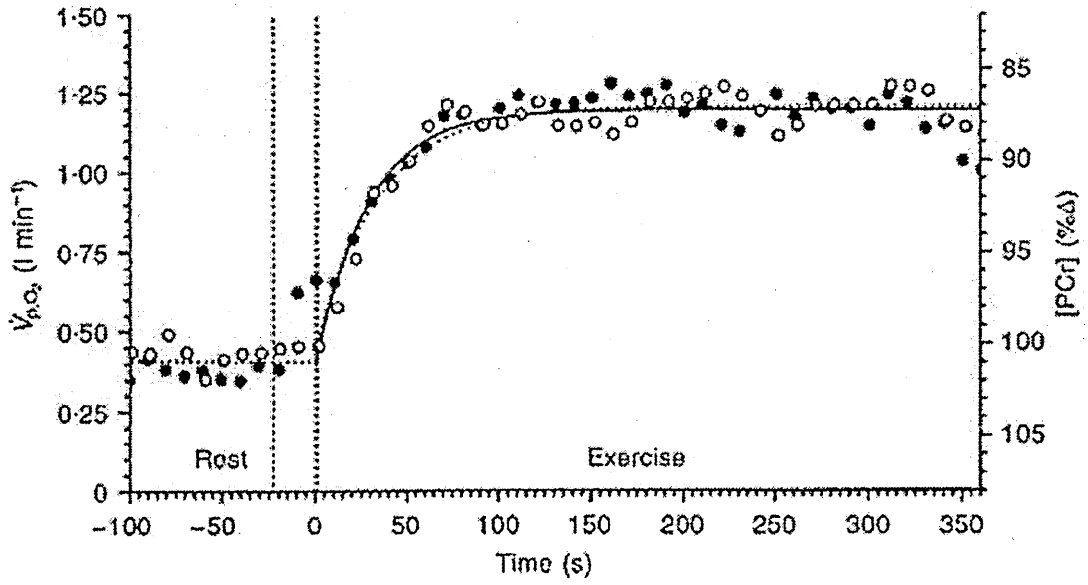


Figure 2.4 Temporal response of $\dot{V}O_2$ (•) and (°) PCr to the onset of moderate-intensity square-wave exercise (Rossitter *et al.*, 1999).

2.7.7 Mechanisms regulating oxidative phosphorylation

2.7.7.1 Exercise onset - Phase II response

Oxidative phosphorylation is increased via both feed forward and feedback mechanisms. In the feed forward system, the release of calcium from the sarcoplasmic reticulum activates the contractile apparatus of the muscle. As a consequence, calcium is believed to be a metabolic switch that triggers ATP hydrolysis and hence oxidative phosphorylation (Meyer and Foley, 1996).

Increase in mitochondrial ADP that occurs from the hydrolysis of ATP has been argued to act in a feedback manner to stimulate additional ATP production. Early research by Chance and Williams (1956) observed the rate of oxygen consumption to vary with ADP concentration in the classic enzyme kinetic model of Michaelis-Menten (Meyer and Foley, 1996). Associated with ADP mediated control of respiration is the ATP/ADP ratio. Adenine nucleotide translocase (ANT) catalyses the transmembrane exchange between ATP generated by oxidative phosphorylation and inter-membrane ADP. As the rate of ANT translocase reaction is determined by ATP, the extra-mitochondrial [ATP]/[ADP] ratio has been proposed as a potential controller of $\dot{Q}O_2$ by determining the rate of ADP delivery to the mitochondrion (Rossiter *et al.*, 2005). As the Km for the resynthesis of ADP to ATP is dependent on both ADP and Pi, it has been suggested that the rate of oxidative phosphorylation might be reliant on the phosphorylation potential of the mitochondria ([ATP]/([ADP] x [Pi]) and/or the intra-mitochondrial redox potential [NADH]/[NAD] (Wilson, 1994). However, recent research has questioned the contribution ADP plays in the regulation of oxidative phosphorylation as the kinetics of ADP during exercise were not found to correspond closely to those of $\dot{V}O_2$ and hence $\dot{Q}O_2$ (Rossiter *et al.*, 2002).

In contrast, the kinetics of PCr degradation and $\dot{V}O_2$ appear to be indistinguishable (Whipp and Mahler, 1980; Rossiter *et al.*, 1999; Rossiter *et al.*, 2002). Such observations have been used to support the creatine shuttle hypothesis of respiratory control. Whereas ADP access to the inner mitochondrial membrane is restricted (see Figure 2.5), PCr and Cr can move relatively freely between intra and extra-mitochondrial sites. A local increase in Cr resulting from energy buffering at the cross bridge is transduced to the inter-mitochondrial membrane where it can accept a high energy phosphate from newly formed ATP to produce PCr. This reaction permits the shuttling of high energy phosphate to the myofibril and elevates the levels of ADP entering the inner mitochondrial membrane, hence increasing the substrate for oxidative phosphorylation. Therefore the rate of delivery of Cr to the mitochondria and rate of PCr hydrolysis provides a feedback control signal for $\dot{Q}O_2$, rather than direct feedback control from ADP.

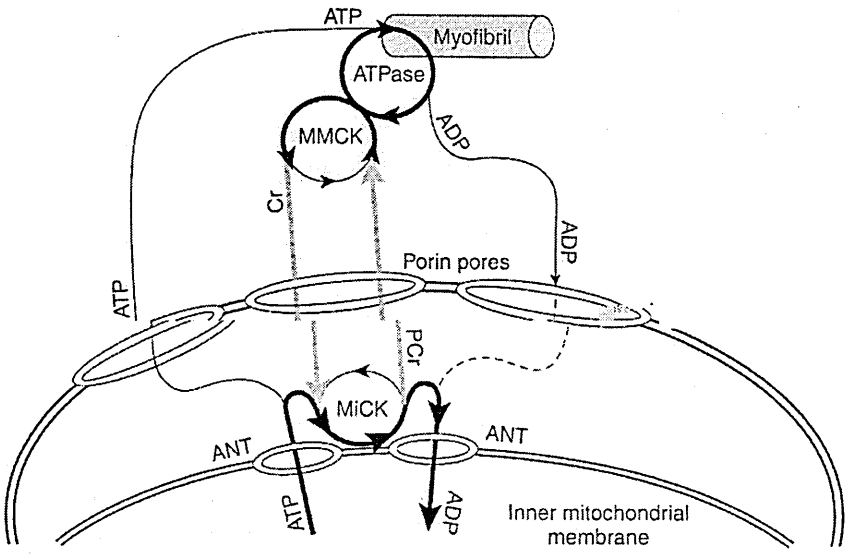


Figure 2.5 A diagram illustrating the PCr, CK and ANT systems involved in respiratory control. Phosphate exchange occurs between Cr and PCr via CK and is shuttled between sites of CK at the mechanical machinery (MMCK) and the mitochondria (MiCK). PCr and Cr entry to the mitochondrial inter-membrane space is relatively uninhibited compared to ADP (dotted line). High energy phosphates are primarily transferred between mitochondrion and myofibrils by the exchange of PCr/Cr rather than ATP/ADP. The weighting of the arrows indicates the relative flux of each pathway during exercise; ATP is thought to be quickly hydrolysed on exiting the inner space via MiCK, producing PCr (adapted from Rossiter *et al.*, 2005).

By measuring PCr degradation and $\dot{V}O_2$ simultaneously, Rossitter *et al.* (2002) suggest that this CK-mediated control of respiration appears to remain simple and linear across moderate- and heavy-intensity exercise. This supports the observations of invariant τ across intensity domains discussed previously in section 2.8.4. However, further research is required to firmly establish if $\dot{V}O_2$ kinetics do express dynamic linearity across exercise intensities as a number investigations have reported phase II τ to

lengthen as exercise intensity increases from moderate to heavy (Paterson and Whipp, 1991; Koga *et al.,* 1999; Carter *et al.*, 2002; Koppo *et al.*, 2004). It has been hypothesised that an increased contribution towards force production from less oxidative type II fibres is the cause of slowed phase II $\dot{V}O_2$ kinetic responses during heavy-intensity exercise (Carter *et al.*, 2002). This theory is underpinned by the observations that type II fibres have up to two thirds fewer mitochondria than type I fibres (Meyer *et al.*, 1985) and also express low oxidative efficiency and slow kinetics in responses to square-wave exercise (Crow and Kushmerick, 1982; Barstow *et al.*, 1996).

2.7.7.2 Exercise onset - Slow component

Several mediators of the slow component have been proposed. During heavy- and severe-intensity exercise, it was hypothesised that the increase in the work of cardiac, respiratory and accessory muscles would generate an additional O_2 cost that would substantially contribute to the development of the slow component (Hagberg, 1980; Poole *et al.*, 1988). However, such theories have been largely disregarded as it has since been demonstrated that ~86% of the $\dot{V}O_2$ of the slow component originates from within the exercising muscle (Poole *et al.*, 1994), an observation supported by the work of Rossitter *et al.* (2002) who found the $\dot{V}O_2$ slow component to be mirrored by a low and gradual decrease in intramuscular PCr.

Several studies have reported a strong association between lactate concentration and $\dot{V}O_2$ slow component development (Casaburi *et al.*, 1987; Barstow 1994; Womack *et al.*, 1995). An increased circulation of lactate could stimulate glyconeogenesis that would result in an increased oxygen cost in skeletal muscle (Barstow *et al.*, 1996). The oxygen cost of glycogen resynthesis is however likely to be small (Whipp, 1994) and

the development of a slow component has been observed in the absence of lactate accumulation (Barstow and Mole, 1991; Poole *et al.*, 1994). Furthermore, the infusion of noradrenaline during exercise has been found to increases lactate substantially without an elevation in $\dot{V}O_2$ (Gaesser *et al.*, 1994). Therefore the relationship between blood lactate accumulation and the $\dot{V}O_2$ slow component could be coincidental rather than causal (Womack *et al.*, 1995; Carter *et al.*, 2002).

An increased O_2 cost due to elevation in temperature via the Q_{10} effect has been proposed as a contributor to slow component development. Recent studies however have observed that an elevation in muscle temperature can occur without an elevation in exercising $\dot{V}O_2$ (Poole *et al.*, 1994; Koga *et al.*, 1997), dismissing the hypothesis.

A theory that has received much attention is that some aspect of fibre type recruitment accounts for the development of the slow component. Several researchers have proposed that an increased recruitment of less oxidative type II muscle fibres to generate and maintain the high forces necessary to perform heavy-intensity exercise will lead to a slow and excessive rise in $\dot{V}O_2$ (Shinohara and Moritani, 1992; Barstow *et al.*, 1996; Carter *et al.*, 2000a). Using integrated electromyography (iEMG), mean power frequency (MPF), which is used as an indicator of type II fibre recruitment, has been found to coincide with the onset of the slow component (Borrani *et. al.*, 2001). In addition an innovative study by Krustrup *et al.* (2004a) demonstrated a reduced slow component amplitude during knee extensor exercise following the selective aerobic training of type II fibres. Krustrup *et al.* (2004b) also reported that a slow component could be developed below the AT by glycogen depleting type I fibres to promote the increased recruitment of type II fibres.

In contrast, other investigations have failed to find any evidence associating an increase in type II fibre type recruitment and the slow component. During repeated bouts of heavy-intensity exercise, Scheuermann *et al.* (2001) observed the iEMG and MPF of the vastus lateralis muscle to remain constant despite the amplitude of the slow component decreasing. These results have been replicated in the rectus femoris, vastus medialis and gastocnemius muscles (Tordi *et al.*, 2003). Poole and Jones (2005) make the observation that the equivocal findings of the above studies could indicate that the $\dot{V}O_2$ cost of force production increases during heavy exercise due to either elevated cytosolic $[Ca^2+]$ or Ca^2+ turnover rates, irrespective of whether additional type II fibres are recruited.

2.7.7.3 Exercise cessation

Less research has been conducted into the control of oxidative phosphorylation during recovery. The metabolic circumstances differ from those at the onset of exercise as the energy demand from exercising muscles has substantially diminished, although it is possible that during the initial stages of recovery muscles involved in respiratory and cardiac functions will still be highly active (Cunningham *et al.*, 2000). This reduction in the demand for ATP hydrolysis will be reflected by a fall in oxidative phosphorylation and hence $\dot{V}O_2$. During recovery, Rossitter *et al.* (2002) reported that the phase II kinetic responses of $\dot{V}O_2$ and PCr resynthesis were closely associated, adequately characterised by a mono-exponential model and independent of exercise intensity. This would suggest that phosphate linked controllers of respiration are active during recovery and demonstrate dynamic linearity across exercise intensities similar to that observed during the onset of exercise.

However, observed asymmetry between the on- and off-transient $\dot{V}O_2$ responses suggests that the control of $\dot{V}O_2$ is not linear between exercise and recovery, implying mechanisms of respiratory control are influenced by the exercise transient in which they are operating. An explanation for asymmetrical phase II $\dot{V}O_2$ responses is provided by the work of Kushmerick (1998), who demonstrated a potential for asymmetry in PCr and so $\dot{V}O_2$ between exercise transitions due to alterations in the CK equilibrium associated with changes in ATP utilisation. Hence the dynamic response of $\dot{V}O_2$ could retain first-order control between transients, but via a transfer function that is dependent on CK equilibrium (Rossitter *et al.*, 2002). Several alternative or contributing explanations for this apparent asymmetry have also been suggested: 1) an involvement of a component of obligatory anaerobisis arising from the dynamics of cardiac output (Yoshida and Whipp, 1994), 2) asymmetries of intramuscular pH (Rossitter *et al.*, 2002), 3) influences of asymmetrical Pi kinetics to respiratory control mechanisms (Bendahan *et al.*, 1990) 4) possibility that constant intensity exercise does not require a constant ATP supply (Bangsbo *et al.*, 2001).

The cause of the small but prolonged $\dot{V}O_2$ slow component and hence oxidative phosphorylation during recovery is unclear. One explanation stems from the hypothesis that the recruitment of type II fibres contributes to the slow component during exercise. In the off-transient, type II fibre recruitment would cease, and without delay mitochondrial oxygen utilisation would contribute to the restoration of PCr. This oxidative metabolism in glycolytic type II fibres will have a slow time course and so might be reflected as a prolonged elevation of $\dot{V}O_2$ during recovery. However, this explanation needs further investigation as simultaneous measurement of PCr resynthesis and slow component development during recovery has not been performed (Rossiter *et al.*, 2002). An alternative or contributing explanation is the metabolic cost of lactate

metabolism. If following heavy-intensity exercise lactate serves as a source of gluconeogenesis there will be an obligatory increase in oxygen consumption. Similarly, any lactate reducing equivalents transported into the mitochondria as an aerobic source that utilises the α-glycerophosphate shuttle rather than the malate-asparate shuttle would also incur an additional oxygen demand during recovery. It was demonstrated by Roth *et al.* (1988) that an additional oxygen cost from the metabolism of lactate might only be substantial when blood lactate exceeds 5 $mmol.l^{-1}$. Similarly, Whipp (1987) showed slow component magnitudes become appreciably larger above these blood lactate values. It has been stated by Gaesser and Brooks (1984) that the process of lactate metabolism and PCr resynthesis will impose only relatively low oxygen costs, which would fit the observation that the amplitude of the slow component during recovery is small. Other processes that might contribute to this prolonged metabolic cost include raised temperature, circulating catchecolamines and cost of increased ventilation and cardiac function during recovery.

2.7.8 Application of $\dot{V}O_2$ kinetics

The application of $\dot{V}O_2$ kinetics is wide reaching as it provides a non-invasive means to obtain information about cellular metabolism. Settings in which it is used range from the study of patients with cardiovascular and peripheral arterial disease (Bauer *et al.*, 1999; Hepple *et al.*, 1999; Brandenberg *et al.*, 1999) to identification of physiological processes that determine elite sports performance (Norris and Peterson, 1998; Demarle *et al.*, 2001).

2.7.8.1 The effects of training on $\dot{V}O_2$ kinetics

The influence of physical training on $\dot{V}O_2$ kinetics has been studied using various intensities and forms of exercise. Endurance training has been a particular area of

interest because of the adaptations it imposes on the aerobic energy system. Studies have reported speeded $\dot{V}O_2$ kinetic responses following endurance training to both on- (Hickson *et al.*, 1978; Ceretelli *et al.*, 1979; Berry and Moritani, 1985; Phillips *et al.*, 1995; Koppo and Bouckaert, 2004) and off- (Hagberg *et al.*, 1980; Phillips *et al.*, 1995) exercise transients.

Oxygen uptake kinetic responses are influenced by the intensity of endurance training. Berry and Moritani (1985) reported that five weeks of heavy-intensity interval training at 85 to 95% of maximum heart rate reserve (HR_{res}) speeded $\dot{V}O_2$ kinetics at the onset of moderate-intensity exercise to a greater extent than less intense continuous training at 65 to 75% HR_{res}. The total distance covered in each condition was the same. Unfortunately the authors did not report the effect the two different training regimes had on other physiological processes, which makes interpretation of the mechanisms responsible for the changes in $\dot{V}O_2$ kinetics difficult.

Endurance training has also been reported to influence $\dot{V}O_2$ kinetic responses to the onset of heavy-intensity exercise (Womack *et al.*, 1996). Carter *et al.* (2000b) reported that six weeks of endurance training reduced the slow component from 321 ± 32 $ml.min^{-1}$ to 217 ± 23 $ml.min^{-1}$ but had no effect on phase II $\dot{V}O_2$ kinetics. Using trained runners, Billat, (2002) reported that eight weeks of high-intensity aerobic training substantially reduced the slow component at the same absolute speed. Such an attenuation of the slow component will have occurred due to the running speed being of a lower intensity relative to the participant's increased aerobic fitness. In addition, if endurance training increases the oxidative potential of type II muscle fibres, then any role they play in the development of the slow component will be reduced. It has also been suggested that a smaller slow component amplitude might be associated with

reduced lactate production during high-intensity exercise following a period of endurance training. However Carter *et al.* (2000b) observed that training induced reductions in blood lactate following a heavy-intensity run were not associated with the decrease in the amplitude of the slow component. There is a scarcity in the research that has investigated the influence endurance training might have on the recovery slow component.

Training programmes that are more anaerobic in nature influence $\dot{V}O_2$ kinetics to a lesser extent. Fukuoka *et al.* (1997) reported that sprint training increased $\dot{V}O_2$ max but not the amplitude of the phase shift response of $\dot{V}O_2$ to a sinusoidal intensity forcing function. Sprint-trained athletes also have slower $\dot{V}O_2$ kinetics than endurance trained, although their $\dot{V}O_2$ max was found to be greater than that expected for a sedentary individual (Edwards *et al.*, 1999). These findings suggest that different mechanisms are involved in the control of $\dot{V}O_2$ kinetics and $\dot{V}O_2$ max. This is supported by Carter *et al.* (2000b) who reported no change in the phase II $\dot{V}O_2$ kinetics after six weeks of endurance training, despite significant increases in $\dot{V}O_2$ max and LT. Anaerobic-type training might be ineffective in augmenting $\dot{V}O_2$ kinetics as it does not improve the aerobic capability of muscle.

The time required to enhance $\dot{V}O_2$ kinetic responses through physical training can be small. Changes in $\dot{V}O_2$ kinetics have been observed after eight hours of endurance training (Yoshida *et al.*, 1992). Phillips *et al.* (1995) noted the mean response time (MRT) to be speeded after four days of aerobic cycling and was found to decrease as the training was extended to 30 days. So it appears $\dot{V}O_2$ kinetics become progressively faster as training is progressed.

2.7.8.2 Mechanisms of training induced adaptations

Several mechanisms have been proposed to explain why endurance training in particular speeds $\dot{V}O_2$ kinetic responses. Aerobic based endurance training increases the mitochondrial content of skeletal muscle, which might contribute to the speeding of $\dot{V}O_2$ kinetics. This would agree with Meyer's (1988) first-order model of respiratory control that suggests τ is a product of mitochondrial resistance (a function of the number and properties of the mitochondria). These explanations could account for the absence of speeded $\dot{V}O_2$ kinetics in sprint trained athletes (Fukuoka *et al.*, 1997; Edwards *et al.*, 1999), as the anaerobic based training they perform will not incur specific adaptations that will increase the aerobic potential of the muscle. A high percentage of type I fibres has also been associated with faster kinetics (Pringle *et al.*, 2003) as they are better equipped to use O_2 than type II fibres.

The role of central delivery mechanisms in the speeding of $\dot{V}O_2$ kinetic responses however should not be disregarded. Phillips *et al.* (1995) showed that a decrease in MRT to the onset and cessation of moderate square-wave cycling after only four days of endurance training was not matched by concomitant increases in the oxidative potential of muscles or $\dot{V}O_2$ max. This initial speeding after only limited training was attributed to an increase in femoral artery blood flow leading to accelerated O_2 transport to active muscle. However, after 30 days of training a further decrease in MRT was accompanied by a 50% increase in citrate synthase activity and hence muscle oxidative potential.

Although changes in oxidative enzymes were not observed in the early stages of training, this cannot exclude an O_2 utilisation mechanism as the reason for the speeding of $\dot{V}O_2$ kinetics. It is possible that other enzymes involved in oxidative phosphorylation

such as pyruvate dehydrogenase (PDH), which were not measured could contribute to the speeding of the $\dot{V}O_2$ kinetic response.

Alternatively, endurance training could result in a reduction in the diffusion distance of O_2. It has been shown that the capillary-to-fibre interface is matched to mitochondrial volume/fibre length with adaptation to training (Poole and Mathieu-Costello, 1996). This would enable O_2 to diffuse from circulating blood and into the mitochondria at a faster rate and contribute to a speeding of $\dot{V}O_2$ kinetics.

It is possible that endurance training brings about a series of central and peripheral adaptations that combine at different time points during a training programme to speed $\dot{V}O_2$ kinetic responses. After initial central adaptations, O_2 utilisation mechanisms appear to be responsible for the speeding of $\dot{V}O_2$ kinetics. If $\dot{V}O_2$ kinetics are related to performance, then it would be beneficial to determine the time course and contribution of central and peripheral mechanisms, as training could then be designed to optimise specific adaptations.

2.7.8.3 Application of $\dot{V}O_2$ kinetics to performance

It has been speculated that speeded $\dot{V}O_2$ kinetics at the onset of exercise might benefit the performance of endurance athletes as it will reduce the reliance on intramuscular energy stores and fatigue inducing anaerobic glycolysis (Poole and Richardson, 1997). The consequence of this behaviour will be a reduced disturbance to muscle homeostasis, particularly in the heavy-intensity exercise domain. Norris and Peterson (1998) reported that during eight weeks of endurance training, reductions in τ coincided with improvements in 40 km time trial performance. By week four of the training programme the reduction in τ was mirrored by increases in $\dot{V}O_2$ max, $\dot{V}O_2$ at GET and

power output at GET. However at week eight, further reductions in τ and 40 km performance time were observed but with no further increases in the other aerobic or performance indices. Demarle *et al.* (2001) made similar observations when they investigated the association between $\dot{V}O_2$ kinetics and running performance. In this study, eight weeks of endurance training increased run time to exhaustion without a concomitant increase in $\dot{V}O_2$ max. Instead the performance improvement was found to be associated with a reduced oxygen deficit as a result of speeded $\dot{V}O_2$ kinetics at exercise onset. Such observations are important as they indicate that $\dot{V}O_2$ kinetics measured at the onset of exercise: 1) play an important role in high-intensity endurance performance and 2) are more sensitive than $\dot{V}O_2$ max and GET to changes in physiological status and performance potential.

The role of $\dot{V}O_2$ kinetic responses during recovery from exercise has received little attention. This could be because the ability to recover in many endurance type sports is irrelevant due to the continuous nature of the event. However, in a prolonged intermittent sport such as soccer, an enhanced ability to recover between bouts of high-intensity exercise could markedly improve performance. Speeded kinetics in the off-transient could be representative of rapid PCr and muscle O_2 store restoration plus metabolism of lactate. Based on these assumptions, the role that $\dot{V}O_2$ kinetics might play during the performance of soccer-specific high-intensity exercise warrants investigation.

CHAPTER 3

METHODS

The following methods relate to the individual studies completed as part of this thesis. This chapter provides an in-depth explanation of: 1) the equipment used and its calibration; 2) the protocols used for the acquisition of anthropometric and physiological data; 3) data preparation and analysis techniques and 4) statistical analyses.

3.1 Equipment and calibration

The equipment used in this thesis can be separated into four distinct categories:

1) ergometry; 2) pulmonary gas analysis; 3) HR monitoring and 4) lactate analysis.

3.1.1 Ergometry

A motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany) was used to measure physiological responses to sub-maximal and maximal running speeds. The treadmill had a top speed of 40 $km.h^{-1}$ (11.11 $m.s^{-1}$), with 7 acceleration/deceleration steps (from 0 to 40 $km.h^{-1}$ in 3 to 131 s). The treadmill belt could be elevated between 0 and 25% (0 - 14°) by increments of 0.1%. Verification for treadmill speed and gradient are provided in appendix 2, page 232.

3.1.2 Pulmonary gas analysis

In the first three studies, pulmonary gas concentrations were measured by mass spectrometry (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA). However, the MGA 1100 became unusable and beyond repair following the third study. To allow the data collection for the thesis to be completed, breath-by-breath pulmonary gas concentrations in study four were measured using rapid-response

zirconian O_2 and infrared CO_2 analysers (CPX/D, Medgraphics, St Paul, MN, USA). Pilot investigations revealed that $\dot{V}O_2$ values measured at 50 W, 100 W, 150 W, 175W and $\dot{V}O_2$ max using the Medgraphics rapid response analysers did not differ ($P>0.05$) to those recorded by the respiratory mass spectrometer. Using the equation of Lamarra *et al.* (1987), it was also observed that the 95% CI for τ estimation were superior for the Medgraphics than the mass spectrometer. These data indicated that the Medgraphics system could be used to provide accurate measures of aerobic fitness and $\dot{V}O_2$ kinetics during the fourth study. Refer to appendix 3 page 234 for more detailed information.

3.1.2.1 MGA 1100 principle of operation

Inspiratory and expiratory gas volumes were determined from the breathing of room air through a low dead-space, low resistance turbine volume transducer (VMM 110, Alpha Technologies, Laguna Niguel, CA, USA). Inspiratory and expiratory gas concentrations were sampled by a capillary tube inserted into the flow volume apparatus. The volume transducer and sample line were both coupled to the MGA 1100 mass spectrometer. Gas concentration and volume signals were fed to a computer (PS325C, Tiko Computer Corporation, Broxburn, UK) via an analogue-to-digital converter, where they were integrated online using First Breath Software v2.0 (First Breath Inc., St Agatha, Ontario, Canada, 1992). Estimates of alveolar gas exchange were based on the algorithm of Beaver *et al.* (1981).

3.1.2.2 CPX/D principle of operation

Expiratory and inspiratory gas volumes were determined from the breathing of room air through a low dead space (20 ml) bi-directional differential pressure pneumotach (Medgraphics Corporation, St Paul, MN, USA). Expiratory gas concentrations were sampled by a capillary tube inserted into the pneumotach. The sample line was

connected to the zirconian O_2 and infrared CO_2 analysers and the pneumotach to a flow module. The flow volume and gas concentration signals were sent to a computer (Elonex PC 466/1, UK) via an analogue-to-digital converter for integration by Breeze3 software *v*1.0 (Medgraphics Corporation, St Paul, Mn, USA). The Medgraphics Corporation will not disclose the algorithm used by the Breeze3 software to estimate alveolar gas exchange.

3.1.2.3 Calibration of pulmonary gas analysis equipment

Both the MGA 1100 and CPX/D were calibrated immediately before and verified immediately after each exercise test in the same systematic order: 1) gas calibration, 2) volume calibration and 3) lag time calibration.

3.1.2.3.1 Gas Calibration

Calibration of the MGA 1100 was performed using two high tolerance (± 0.03%) gases (Medgraphics Corporation, St Paul, MN, USA) of fixed concentrations (Reference gas, 21% O_2, 0% CO_2, Bal N_2 and Calibration gas, 12% O_2, 5% CO_2, Bal N_2). A two-point calibration was performed, 21% and 12% for O_2 and 5% and 0% for CO_2. Gas was delivered to the analysers at a pressure of 15 PSI along the capillary sample line. A successful calibration resulted in measurement of the reference gas to within ± 0.03%. To verify the gas calibration, a pre-test check was completed using the reference gas. Calibration of the O_2 and CO_2 rapid response analysers of the CPX/D followed the same procedure as used for the MGA 1100.

3.1.2.3.2 Volume calibration

Irrespective of the method of pulmonary gas analysis, when making comparisons between tests carried out under different atmospheric conditions, it was necessary to

apply a correction factor to account for the effects of differences in ambient temperature, pressure and water vapour on measures of volume. Standard temperature and pressure dry (STPD) was used for all metabolic measurements and was calculated as a dry gas at a temperature of 273 K and a pressure of 760 mmHg. When referring to a physiological measure such as $\dot{V}$E, body temperature and pressure saturated (BTPS) was used, where temperature was 310 K and pressure was ambient and saturated with water vapour.

The gas volume turbine of the MGA 1100 (VMM 110, Alpha Technologies, Laguna Niguel, CA, USA) was calibrated using a precision 3 l syringe (Hans Rudolph Inc, Kansas City, MO, USA) to pump room air at a rate representative of human ventilation during exercise (~ 2 $l.s^{-1}$) through the turbine The accuracy of the turbine volume determination was deemed suitable if the mean of five inspiratory and expiratory volumes was within ± 1% (30 ml) of 3 l.

The bi-directional differential pressure pneumotach (Medgraphics Corporation, St Paul, MN, USA) of the CPX/D was calibrated with the same precision 3 l syringe (Hans Rudolph USA). Following the manufacturer's guidelines, room air was pumped through the pneumotach at five inspiration and expiration flow rates ranging from 0.5 to 6 $l.s^{-1}$. The calibration was successful if the inspiration and expiration volumes for each flow rate were within ± 1% of 3 l.

3.1.2.3.3 Lag-Time Calibration

Measures of gas volume are provided almost instantaneously, whereas the measurement of fractional gas concentrations is delayed by the transport of expired gases from the mouthpiece to the gas analysis system and the response of the gas analysis system to a

change in gas concentration. Integration of these two signals via a lag time calibration is necessary for the accurate determination of $\dot{V}O_2$ and $\dot{V}CO_2$.

The system lag-time of the MGA 1100 was determined by exhaling through the assembled breathing apparatus at a constant rate and then inhaling rapidly. An algorithm (First Breath software *v*2.0, First Breath Inc, St Agatha, Ontario, Canada, 1992) was then applied to calculate the lag time between the signal change at the start of inspiration detected by the volume turbine and the time taken by the MGA 1100 to measure the exponential increase (τ) in end tidal CO_2. The time lag was found stable at ~300 ms for each exercise test.

Lag-time calibration for the CPX/D was performed autonomously by the Breeze3 software *v*1.0 (Medgraphics Corporation, St Paul, Mn, USA). Using the reference and calibration gases described in section 3.2.1.2.3, the software recorded the time for the rapid response analysers to measure near square-wave changes in O_2 (21% to 12%) and CO_2 (0% to 5%). The calibration was successful if the time taken for the analysers to measure these changes in O_2 and CO_2 concentrations was within 0.1 to 0.6 s. The Breeze3 software then aligned the response time of the analysers with that of the pneumotach.

3.1.2.4 Estimation of pulmonary gas exchange

The First Breath software of the MGA 1100 employed the algorithms described by Beaver *et al.* (1981) to estimate pulmonary gas exchange. The values for gas exchange measured at the mouth are corrected for changes in lung gas stores which are dependent on changes in alveolar gas concentration and functional residual capacity change. To

provide a measure of alveolar gas exchange these corrections are then applied to total lung gas exchange that is obtained by subtracting expired from inspired gas quantities.

The CPX/D only measures expired gas concentrations, which prevents the Breeze3 software from using the algorithm of Beaver (1981) described above. Unfortunately, the Medgraphics Corporation will not disclose the actual algorithm used by the Breeze3 software to estimate pulmonary gas exchange from the CPX/D. Therefore this section of the methods chapter is limited due to factors beyond the researcher's control.

.3.1.3 Heart rate monitoring

During all exercise tests, HR was continuously recorded every 5 s using a short-range telemetric HR monitor (Accurex Plus, Polar Electro Oy, Kempele, Finland). An electrode belt worn around the chest measured the time between each R-R interval of the heart's sinus rhythm. This information was telemetrically transmitted to a receiver and displayed in $b.min^{-1}$. Previous research (Leger and Thivierge, 1988) has demonstrated that the accuracy of Polar HR monitors is comparable to that of electrocardiograms (ECG).

3.1.4 Blood lactate analysis

Blood lactate was determined from a single sample of whole capillary blood taken at the fingertip. The skin of the fingertip was punctured using a lancet (Soft Clix Pro, Roche, Sussex, UK). Whole capillary blood was then drawn into a 25 µl sample tube (YSI Inc, Yellow Springs, OH, US) via capillary action. The sample was immediately analysed using an automated lactate analyser (1500 Sport, YSI Inc, Yellow Springs, OH, USA) that uses immobilised enzyme electrode technology. A thin film of lactate enzyme is immobilised within a membrane. Hydrogen peroxide is produced when the lactate in the injected blood sample diffuses through the membrane. The hydrogen peroxide measured

at a platinum electrode is proportional to the lactate in the sample. The measurement range of the YSI 1500 sport is 0 to 30 mmol.l^{-1}, with a precision of 2% of the reading or 0.1 mmol.l^{-1}, whichever is larger.

3.1.4.1 Calibration of the lactate analyser

The lactate analyser was calibrated before each exercise test and every 10 samples thereafter using 25 µl of a 5 mmol.l^{-1} lactate standard (YSI Inc, Yellow Springs, OH, USA). Calibration was deemed acceptable if values were within ± 0.25 mmol.l^{-1} (5%) of the 5 mmol.l^{-1} standard. If the value for the calibration check was outside this range then the calibration procedure was repeated. Calibration was verified by injecting 25µl of a lactate standard that would have a similar concentration to that of subsequent blood samples (Table 3.1). Reproducibility data for the YSI 1500 sport is shown in appendix 5 page 237.

Table 3.1 Lactate standard concentrations used to verify the calibration of the lactate analyser.

Physical activity	**Lactate standard concentration (mmol.l^{-1})**
Rest / moderate exercise	2.5
Heavy exercise	5
Maximal exercise	10

3.2 Exercise testing procedures and protocols

3.2.1 Pre-exercise test procedures

3.2.1.1 Ethics

Prior to each study, ethics approval was sought and granted by the School of Sport and Leisure Management Research Ethics Committee, Sheffield Hallam University, in accordance with the declaration of Helsinki.

3.2.1.2 Informed consent

Before each investigation, participants were given clear and concise information explaining the purpose, procedures and requirements of the study. Any questions the participants had about the study were answered verbally on a one-to-one basis. Examples of the informed consent forms are provided in appendix 7 page 240.

3.2.1.3 Pre-exercise screening

All participants were required to complete a pre-exercise medical questionnaire (appendix 6, page 238) to screen for previous and/or current medical conditions or musculo-skeletal injuries.

3.2.1.4 Pre-test instructions / requirements

Prior to the undertaking of any laboratory or field based exercise test, participants were instructed to: 1) be in a 3 hour post absorptive state, 2) have maintained normal dietary intake 3) not consumed alcohol or caffeine in the 12 hours preceding the test and 4) abstained from strenuous physical activity in the 48 hours preceding the exercise test.

3.2.2 Stature

Stature was measured using a wall mounted stadiometer (Holtain, Crymych Dyfed, UK). In bare feet, participants were required to stand with heels, buttocks and shoulder blades touching the back board of the stadiometer, while their head was positioned in the Frankfort plane. Once in the correct position, participants were instructed to inhale fully while light pressure was applied to the mandibles. Stature was then recorded to the nearest mm.

3.2.3 Body mass

Body mass was recorded using a beam balance type scale (Weylux, UK) that incremented in 0.05 kg. Male participants were required to wear only shorts and female participants shorts and T-shirt.

3.2.4 Incremental exercise test

A continuous and maximal incremental exercise test was performed to volitional exhaustion on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany) for the determination of $\dot{V}O_2$ max and GET via breath-by-breath analysis of pulmonary gas exchange. Prior to the test, all participants were accustomed to running on a motorised treadmill, the pulmonary gas exchange and HR apparatus. It was the aim of the incremental exercise test to elicit $\dot{V}O_2$ max within 8 to 12 min. Participants performed a 5 min warm-up at a running speed that elicited a HR of approximately 150 $b.min^{-1}$. The initial running speed for the incremental exercise test was 8 $km.h^{-1}$ and was increased by 1 $km.h^{-1}$ every min until volitional exhaustion. On cessation of the test, treadmill speed was reduced to 4 $km.h^{-1}$ for 5 min to allow the participant an active recovery. Run time to exhaustion was noted (s).

3.2.4.1 Determination of $\dot{V}O_2$ max

Breath-by-breath pulmonary gas exchange data collected during the incremental exercise test were averaged on a 30 s basis. The highest $\dot{V}O_2$ attained during the incremental exercise test was taken as $\dot{V}O_2$ max if a plateau in the $\dot{V}O_2$ / exercise intensity relationship was evident, or $\dot{V}O_2$ increased by no more than 2 $ml.kg^{-1}.min^{-1}$ with a further increase in exercise intensity (BASES, 1997). If a plateau was not present, $\dot{V}O_2$ max was only reported to have been achieved if two of the following secondary criteria (BASES, 1997) were observed: 1) HR within ± 10 beats of age predicted maximum HR (220 – age); 2) a plateau in HR ($\pm$ 2 $b.min^{-1}$) with an increase in exercise intensity; 3) a respiratory exchange ratio (RER) $\geq$ 1.15.

3.2.4.2 Determination of GET

The GET was determined from the breath-by-breath pulmonary gas exchange data collected during the incremental exercise test. Using the V-slope method (Beaver, 1986), $\dot{V}O_2$ was plotted against $\dot{V}CO_2$. The data were visually inspected and GET was taken as the transition in the relationship between $\dot{V}O_2$ and $\dot{V}CO_2$ caused by the buffering of lactic acid by HCO_3. When the transition point was difficult to discern, additional analysis of the pulmonary gas exchange data was performed (ventilatory equivalent, Whipp *et al.*, 1981) to aid the identification of GET. This involved the construction of individual graphs: $\dot{V}E/\dot{V}O_2$, $\dot{V}E/\dot{V}CO_2$, end tidal PO_2 ($PETO_2$) and end tidal PCO_2 ($PETCO_2$) against $\dot{V}O_2$. Gas exchange threshold was identified as the nadir of the $\dot{V}E/\dot{V}O_2$ and $PETO_2$ graphs before they began to increase consistently without a concomitant increase in $\dot{V}E/\dot{V}CO_2$ or a decrease in $PETCO_2$.

3.2.4.3 Determination of moderate- and heavy-intensity running speeds

The running speed / $\dot{V}O_2$ relationship generated from the incremental exercise test was used to identify the speeds that would elicit $\dot{V}O_2$ values corresponding to moderate- (80% of GET), heavy- (halfway between GET and $\dot{V}O_2$ max, 50%Δ) and very heavy- (80% of the way between GET and $\dot{V}O_2$ max, 80%Δ) intensity exercise. To take into account the time required for $\dot{Q}O_2$ to meet the metabolic demand of an increase in exercise intensity and for this increase in $\dot{Q}O_2$ to be measured at the lungs, a specific $\dot{V}O_2$ value (e.g. 80%GET) was elicited by selecting the preceding running speed from the incremental exercise test.

3.2.4.3.1 Verification of intensity domain specific running speeds

A treadmill protocol was devised to verify how successfully running speeds that elicit $\dot{V}O_2$ values corresponding to different intensity domains could be identified from the running speed / $\dot{V}O_2$ relationship of the incremental exercise test. Blood lactate was measured before a 5 min warm-up on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany) at 8 $km.h^{-1}$. Pulmonary gas exchange was then recorded on a breath-by-breath basis while participants ran for 6 min at a speed estimated to elicit a $\dot{V}O_2$ response that corresponded to 80% GET. On completion of the run, blood lactate was measured and a 5 min cool down was performed. Following a further 5 min of passive recovery the procedure was repeated, with the running speed increased to one estimated to elicit a $\dot{V}O_2$ response that corresponded to 50%Δ. Following the test, participants were allowed to cool down for at least 5 min at a self selected running speed.

3.2.5 Repeated square-wave transition treadmill protocols

3.2.5.1 Moderate- and heavy-intensity protocol

A square-wave treadmill protocol was devised for the measurement of $\dot{V}O_2$ kinetics at the onset and cessation of moderate- and heavy-intensity exercise. On arrival to the laboratory, participant's resting $\dot{V}O_2$, HR and blood lactate were recorded after being seated for 5-min. Before the protocol, participants performed a 5-min warm-up on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany HP) at 8 $km.h^{-1}$. The protocol was continuous and consisted of 3, 6 min runs at a speed corresponding to 80%GET and a 6 min run at a speed corresponding to 50%Δ (Figure 3.1). Each run was preceded by a 6 min walk at 4 $km.h^{-1}$. Following the 50%Δ run, participants walked for a further 12 min at 4 $km.h^{-1}$. The time taken to change between running speeds ($\leq$ 1.5 s) allowed for near square-wave transitions in exercise intensity. A blood lactate measurement was taken immediately after the 50%Δ run while breath-by-breath pulmonary gas exchange was measured continuously. After 30 min of passive recovery, resting measures of $\dot{V}O_2$, HR and blood lactate were taken to verify that participants' metabolism had returned to pre-exercise levels (Bernard *et al.*, 1998). The protocol was then repeated to provide 6 moderate and 2 heavy exercise transitions in total. During the recovery period, the accuracy of the pulmonary gas analysis equipment was verified, and if necessary recalibration was performed.

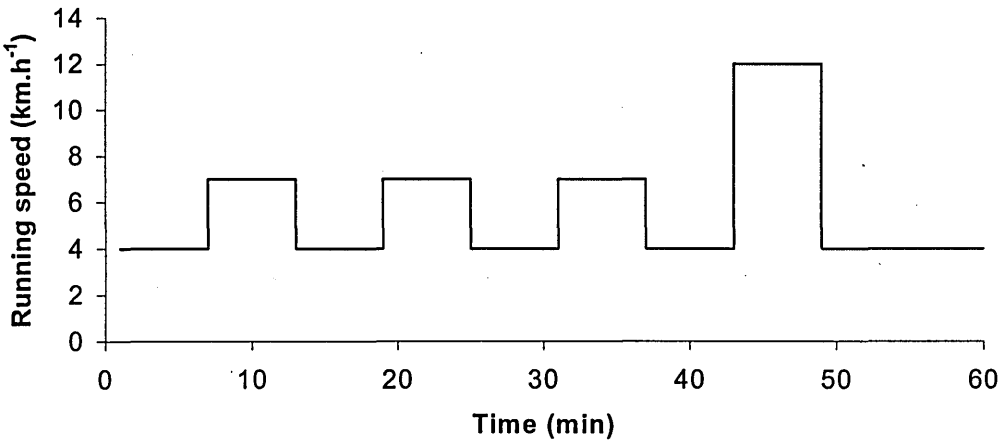


Figure 3.1 Graphical representation of the square-wave treadmill protocol used to measure $\dot{V}O_2$ kinetic responses to the onset and cessation of moderate- and heavy-intensity exercise. The protocol was repeated following a 30 min recovery period to provide six moderate- and two heavy-intensity running transitions in total.

3.2.5.2 Very heavy-intensity protocol

Participants performed a 5 min warm up on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany HP) at 8 $km.h^{-1}$. The protocol was continuous and involved walking for 2 min at 4 $km.h^{-1}$ followed by a square-wave transition to a speed that elicited a $\dot{V}O_2$ response corresponding to 80%Δ for 6 min. On completion of the run, blood lactate was measured and treadmill speed was reduced to 4 $km.h^{-1}$ for a further 12 min (Figure 3.2). Pulmonary gas exchange was measured on a breath-by-breath basis throughout the protocol. Following 30 min of passive recovery, blood lactate and HR were recorded to ensure the participants' metabolism had returned to pre-exercise levels. This procedure was repeated a further 3 times, providing 4 transitions of an 80%Δ run. In between each repetition the accuracy of the pulmonary gas analysis equipment were verified, and if necessary recalibrated.

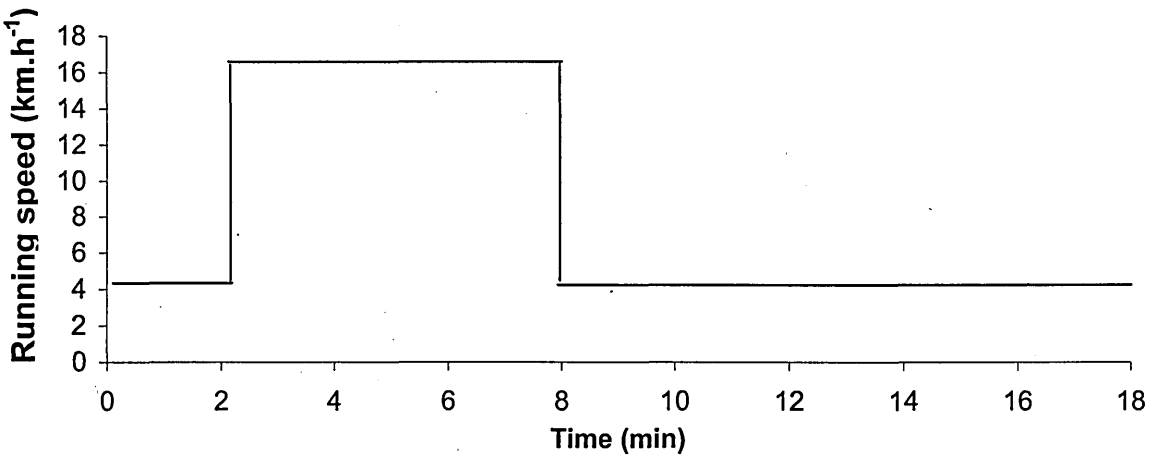


Figure 3.2 Graphical representation of the square-wave treadmill protocol for the measurement of $\dot{V}O_2$ kinetics at the onset and cessation of very heavy-intensity running. The protocol was performed four times, each separated by a 30 min recovery period.

3.2.5.3 Data Analysis

The $\dot{V}O_2$ responses generated from the intermittent running protocol were characterised by either a single or double exponential model, which used a non-linear least-squares fitting procedure (Excel, Microsoft, USA). The first 20 s (phase I) were removed from the fitting field for both the on- and off-transients. Although the duration of phase I is likely to be less in recovery, as blood flow is higher in the off-transient than the on, little is known about this duration and so 20 s was deemed a suitable time period to eliminate any influence of phase I on subsequent kinetics (Özyener *et al.*, 2001).

For the on $\dot{V}O_2$ kinetic transient the following models were used (Özyener *et al.*, 2001):

Moderate-intensity exercise: $\Delta\dot{V}O_{2(t)} = A_1 (1\text{-}e^{-(t-\delta_1)/\tau_1})$ (7)

Heavy-intensity exercise: $\Delta\dot{V}O_{2(t)} = A_1 (1\text{-}e^{-(t-\delta_1)/\tau_1}) + A_2(1\text{-}e^{-(t-\delta_2)/\tau_2})$ (8)

where $\Delta\dot{V}O_{2(t)}$ is the change in $\dot{V}O_2$ above base line, t is the time after the onset of exercise, and τ, δ and A are the associated time constant, time delay and amplitude terms. A two component model was used for heavy exercise so that the fast and slow components could be characterised.

For the off-transient:

Moderate exercise: $\Delta\dot{V}O_{2(t)} = A_1 e^{-(t-\delta_1)/\tau_1}$ (9)

Heavy and very heavy exercise: $\Delta\dot{V}O_{2(t)} = (A_1 e^{-(t-\delta_1)/\tau_1}) + (A_2 e^{-(t-\delta_1)/\tau_2})$ (10)

In the case of equation 10, the fundamental and slow components were constrained to begin at the same time delay, it being logical to assume that these were both in operation at the start of recovery (Özyener *et al.*, 2001)

The amplitude of the slow component was calculated as the difference between the asymptotic $\dot{V}O_2$ values of phase II and phase III. A rigid time frame for the identification of the slow component was not used because mathematical modelling has shown that the onset of the slow component varies among individuals (Bearden and Moffatt, 2001).

3.2.5.4 Filtering of the oxygen uptake response to reduce noise

Prior to data analysis, spurious (non physiologic) $\dot{V}O_2$ values were removed from the data set. The method used to identify non physiologic $\dot{V}O_2$ values was that outlined by Claxton (2000), where mean breath-to-breath change in $\dot{V}O_2$ is first calculated. Any breath-to-breath variation greater than 3 standard deviations of the mean breath-to-breath difference was removed.

3.2.5.5 Calculation of 95% Confidence intervals

The variability inherent in breath-by-breath measures of pulmonary gas exchange can influence the accuracy of $\dot{V}O_2$ kinetic measures calculated from exponential modelling techniques. For the estimation of τ, Lamarra *et al.* (1987) proposed two equations to determine the 95% confidence intervals (95% CI) for an individual's $\dot{V}O_2$ kinetic response. The accuracy of the non-linear least squares estimation of τ is directly proportional to the SD of the noise (S_o). This allows *apriori* determination to be made of the number of transitions required to achieve a desired 95% CI in the estimated parameter for a given participant. The 95% CI for the τ on was calculated as follows:

$$K_1 = \hat{L} \frac{S_o}{\Delta Y_{ss}} \qquad (11)$$

Where K_1 is the CI, S_o is the SD of the noise, ΔY_{ss} is the amplitude of $\dot{V}O_2$ above baseline and $\hat{L}$ is a constant, as described in Lamarra *et al.* (1987). For the superposition of n independent transitions, the effective noise variance is reduced by a factor of n if the noise is assumed to be Gaussian and uncorrelated between transitions. Hence, the confidence interval (K_n) is reduced by the factor of $\sqrt{n}$. The number of transitions (n) required for a desired 95% CI (K_n) is given by:

$$n = \left[\frac{\hat{L} \cdot S_o}{K_n \cdot \Delta Y_{ss}} \right]^2 \quad (12)$$

3.2.5.6 Calculation of the mean sum of squares

To assess how closely the mono- and double-exponential models fitted the $\dot{V}O_2$ data, the mean sum of squares was calculated for the last 100 breaths of the $\dot{V}O_2$ response for each phase during the moderate- and heavy/very heavy-intensity exercise transitions as follows:

$$\frac{\text{Breath 1 } ((\text{Model-}\dot{V}O_2)^2) + \text{Breath 2 } ((\text{Model-}\dot{V}O_2)^2) + \ldots\ldots \text{Breath 100 } ((\text{Model-}\dot{V}O_2)^2)}{100} \quad (13)$$

3.2.5.7 Calculation of oxygen deficit

The oxygen deficit (DO_2) for the phase II and phase III $\dot{V}O_2$ responses were calculated separately (Demarle *et al.*, 2001) and then combined to provide a measure of the total DO_2 deficit incurred during the 80%Δ run:

$$\text{Phase II } DO_2 = (A_1 \times TD_1) + (A_1 \times \tau_1) \quad (14)$$

$$\text{Phase III } DO_2 = (A_2 \times TD_2) + (A_2 \times \tau_2) \quad (15)$$

$$\text{Total } DO_2 = ((A_1 \times TD_1) + (A_1 \times \tau_1)) + ((A_2 \times TD_2) + (A_2 \times \tau_2)) \quad (16)$$

3.2.6 Maximal Anaerobic Running Test (MART)

The MART (Maxwell and Nimmo, 1996) was performed on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany HP) to obtain an indirect estimation of anaerobic capacity. All participants were accustomed to running at high speeds on a motorised treadmill while wearing a safety harness. The pre-test warm-up consisted of 4 min at 8 $km.h^{-1}$ interspersed by 30s at 14.3 $km.h^{-1}$. The MART is a discontinuous incremental protocol in which participants perform a series of 20 s runs separated by 100 s of passive recovery. The gradient of the treadmill was kept at 10.5% and the initial running speed was 14.3 $km.h^{-1}$. For each subsequent run the speed was increased by 1.2 $km.h^{-1}$ until volitional exhaustion. Holding onto the handrails of the treadmill was permitted while stepping onto the treadmill belt but the 20 s count did not start until the participant was running unsupported. The measure provided by the test was anaerobic power expressed in ml O_2. $kg^{-1}.min^{-1}$ and calculated using the following equation (ACSM, 1986):

$$\dot{V}O_2 = 3.5 + 12v + 54gv \qquad (17)$$

where v is the highest treadmill speed in $m.s^{-1}$ that could be performed for 20 s, g is the gradient expressed as a fraction and the value 3.5 represents resting $\dot{V}O_2$. If participants became exhausted and stopped 10 s into a 20 s running bout, 1 $ml.kg^{-1}.min^{-1}$ was added to the anaerobic power value calculated from the above equation (13). Each additional 2 s completed in the 20 s bout thereafter accounted for another 1 $ml.kg^{-1}.min^{-1}$ being added to the calculated anaerobic power value. At the end of the protocol blood lactate was measured and a 5 min cool down at a self selected speed was performed.

3.2.7 Yo Yo Intermittent Recovery Test Level 2 (YIRT2)

The YIRT2 (Bangsbo, 1996) is an incremental test of high-intensity intermittent running capacity. The course of the test is presented below in Figure 3.3. All participants were accustomed to the course and practised it during a 5 min warm-up. After a 5 s countdown the test began; participants were required to perform a shuttle that consisted of running back and forth between markers A and B, adjusting their running speed so that they reached each marker in time with an audible signal generated from a cassette tape. On returning to marker A, participants had 10 s to jog around marker C and back to marker A, before the next audible signal sounded and the shuttle between markers A and B was repeated. This intermittent running pattern remained constant throughout the test. The running speed and number of shuttles run for each of the 12 incrementing levels of the YIRT2 is depicted below in Figure 3.4. Participants were stopped when they could not maintain the running speed between markers A and B dictated by the audible signals. The first time a marker was not reached a warning was given and the second time the participant was withdrawn from the test. The performance measure provided by the test was distance run (m).

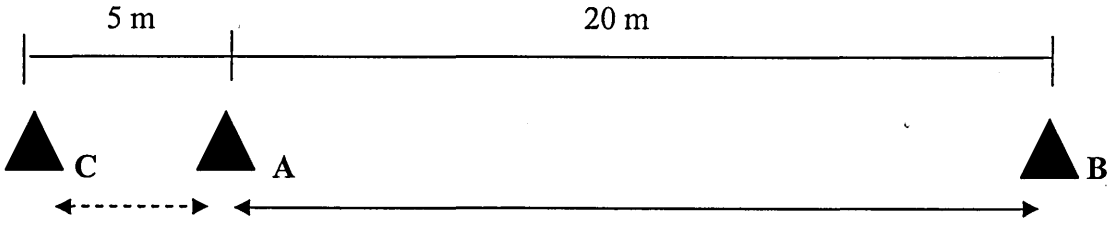


Figure 3.3 The course layout for the YoYo Intermittent recovery test.

A 60 s interval on the cassette tape was timed before each test to verify the speed of the cassette player (X-670, Sony, Japan). The speed of the cassette player was deemed acceptable if the 60 s interval was within ± 1 s. A pilot investigation found the distance

run in the YIRT2 to be reproducible on a test-retest basis, refer to appendix 4 page 236 for detailed information.

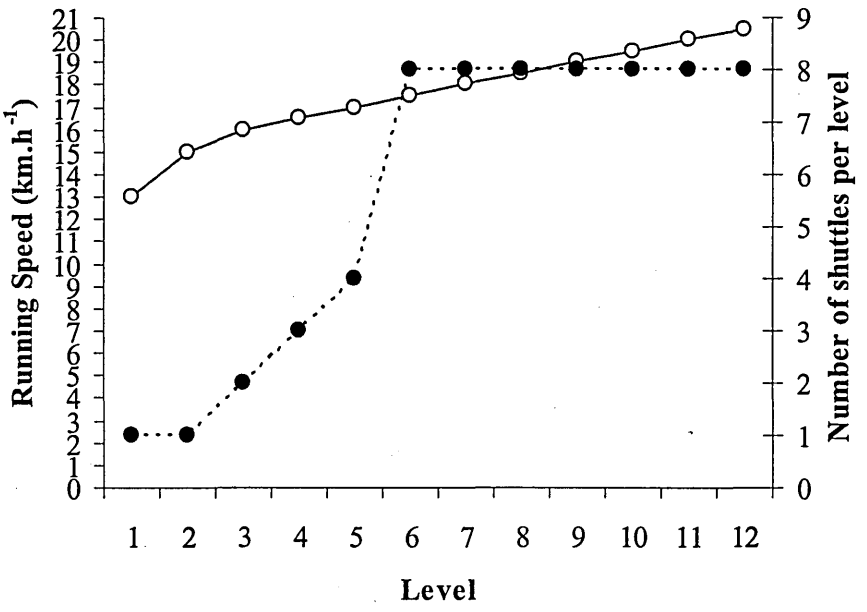


Figure 3.4 Running speed o— and number of shuttles •--- for each level of the YIRT2.

3.2.8 Repeated Sprint Test (RST)

A RST (Bangsbo, 1994) was used to determine a participant's ability to perform repeated bouts of maximal intensity exercise. The 30 m course used for the RST is depicted below in Figure 3.5. Prior to the RST participants were accustomed to the course and practised it during a 5 min warm-up. The RST involved sprinting from point A to point B, performing a 5 m deviation to the left after 10 m. On completing the sprint participants had 25 s to jog back to point A. This procedure was performed 7 times. Photo-electric timing gates (Brower Timing Systems, Salt Lake City, Utah, USA) were positioned at points A and B to record the time taken for the sprint. The 25 s jog was recorded using a manual stop watch (W-42H, Casio, China). The recorded sprint times

were used to identify the quickest sprint and to calculate the mean time of the 7 sprints. Fatigue index for the test was also calculated by subtracting the slowest from the quickest sprint.

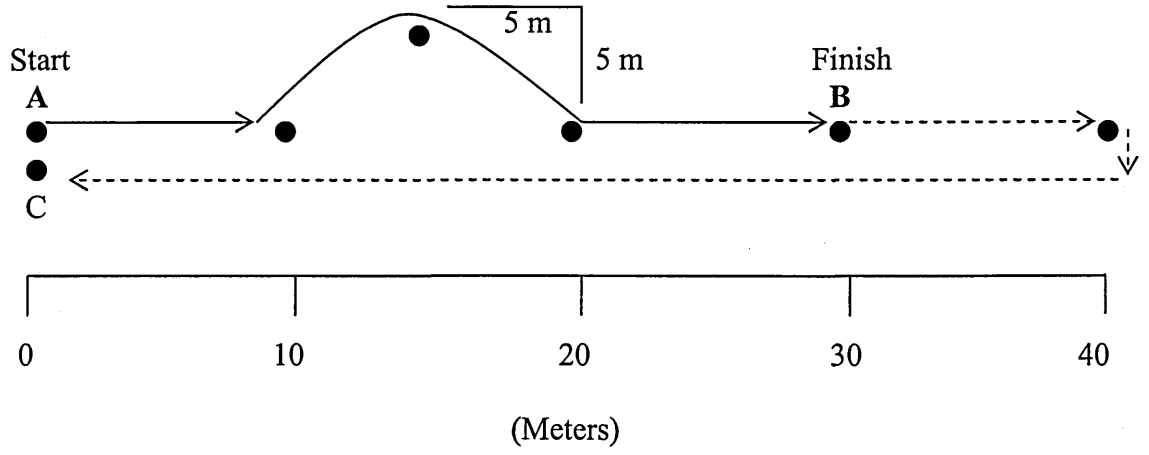


Figure 3.5 Representation of the running course used in the RST. Solid and dashed lines indicate where the participants must sprint and jog respectively.

3.3 Statistical analyses

Various methods of statistical analysis have been used in this thesis to determine: 1) the reproducibility for certain physiological measures; 2) the strength of association between measures of performance and physiological function and 3) the difference between the various measures. All the statistical tests were performed using commercially available statistical software (SPSS for Windows v11.0; SPSS Inc., Chicago, IL., USA).

3.3.1 Limits of agreement

A popular assessment of test-retest reproducibility is the 95% limits of agreement (LOA) first described by Bland and Altman (1986). The LOA is used to determine the

difference between two measures (measurement errors) using simple calculations and graphical techniques. The major assumption of LOA is that the difference (error) between measures is homoscedastic. That is, the differences are of the same magnitude regardless of the magnitude of the measure. A simple check for homeoscedasticity can be obtained from a scatter plot of the differences between the two tests against the grand mean of the two tests. If a relationship is visually detected between the two variables, confirmation can be achieved by calculating the correlation coefficient between the absolute differences between the two tests and the grand mean. If it is found that larger errors are associated with larger measurement means, the measurement error is heteroscedastic, which requires logarithms of each measurement be performed before LOA can be applied (Atkinson and Nevill, 1998). It has been demonstrated that heteroscedastic errors are common among measurements in sport and exercise science recorded on a ratio scale (Atkinson and Nevill, 1998). However, provided that the previously stated assumption has been checked and the differences are homeoscedastic, the LOA can be calculated without the need for logarithmic transformation, as:

$$\pm 95\% \text{ LOA} = 1.96 \times SD_{diff} \quad (18)$$

Where SD_{diff} is the SD of the differences between test 1 and test 2. The LOA in proportion to the grand mean of test 1 and 2 is calculated as:

$$\text{Measurement error (\%)} = \frac{1.96 \times SD_{diff}}{\text{Grand mean}} \times 100 \quad (19)$$

Where grand mean is (mean of test 1 + mean of test 2)/2.

Whether the calculated LOA reflect a reproducible measure is a subjective decision that must be made by the researcher. An advantage of using LOA to assess reproducibility is that when the error is homeoscedastic, the calculated values are in the original units of measurement, hence allowing direct application to the measure being assessed.

3.3.2 Coefficient of variation

An alternative and more traditionally used measure of reproducibility is the coefficient of variation (CV). This statistical test was performed to allow greater cross study comparisons than would be permitted by using LOA alone. The CV (%) was calculated as follows:

$$CV\ (\%) = \frac{SD}{\overline{X}} \times 100 \qquad (20)$$

Where SD is the standard deviation and $\overline{X}$ is the mean.

3.3.3 Method Error

The calculation of CV requires several measures to be taken either on the same day or over several days. This is not practical in the study of $\dot{V}O_2$ kinetics where exercise tests can involve maximal efforts or be long in duration. It is conceivable that performing such exercise over several occasions could have a training effect on the physiological or performance variable being measured. A suitable alternative to CV is the calculation of the Method Error (ME). It has been demonstrated that the difference between two measures would have a mean of zero and that the SD_{diff} would be equal to the SD of an individual (SD_{indiv}) divided by the square root of two (Gore, 2000). As infinite measures on infinite participants is impossible, Dahberg (1940) proposed the use of duplicate

measures on a group of participants to approximate the standard error of a single determination (cf. Gore, 2000). This has been termed method error and is calculated as:

$$\text{Method error (ME)} = \frac{SD_{diff}}{\sqrt{2}} \quad (21)$$

3.3.4 t-tests

To assess whether the mean of two samples differed, t-tests were performed. If the two means were generated from the same participant sample under different experimental conditions a paired sample t-test was used. When the two means came from two different sample groups an independent t-test was used. For the t-test to be used the following assumptions outlined by Vincent (1995) were checked: 1) participants are randomly sampled; 2) data are normally distributed and parametric and 3) there is homogeneity of variance (i.e. the variance between groups is equal), assessed using Levene's test for equal variances. Providing the test result is non-significant, the variances can be assumed to be homogeneous.

3.3.5 Analysis of variance

Analysis of variance (ANOVA) is a set of tests used to identify if 3 or more means differ (Kinnear and Gray, 2000). The type of ANOVA used depends upon whether the experiment has a within (repeated measures from one sample group), between (individual measures from several sample groups) or mixed (a combination of within and between measures) design. It has been suggested that it is preferable to run an ANOVA rather than multiple t-tests because: 1) multiple t-tests increase the chance of type I error (probability of falsely rejecting the null hypothesis); 2) separate tests do not combine all of the available information about the population, and might lead to additional errors of inference and 3) provides more informative results i.e. the

interaction term. In addition to checking the standard assumptions that must be met to perform a parametric test, the sphericity of the data was also checked. This refers to whether there is compound symmetry of homogeneity of covariance (i.e. correlations between all groups are similar) and homogeneity of variance (i.e. variances of all groups are similar). Sphericity of the data was assessed using Maulchly's test of sphericity, where sphericity could be assumed if $P>0.05$. Levene's test of homogeneity of variance was also performed.

3.3.6 Bivariate correlations

According to Vincent (1995), correlation is a numerical coefficient that indicates the extent to which two variables are related or associated. Theoretically this can range in either a negative or positive direction from zero, no relationship, to 1, a perfect relationship. The association between physiological and performance measures in this thesis was assessed using Pearson's product moment correlation coefficient (r). The assumptions that were assessed prior to the test being performed were: 1) visual inspection of the association between the two variables using a scatter plot; 2) the data were parametric and 3) the data were normally distributed.

CHAPTER 4

The reproducibility and identification of aerobic markers used to set exercise intensities in the study of $\dot{V}O_2$ kinetics

4.1 Introduction

The characteristics of $\dot{V}O_2$ kinetics are dependent upon the intensity domain in which exercise is performed (Whipp and Wasserman, 1972; Linnarsson, 1974; Whipp *et al.*, 1982). In the moderate-intensity domain, which encompasses all intensities below the AT, constant rate exercise causes $\dot{V}O_2$ to increase exponentially to a steady-state, usually within 3 min (Mahler, 1980). The speed of this primary $\dot{V}O_2$ response appears to be invariant of exercise intensity within the moderate-intensity domain (Barstow and Mole, 1991).

In contrast, constant rate exercise in the heavy-intensity domain, which comprises exercise intensities that lie between an individual's AT and critical power (the asymptote of the power-duration curve for high-intensity exercise, Jones and Poole, 2005), leads to the primary $\dot{V}O_2$ response being complicated by the development of an additional and delayed O_2 cost termed the slow component. This additional energy cost causes $\dot{V}O_2$ to rise above the predicted value to either a deferred attainment of steady-state or exhaustion (Whipp, 1994). There is also evidence to suggest the primary $\dot{V}O_2$ response is slowed in the heavy-intensity domain (Paterson and Whipp, 1991; Koga *et al.,* 1999; Carter *et al.*, 2002), although there is a lack of consensus among the available literature to support this statement (Barstow and Mole, 1991; Barstow *et al.*, 1996).

In an attempt to ensure the desired $\dot{V}O_2$ kinetic response is derived from a square-wave exercise transition, aerobic markers have been used to define the different intensity

domains. Several investigations (Barstow *et al.*, 1994; Jones and McConnell, 1999; Carter *et al.*, 2000) have used 80%AT (80% of AT) and 50%Δ (mid-point between AT and $\dot{V}O_2$) to denote moderate- and heavy-intensity exercise respectively.

Although 80%AT and 50%Δ are commonly used within the study of $\dot{V}O_2$ kinetics as indicators of exercise intensity domains, there seems to be little published data on the reproducibility of the two measures. There has, however, been research into the reproducibility of $\dot{V}O_2$ max and GET from which 80%AT and 50%Δ are derived. Repeated measures have been performed using different forms of ergometry as well as participants of differing age and fitness. Coefficients of variation for $\dot{V}O_2$ max and GET have been reported to range from 4.4% to 16% and 5.5% to 31% respectively (Meyer *et al.*, 1997; Baba *et al.*, 1999; Skinner *et al.*, 1999). Such observations suggest that both measures can be highly variable on a day-to-day basis, which could influence how accurately 80%AT and 50%Δ can be identified. The consequence of which would be an increased chance of incursion into an undesired intensity domain and the measurement of inappropriate $\dot{V}O_2$ responses. However, a major limitation of these investigations is that CV does not necessarily assess both fixed and proportional biases and the possible interaction that may occur between the two (Mullineaux *et al.*, 1999). This can lead to an incorrect conclusion about the reproducibility of a measure.

The exercise intensities that elicit $\dot{V}O_2$ values corresponding to 80%AT and 50%Δ are typically identified from the $\dot{V}O_2$ / exercise intensity relationship obtained from an incremental exercise test to exhaustion. A recent approach has been to measure pulmonary gas exchange during a series of incrementing four min sub-maximal stages (Burnley *et al.*, 2000; Carter *et al.*, 2000b; Carter *et al.*, 2002), where blood lactate is

measured at the end of each stage for the identification of LT and hence AT. Once the criteria for LT is met, exercise intensity is increased every min until volitional exhaustion for a measure of $\dot{V}O_2$ max. An advantage of this method is that the four min exercise bouts permit a steady-state $\dot{V}O_2$ / exercise intensity relationship to be established, which will allow for accurate identification of moderate exercise intensities (Carter *et al.*, 2000a).

Such a protocol however can be both invasive and time consuming. An alternative approach is to measure pulmonary gas exchange during a ramp type incremental protocol (Barstow *et al.*, 1994; Barstow *et al.*, 1996), where exercise intensity is increased every min until exhaustion. From such a test, GET can be used as the indicator of AT for the calculation of moderate- and heavy-intensity exercise domains. However, such a protocol will not allow steady-state $\dot{V}O_2$ responses to be achieved in the moderate-intensity domain due to the rapid increments in exercise intensity. The consequence of which is that the $\dot{V}O_2$ at a given exercise intensity will be an underestimation of the energy demand for that exercise (Whipp, 1987). A method for establishing an appropriate $\dot{V}O_2$ / exercise intensity relationship from a quickly incrementing exercise test needs to be established if such a protocol is to be used in future studies of this thesis. A possible solution could be provided from the τ of $\dot{V}O_2$ responses to changes in exercise intensity. For example, if the phase II τ for recreationally active individuals at the onset of exercise is taken to be ~ 30 s, then it could be predicted that after 60 s (~ 2 x τ), 86% of the $\dot{V}O_2$ response is attained (Whipp, 1987; Jones and Poole, 2005). Therefore, during a quickly incrementing exercise test where exercise intensity increases every 60 s, it is conceivable that a given $\dot{V}O_2$ value will be largely attributable to the increment in exercise intensity that

occurred 60 s earlier. Subsequent use of this exercise intensity should not lead to $\dot{V}O_2$ exceeding the predicted $\dot{V}O_2$ value for the moderate-intensity domain.

4.1.1 Aims

1. To assess the reproducibility of $\dot{V}O_2$ max, GET, 80%GET and 50%Δ.

2. To determine if the running speeds intended to elicit specific $\dot{V}O_2$ responses can be accurately determined from a ramp-type incremental exercise test.

4.2 Participants and Methods

4.2.1 Participants

With institutional ethics approval, nine participants (8 male, 1 female) mean ± SD: age 24.4 ± 4.1 years, stature 176.6 ± 9.2 cm, body mass 67.8 ± 4.5 kg took part. All participants were healthy and performed physical activity on a regular basis. Prior to the administration of any test, participants were screened for existing medical conditions that might become aggravated during the testing procedure (appendix 6, page 238). Pre-test instructions can be seen in chapter 3.2.1.4.

4.2.2 Experimental design

Participants performed three laboratory based physiological assessments over a period of two weeks. Two incremental tests to exhaustion were performed in week one, five days apart. Continuous sub-maximal treadmill running was performed once in week two, at speeds identified from the first incremental exercise test to elicit $\dot{V}O_2$ responses corresponding to 80%GET and 50%Δ. On each visit to the laboratory participants' stature and body mass were measured and heart rate was recorded at 5 s intervals during each assessment. All assessments were performed at the same time of day to reduce the effects of diurnal variation and the temperature of the laboratory was kept within 20°C ± 1°C.

4.2.3 Experimental protocols

All exercise tests were performed on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany). Pulmonary gas exchange (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA) was measured on a breath-by-breath basis during an incremental exercise test to volitional exhaustion for the identification of $\dot{V}O_2$ values and running speeds that corresponded to $\dot{V}O_2$ max,

GET, 80%GET and 50%Δ (see chapter 3.2.4). To verify whether the specific running speeds identified from the GXT elicited $\dot{V}O_2$ values corresponding to 80%GET and 50%Δ, a sub-maximal running protocol was performed (see chapter 3.2.4.3.1). In summary, this consisted of a 6 min run at a speed calculated to elicit a $\dot{V}O_2$ value corresponding to 80%GET (see chapter 3.2.4.3). Participants were then allowed 10 min of rest before performing a 6 min run at a speed calculated to elicit a $\dot{V}O_2$ corresponding to 50%Δ (see chapter 3.2.4.3). Pulmonary gas exchange was measured during both 6 min runs on a breath-by-breath basis (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA). Blood lactate was measured at rest and immediately after the 80%GET and 50%Δ runs.

4.2.4 Data analysis

Breath-by-breath pulmonary gas exchange data collected during the incremental exercise tests and sub-maximal running protocol were analysed following the procedures outlined in chapter 3.2.4.1 and 3.2.5.3 respectively.

4.2.5 Statistical analyses

To identify whether a difference exists between the test-retest measurements a paired sample t-test was performed. Statistical significance was set at $P \leq 0.05$. To assess the reproducibility of the repeated measures LOA (95% confidence interval) calculations were performed. Coefficients of variation were also calculated so that results could be compared with those of previous investigations.

4.3 Results

4.3.1 Incremental exercise test performance

The maximum speed attained in the first incremental exercise test was 17.8 ± 1.8 km.h^{-1}, which did not differ (P=0.242) to the 17.6 ± 1.6 km.h^{-1} achieved in test 2. Consequently, time to exhaustion in tests 1 (8.79 ± 0.82 min) and 2 (8.74 ± 0.7 4min) did not differ (P=0.273).

4.3.2 Reproducibility of aerobic markers of exercise intensity

Paired sample t-tests revealed no differences to exist between test-retest values (Table 4.1) for any of the aerobic markers ($\dot{V}O_2$ max P=0.271; GET P=0.214; 80%GET P=0.296; 50%Δ P=0.232) measured from the graded exercise test to exhaustion.

Table 4.1 Mean (± SD) test-retest values of the aerobic markers (n = 9).

Measure	Test 1	Test 2
$\dot{V}O_2$ max (ml.kg.min^{-1})	51.5 ± 4.2	52.1 ± 4.9
GET (ml.kg^{-1}.min^{-1})	32.9 ± 3.5	31.9 ± 4.7
80%GET (ml.kg^{-1}.min^{-1})	24.3 ± 2.0	25.5 ± 3.2
50%Δ (ml.kg^{-1}.min^{-1})	41.6 ± 3.6	41.9 ± 3.6

The measures of reproducibility summarised in Table 4.2 indicate that the day-today variability in the aerobic markers of exercise intensity is low. Method error and CV for all measures did not exceed 5%. The largest measurement errors obtained from the LOA calculations was 12.3% and 11.9% for GET and 80%GET respectively, while the measurement errors for $\dot{V}O_2$ max and 50%Δ were lower at 5.5% and 6.7%.

Table 4.2 The 95% LOA, method error and CV for the aerobic measures determined from the two incremental exercise test ($n = 9$).

Measure	Mean of test 1 and 2	Mean ± s difference (x 1.96)	95% LOA	Measurement error (%)	Method error	CV (%)
VO_2 max ($ml.kg.min^{-1}$)	52.3 ± 4.7	-0.12 ± 2.9	-3.0 to 2.81	5.5	3.5	1.0
GET ($ml.kg^{-1}.min^{-1}$)	32.4 ± 4.1	0.04 ± 3.9	-3.86 to 3.94	12.3	1.0	4.5
80%GET ($ml.kg^{-1}.min^{-1}$)	24.9 ± 2.6	-0.04 ± 1.6	-1.65 to 1.57	11.9	0.8	4.4
50%Δ ($ml.kg^{-1}.min^{-1}$)	41.8 ± 3.6	-0.03 ± 2.9	-2.93 to 2.87	6.7	0.7	3.6

4.3.3 Prediction and measurement of 80%GET and 50%Δ

During the sub-maximal treadmill protocol, a paired sample t-test demonstrated that running at the calculated 80%GET speed of 9.5 ± 1.5 $km.h^{-1}$ produced a $\dot{V}O_2$ of 26.4 ± 3.2 $ml.kg^{-1}.min^{-1}$, which was not different (P=0.194) from the predicted 80%GET value of 24.3 ± 2.0 $ml.kg^{-1}.min^{-1}$. Similarly, the calculated 50%Δ speed of 14.5 ± 2.0 $km.h^{-1}$ produced a $\dot{V}O_2$ of 41.6 ± 3.6 $ml.kg^{-1}.min^{-1}$, which did not differ (P= 0.257) from the predicted 50%Δ $\dot{V}O_2$ of 42.2 ± 3.6 $ml.kg^{-1}.min^{-1}$.

The blood lactate values measured after the 80%GET and 50%Δ running speeds were different (P=0.002), with means of 1.39 ± 0.21 $mmol.l^{-1}$ and 2.26 ± 0.54 $mmol.l^{-1}$ respectively. A mean slow component value of 246 ± 97 $ml.min^{-1}$ was produced during the 50%Δ running speeds, which would indicate the run took place in the heavy-intensity domain.

4.4 Discussion

4.4.1 Reproducibility of aerobic markers of exercise intensity

The results suggest that the four aerobic measures determined from the incremental exercise test are reproducible. The $\dot{V}O_2$ max was the most reproducible, indicated by the small CV (1%) and narrow LOA (-0.12 ± 2.9 $ml.kg^{-1}.min^{-1}$). The lower reproducibility observed for GET, demonstrated by a greater CV (4.5%) and wider LOA (0.04 ± 3.9 $ml.kg^{-1}.min^{-1}$) is similar to that reported previously (Meyer *et al.*, 1997; Baba *et al.*, 1999; Skinner *et al.*, 1999). Repeated identification of the specific changes in pulmonary gas exchange behaviour that correspond to GET is possibly impaired by the large amount of noise (Lamara *et al.*, 1987) inherent in breath-by-breath measurements of pulmonary gas-exchange. The lower level of reproducibility for GET could be problematic as it is widely used in the study of $\dot{V}O_2$ kinetics to provide a reference point for the setting of exercise intensities. A solution could be to average the breath-by-breath data to reduce the impact of the noise on the underlying signal. However, averaging a $\dot{V}O_2$ response can artificially delay the occurrence of GET (Whipp, 1987), which would lead to the setting of inappropriate exercise intensities. Despite the observations that GET is less reproducible than $\dot{V}O_2$ max, the variability in GET identification in this study using unaveraged data is such (LOA, -3.86 to 3.94 $ml.kg^{-1}.min^{-1}$) that the chance of exercise being performed in the wrong intensity domain is small (see Figure 4.1).

As 80%GET and 50%Δ were derived from the identification of GET, they also had low test-retest CV (80%GET, 4.4%; 50%Δ, 3.6%) and narrow LOA (80%GET, -0.04 ± 1.61 $ml.kg^{-1}.min^{-1}$: 50%Δ, -0.03 ± 2.9 $ml.kg^{-1}.min^{-1}$). The calculations show that 50%Δ is more reproducible than 80%GET. A reason for the discrepancy in the reproducibility of the two measures is that 80%GET is calculated solely from GET, whereas 50%Δ is

calculated from both GET and the less variable measure of $\dot{V}O_2$ max. A second explanation is that the corresponding running speed for 80%GET (9.5 ± 1.5 km.h^{-1}) was too slow in some cases for the participants to perform a natural running style, which could have inhibited the standardisation of the activity. Conversely, the 50%Δ run (14.5 ± 2.0 km.h^{-1}) was at a higher speed that allowed participants to perform a more consistent and natural stride pattern.

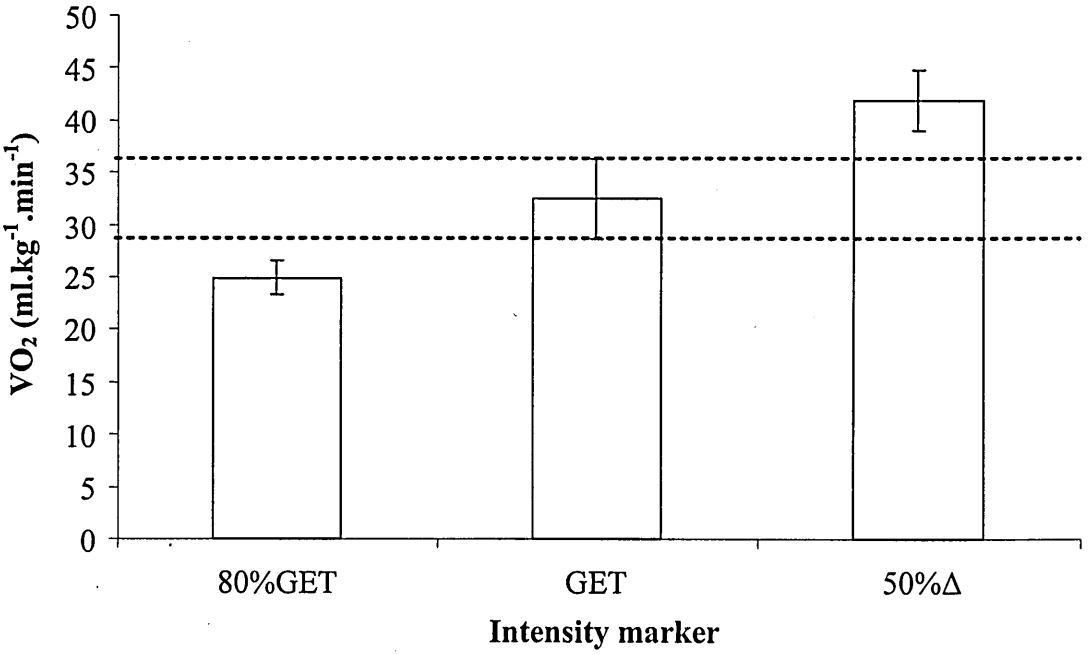


Figure 4.1 The mean 80%GET, GET and 50%Δ values from the two incremental exercise tests (n=9), incorporating the LOA ± 95% spread of differences (I). The dashed lines denote the upper and lower 95% spread of differences for GET, marking the boundaries of the heavy- and moderate-intensity domains.

4.4.2 Identification of moderate- and heavy-intensity running speeds

Running speeds intended to elicit $\dot{V}O_2$ values corresponding to 80%GET and 50%Δ were successfully identified from the speed / $\dot{V}O_2$ relationsip established from the incremental exercise test. The predicted $\dot{V}O_2$ value of 24.3 ± 2.0 ml.kg^{-1}.min^{-1} for 80%

GET did not differ from the actual $\dot{V}O_2$ value of 26.4 ± 3.2 $ml.kg^{-1}.min^{-1}$. The same was found during the 50%Δ run (42.2 ± 3.6 vs. 41.8 ± 3.7 $ml.kg.min^{-1}$). The blood lactate measured after the 80%GET (1.39 ± 0.21 $mmol.l^{-1}$) and 50%Δ (2.26 ± 0.54 $mmol.l^{-1}$) runs fell below and above 2 $mmol.l^{-1}$ respectively, which is of significance as 2 $mmol.l^{-1}$ has previously been used an arbitrary marker of AT (MacDougall *et al.*, 1979). Further analysis also revealed that a slow component response of 246 ± 97 $ml.min^{-1}$ was evident during the 50%Δ. Such data indicates that aerobic markers of exercise intensity can be accurately determined from the incremental exercise test used in this investigation if a 60 s time delay (2 x ~τ) is incorporated into the $\dot{V}O_2$ / exercise intensity relationship. This would suggest that prolonged and more invasive protocols are not required to reliably and accurately calculate aerobic makers of exercise intensity such as 80%GET and 50%Δ.

4.5 Conclusion

The LOA calculations indicated that 80%GET and 50%Δ are reproducible and can be used to set exercise intensities, for at least a week after an incremental test. Over this time period the variability is such that moderate-intensity exercise would be approximately within 68 to 92% GET, and heavy-intensity exercise within 44 to 57%Δ. These data suggest that the possibility of incursion into undesired exercise intensity domains is small. The $\dot{V}O_2$ and blood lactate measurements taken during the 80%GET and 50%Δ runs confirmed that appropriate exercise intensities can be identified from a quickly incrementing exercise test.

CHAPTER 5

The development of a treadmill protocol for the measurement of $\dot{V}O_2$ kinetics during the on- and off- transients of moderate- and heavy-intensity running

5.1 Introduction

The characteristics of $\dot{V}O_2$ kinetic responses during the on- and off-transients of moderate- and heavy-intensity exercise are unclear. Several moderate-intensity cycling based studies (Linnarson, 1974; Whipp *et al.*, 1982; Paterson and Whipp, 1991; Özyener *et al.*, 2001) have reported that phase II τ and A are symmetrical between the on- and off-transients. Conversely, other investigations have noted phase II τ to be slower during the off- than the on-transient of moderate-intensity cycling (Hughson *et al.*, 1988; Carter *et al.*, 2000a; Rossiter *et al.*, 2002) and running (Carter *et al.*, 2000b; Kilding *et al.*, 2003).

In the heavy-intensity domain, Barstow *et al.* (1994) observed symmetry to exist between the on and off $\dot{V}O_2$ kinetic responses for cycling and stated that the primary and slow components represented distinct metabolic processes that retained their distinction in recovery. The findings of several other investigations however contradict this statement (Henry and DeMoor, 1974; Engelen *et al.*, 1996; Cunningham *et al.*, 2000), as they have shown the off- $\dot{V}O_2$ transient of heavy-intensity cycling to have a much longer τ and smaller A than the corresponding on-transient. This would suggest off-transient kinetics are independent of the metabolic profile during the preceding exercise (Cunningham *et al.*, 2000). However, in the literature, information about the characteristics of $\dot{V}O_2$ responses during both the on- and off-transient of heavy-intensity treadmill running is limited. This is surprising, as knowledge of how quickly a person's aerobic system can recover from moderate- and heavy-intensity runs might

provide a useful insight into what limits performance during intermittent sports such as soccer, where athletes are repetitively required to respond and recover from bouts of exercise differing in intensity.

Considering that $\dot{V}O_2$ kinetics could have such important implications for sports performance, there are relatively few studies that have investigated the reproducibility of $\dot{V}O_2$ kinetic parameters at the onset and cessation of different exercise intensities. The majority of reproducibility studies that have been conducted have concentrated on moderate-intensity cycling. Berg (1947) reported the reproducibility of $\dot{V}O_2$ kinetics responses to the cessation of single transitions of moderate-intensity cycling performed on consecutive days. The test-retest correlation was 0.55 and the standard error of the measurement was ± 4.5 s, which equated to 15% of the mean. Berry and Moritani (1985) later reported the test-retest reproducibility for the time course of $\dot{V}O_2$ during the onset of moderate-intensity cycling. The test-retest values were correlated (0.87; $P<0.01$), with a mean difference of only 0.73 s, suggesting the level of reproducibility was satisfactory. In a more recent study (Puente-Maestu *et al.*, 2001), patients with chronic obstructive pulmonary disease (COPD) performed two repetitions of cycling at 80%LT or 50% of $\dot{V}O_2$ peak if LT was insufficiently differentiable. Test-retest correlation coefficients higher than 0.97 were reported for τ_1 and A_1. The statistical techniques employed in these studies lack the ability to assess fixed and proportional bias as well as only measuring error in one measurement. Interestingly, when the reproducibility of test-retest $\dot{V}O_2$ kinetic parameters has been assessed via LOA (95% confidence), large measurement errors and substantial intra-participant variability between tests has been observed (Kilding *et al.*, 2003), indicating phase II kinetic parameters are poorly reproducible.

Studies into the reproducibility of $\dot{V}O_2$ kinetic responses to heavy-intensity exercise are extremely limited. Özyener *et al.* (2001) assessed the reproducibility of on- and off-transient kinetics for both sub and supra GET exercise (moderate, heavy, very heavy and severe), τ was found to typically vary by up to 10% across all intensity domains, with greater variation for τ and A being observed for the on- than the off-transients.

As it is intended that future studies of this thesis will assess the relationship between $\dot{V}O_2$ kinetics and soccer-specific exercise capability, the lack of information regarding the characteristics and reproducibility of $\dot{V}O_2$ kinetics responses to the on- and off-transients of moderate- and heavy-intensity treadmill running must be addressed. To do this it will be necessary to devise a specific square-wave treadmill protocol. A single protocol that consists of sufficient moderate and heavy exercise transitions to allow confident parameter estimation would be advantageous, as it would reduce the number of times a participant was required to visit the laboratory. This would make the study of $\dot{V}O_2$ kinetics less time consuming and hence more practical. The use of such a protocol in previous investigations (Carter *et al.*, 2000a) has tended to involve the performance of several moderate-intensity exercise transitions followed by a single heavy exercise transition, with each transition being separated by ~6 min. Following a recovery period the procedure is often repeated to increase the number of transitions for each intensity in an attempt to improve the accuracy of parameter estimation.

When performing such repeated square-wave exercise transitions, the influence prior exercise might have on subsequent $\dot{V}O_2$ responses must be taken into consideration. Previous cycle based research (Gerbino *et al.*, 1996; Burnley *et al.*, 2000; Koppo and Bouckaert, 2001) has demonstrated a prior bout of heavy-intensity exercise reduces the amplitude of the slow component during a subsequent bout of heavy-intensity exercise. This reduction in the amplitude of the slow component speeds the overall $\dot{V}O_2$

response, although the speed of phase II kinetics remains unchanged. Later Burnley (2001) also noted that prior heavy exercise increases the phase II amplitude during a second heavy exercise transition. In relation to the design of a repeated square-wave transition protocol, such findings suggest sufficient time must be allowed between repeated bouts of heavy-intensity exercise to prevent distortion of the $\dot{V}O_2$ response during the second heavy-intensity transition. Otherwise, the ensemble average of the two heavy-intensity transitions could lead to inaccurate estimates of phase III parameters. However, the time required for the reversal of the physiological adaptations that are associated with a reduction in the slow component has not been clearly defined.

5.1.1 Aims

1. To develop a protocol for the measurement of $\dot{V}O_2$ kinetics at the onset and cessation of moderate- and heavy-intensity treadmill running.

2. To identify the influence prior bouts of moderate and heavy-intensity exercise have on $\dot{V}O_2$ kinetic parameters during a repeated square-wave treadmill protocol.

3. To determine the reproducibility of $\dot{V}O_2$ kinetics measured at the onset and cessation of moderate- and heavy-intensity treadmill running.

4. To determine $\dot{V}O_2$ kinetic characteristics measured at the onset and cessation of moderate- and heavy-intensity treadmill running.

5.2 Participants and Methods

5.2.1 Participants

With institutional ethics approval eight males (mean ± SD): age 23.5 ± 1.3 years, body mass 77.1 ± 12.2 kg, stature 179.9 ± 7.5 cm) took part. All participants were healthy and performed physical activity on a regular basis. Prior to the administration of any test, participants were screened for existing medical conditions that might become aggravated during the testing procedure (appendix 6, page 238). Pre-test instructions can be seen in chapter 3.2.1.4.

5.2.2 Experimental design

Participants performed three laboratory based physiological assessments, each separated by three days. The first assessment was an incremental exercise test to exhaustion, on the other two occasions participants performed a repeated square-wave transition treadmill protocol. On each visit to the laboratory the participants' stature and body mass were measured and heart rate was recorded at 5 s intervals during each assessment. All assessments were performed at the same time of day to reduce the effects of diurnal variation and the temperature of the laboratory was kept within 20°C ± 1°C.

5.2.3 Experimental protocols

All exercise tests were performed on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany). Pulmonary gas exchange (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA) was measured on a breath-by-breath basis during an incremental exercise test to volitional exhaustion for the identification of $\dot{V}O_2$ values and running speeds that corresponded to $\dot{V}O_2$ max, GET, 80%GET and 50%Δ (see chapter 3.2.4). These running speeds were then used to

design a repeated exercise transition treadmill protocol that consisted of 3, 6 min moderate-intensity runs and 1, 6 min heavy-intensity run (part A). Following a 30 min recovery period the protocol was repeated (part B), providing 6 moderate-intensity and 2 heavy-intensity square-wave transitions in total (see chapter 3.2.5.1). Pulmonary gas exchange was measured through out the test (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA) to determine $\dot{V}O_2$ kinetics during the onset and cessation of moderate- and heavy-intensity treadmill running. Blood lactate was measured before parts A and B of the protocol (see chapter 3.2.5.1). The protocol was performed three and six days after the incremental exercise test to generate test-retest measures of the $\dot{V}O_2$ kinetic responses.

5.2.4 Data Analysis

Breath-by-breath pulmonary gas exchange data collected during the incremental exercise tests and repeated square-wave transition protocol were analysed following the procedures outlined in chapter 3.2.4.1 and 3.2.5.3 respectively.

5.2.5 Statistical Analyses

Statistical significance was set at $P \leq 0.05$. The reproducibility of the test-retest $\dot{V}O_2$ kinetic measures generated from the intermittent treadmill protocol was assessed using method error, CV and LOA (95% confidence). To determine if test-retest data differed, two-way analysis of variance with repeated measures was performed. Paired sample t-tests were used to detect for any differences in physiological measures taken at rest before parts A and B of the intermittent treadmill protocol.

To determine if a repeated square-wave transition protocol could be used to provide accurate measures of $\dot{V}O_2$ kinetics, the influence of previous moderate- and heavy-

inensity exercise on $\dot{V}O_2$ kinetics and physiological status was assessed. To check this the following analysis was performed:

1. The $\dot{V}O_2$ data collected for the three moderate-intensity transitions (1-3) from part A of test 1 was ensemble averaged with the $\dot{V}O_2$ data collected during the corresponding transitions from part A of test 2 (retest). This procedure was repeated for the test-retest moderate-intensity transitions (4-6) from part B of the protocol. The two sets of $\dot{V}O_2$ data were then characterised by a single exponential model. The heavy-intensity transitions from parts A and B of test 1 were also ensemble averaged with their corresponding retest transitions and characterised by a double exponential model. Using paired sample t-tests it was then possible to see if the $\dot{V}O_2$ kinetic parameters measured during the on- and off-transients of moderate- and heavy-intensity exercise differed between parts A and B of the protocol. The test-retest transitions were combined to increase confidence in parameter estimation by reducing noise in the $\dot{V}O_2$ response.

2. To check the participant's $\dot{V}O_2$ had returned to baseline following a moderate-intensity exercise transition before the next exercise transition commenced, transitions 1-3 from part A and 4-6 from part B of test 1 were separated and analysed individually. This analysis required determining the actual $\dot{V}O_2$ for the 2 min period preceding each 80%GET transition. One-way analysis of variation with repeated measures was then performed to reveal whether the $\dot{V}O_2$ before transition 1 (baseline $\dot{V}O_2$) differed from the $\dot{V}O_2$ preceding any other transition during the protocol.

5.3 Results

5.3.1 Incremental exercise test performance

The mean (± SD) $\dot{V}O_2$ max was 51.3 ± 3.1 ml.kg^{-1}.min^{-1}, or expressed in absolute terms was 3955 ± 285 ml.min^{-1}. The GET occurred at 37.4 ± 3.7 ml.kg.min^{-1} (74.3 ± 3.4 % of $\dot{V}O_2$ max) or 2884 ± 131 ml.min^{-1}. The mean running speed at $\dot{V}O_2$ max was 17.5 ± 1.2 km.h^{-1}, with time to exhaustion being recorded at 11.34 ± 1.1 min. Maximal HR was 194 ± 7 b.min^{-1}.

5.3.2 Influence of repeated square-wave exercise transitions on $\dot{V}O_2$ kinetics

Paired sample t-tests revealed τ_1 (Onset, P=0.297: Cessation, P=0.311) and A_1 (Onset, P=0.156: Cessation, P= 0.183) values derived from parts A and B of the multi square-wave protocol (Table 5.1) to not differ for either exercise transient. Such data suggests that phase II $\dot{V}O_2$ kinetic parameters during the moderate exercise transitions in part B of the protocol are unaffected by the preceding bouts of moderate- or heavy-intensity exercise in part A. A comparison of the phase III parameters (Table 5.1) revealed that τ_2 and A_2 were slightly faster and smaller respectively during the on-transient of part B, but the difference was not significant (τ_2, P=0.192; A_2, P=0.231). However, analysis did reveal τ_2 and A_2 to be larger durng the off-transient (τ_2, P=0.023: A_2, P=0.028) for part B of the protocol. The blood lactate measured immediately before parts A (1.23 ± 0.18 mmol.l^{-1}) and part B (1.18 ± 0.12 mmol.l^{-1}) of the protocol did not differ (P=0.141). In addition, the mean resting $\dot{V}O_2$ (Part A, 446 ± 122 ml.min^{-1}; Part B, 443 ± 104 ml.min^{-1}) and HR (Part A, 59 ± 4 b.min^{-1}; Part B, 60 ± 3 b.min^{-1}) values were not different before parts A and B of the protocol ($\dot{V}O_2$, P=0.162; HR, P=0.125). The blood lactate concentrations measured immediately after the heavy-intensty runs were not different (P=0.253) between parts A (3.22 ± 0.34 mmol.l^{-1}) and B (3.16 ± 0.26 mmol.l^{-1}). A one-way analysis of variance with repeated measures showed that there

was no difference in $\dot{V}O_2$ before transition 1 and any other transition (P=0.083). This suggests $\dot{V}O_2$ had returned to its pre-exercise baseline after each bout of 80%GET exercise.

Table 5.1 The $\dot{V}O_2$ kinetic measures (mean ± SD) calculated for parts A and B of the multi square-wave treadmill protocol (n = 8).

Measure	Part A	Part B
τ_{1on} 80%GET (s)	23.2 ± 1.7	23.8 ± 1.5
τ_{1off} 80%GET (s)	28.1 ± 2.4	27.9 ± 1.8
τ_{1on} 50%Δ (s)	23.6 ± 1.1	23.9 ± 1.3
τ_{1off} 50%Δ (s)	27.3 ± 1.2	27.6 ± 1.8
A_{1on} 80%GET (ml.min^{-1})	852 ± 39	861 ± 46
A_{1off} 80%GET (ml.min^{-1})	811 ± 47	823 ± 37
A_{1on} 50%Δ (ml.min^{-1})	2073 ± 212	2043 ± 205
A_{1off} 50%Δ (ml.min^{-1})	2106 ± 241	2127 ± 216
τ_{2on} (s)	176.3 ± 29.4	164.7 ± 35.8
τ_{2off} (s)	394.6 ± 54.3	424.2 ± 48.7*
A_{2on} (ml.min^{-1})	283 ± 27	277 ± 32
A_{2off} (ml.min^{-1})	123 ± 48	146 ± 56*

* Differences (P<0.05) between parts A and B of the protocol.

5.3.3 Reproducibility of $\dot{V}O_2$ kinetics

5.3.3.1 Time constants

Two-way ANOVA with repeated measures revealed no differences or interaction (P>0.05) to exist between any of the test-retest on- or off-transient kinetic measures for both moderate- and heavy-intensity exercise (see appendix 10.1, page 244). All the test-

retest measures of τ_1 demonstrated low levels of variability, with method error not exceeding ± 1.6 s and CV remaining under 6% (Table 5.2). In addition, narrow LOA were observed for all τ_1 measures, with τ_1 for test 1 being slightly longer than for test 2 for all exercise conditions, indicating a small positive fixed bias (Table 5.3). The τ_2 was found to be more variable during the off- than on-transient of exercise, revealed by both method error (Off, ± 2.1 s v On, ± 1.2 s) and CV (Off, 6.3% v On, 4.4%). This was further reflected by broader LOA during the off- (-7.8 ± 90.2 s) than on-transient (-5.8 ± 37.3 s), which indicate a negative bias of τ_2 being quicker during test 1 than 2. In proportion to the grand mean, the measurement error for τ_2 during the off-transient (24%) was similar to that for the on-transient (21%).

5.3.3.2 Amplitudes

During all exercise conditions, the test-retest variability for A_1 was low, with the method error not exceeding ± 26 ml.min^{-1} (Table 5.2) and the CV values being 2.1% or less. The LOA analysis revealed small positive and negative biases between test-retest measures of A_1 for the on- (33 ± 43 ml.min-^{1}) and off-transients (–16 ± 37 ml.min^{-1}) of 80%GET exercise respectively. In contrast, the direction of the bias was reversed for A_1 measured during the on (–16 ± 179 ml.min^{-1}) and off-transients (58 ± 139 ml.min^{-1}) of 50%Δ running. The measurement error of the grand mean was lower for 80%GET (On, 4.9%; Off, 3.8%) than 50%Δ (On, 8.8%; Off 6.4%) measures of A_1.

The method error for A_2 during exercise (± 24.7 ml.min^{-1}) and recovery (± 16.1 ml.min^{-1}) over the two tests was similar to that reported for A_1. However the CV for A_2 was greater, calculated at 8.4% and 13.3% for the onset and cessation of exercise respectively. The LOA revealed a positive bias between the test-retest measures for A_2 during both the on- (23 ± 52 ml.min^{-1}) and off-transient (25 ± 50 ml.min^{-1}) of exercise.

The measurement error of the grand mean for A_2 was large for both exercise transients (On, 24%; Off, 41%).

Table 5.2 Method error calculated for τ (s) and A (ml.min^{-1}) for sub and supra GET running (n = 8).

Measure	Method Error (±)	Coefficient of variation (%)
80%GET On T_{D1} (s)	0.4	6.0
80% GET Off T_{D1} (s)	0.5	5.4
50%Δ On T_{D1} (s)	1.1	12
50%Δ Off T_{D1} (s)	0.7	10.5
50%Δ On T_{D2} (s)	10.2	12.5
80%GET On τ_1 (s)	1.4	5.6
80%GET Off τ_1 (s)	0.3	1.2
50%Δ On τ_1 (s)	0.8	3.4
50%Δ Off τ_1 (s)	1.5	5.2
50%Δ On τ_2 (s)	1.2	4.4
50%Δ Off τ_2 (s)	2.1	6.3
80%GET A_1 On (ml.min^{-1})	17.5	2.1
80%GET Off A_1 (ml.min^{-1})	11.2	1.2
50%Δ On A_1 (ml.min^{-1})	25.4	1.3
50%Δ Off A_1 (ml.min^{-1})	17.0	0.7
50%Δ On A_2 (ml.min^{-1})	24.7	8.4
50% Off A_2 (ml.min^{-1})	16.1	13.3

Table 5.3 The 95% LOA for the on- and off-transients of moderate and heavy-intensity treadmill running (n = 8).

Measure	Mean ± SD Difference x 1.96	95% LOA	Measurement Error
80%GET On T_{D1} (s)	-0.2 ± 1.6	-1.8 to 1.4	24.2
80% GET Off T_{D1} (s)	-0.4 ± 1.3	-1.7 to 0.9	18.6
50%Δ On T_{D1} (s)	0.3 ± 1.5	-1.2 to 1.8	19.3
50%Δ Off T_{D1} (s)	0.3 ± 1.7	-1.4 to 2.0	21.2
50%Δ On T_{D2} (s)	-5.1 ± 9.9	-15.0 ± 4.8	8.4
τ_1 80% GET On (s)	1.3 ± 2.4	-1.1 to 3.7	10.8
τ_1 80% GET Off (s)	0.8 ± 2.9	-2.2 to 3.7	10.7
τ_1 50%Δ On (s)	0.4 ± 2.5	-2.1 to 2.9	10.6
τ_1 50%Δ Off (s)	2.1 ± 2.6	- 0.6 to 4.7	10.6
τ_2 50%Δ On (s)	-5.8 ± 37.3	-43.1 to 31.5	21.1
τ_2 50%Δ Off (s)	-7.8 ± 90.2	-97.0 - 82.5	24.0
A_1 80%GET On ($ml.min^{-1}$)	33 ± 44	-10.5 to 77.2	4.9
A_1 80%GET Off ($ml.min^{-1}$)	-16 ± 37	-53.7 to 21.2	3.8
A_1 50%Δ On ($ml.min^{-1}$)	-16 ± 179	-195.2 to 163.4	8.8
A_1 50%Δ Off ($ml.min^{-1}$)	58 ±139	-81.1 to 197.5	6.4
A_2 50%Δ On ($ml.min^{-1}$)	23 ± 52	-75.8 to 29.5	24.2
A_2 50% Off ($ml.min^{-1}$)	25 ± 50	-25 to 75	41.6

5.3.4 On-transient kinetic characteristics

Typical $\dot{V}O_2$ responses for a representative participant (2) to the on-transients of moderate- and heavy-intensity treadmill running are presented below in Figure 5.1 (A and B). The $\dot{V}O_2$ during the on-transients of 80%GET running displayed the 3 phase response expected for sub-AT exercise and was successfully characterised by a mono-exponential model with a time delay. The $\dot{V}O_2$ response to the on-transient of 50%Δ running demonstrated the development of a delayed slow component response that has been reported previously for heavy-intensity exercise and was therefore better characterised by a double-exponential model with a time delay (see chapter 3.2.5.3).

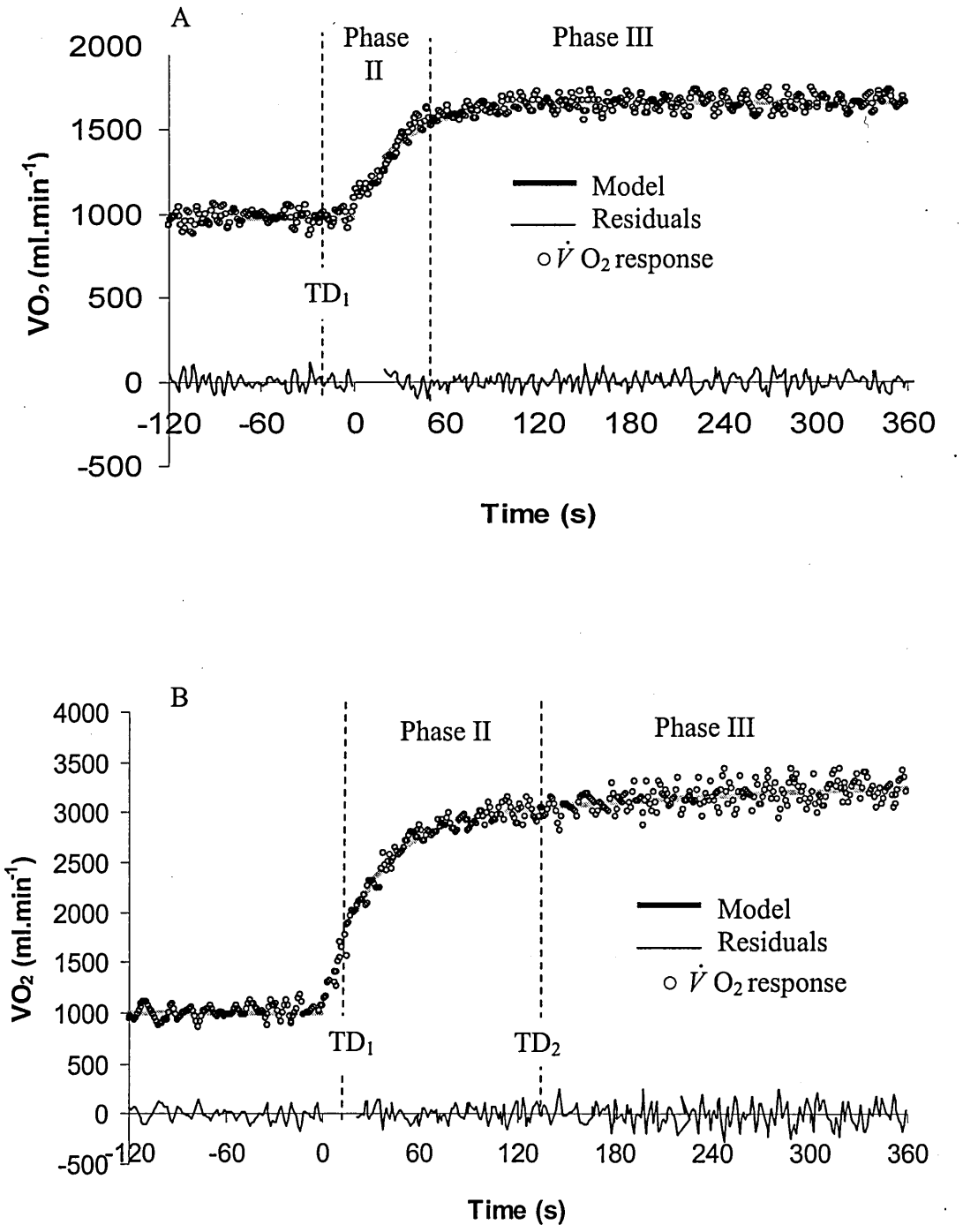


Figure 5.1 A and B. The $\dot{V}O_2$ response from a representative participant (2) during the onset of moderate- (A) and heavy- (B) intensity treadmill running. The $\dot{V}O_2$ responses to moderate- and heavy-intensity running are characterised by a mono-exponential and double exponential model respectively. The residuals (Model - $\dot{V}O_2$ response) for phases II and III have been plotted for each exercise intensity to indicate how closely the model fits the $\dot{V}O_2$ data during different phases of the response (n=8).

Using the speed / $\dot{V}O_2$ relationship generated from the incremental exercise test, the mean 80%GET value of 2307 ± 119 ml.min^{-1} was calculated to occur at a speed of 9.3 ± 1.2 km.h^{-1}, whereas the mean 50%Δ value of 3420 ± 186 ml.min^{-1} was expected to occur at the higher speed of 15.1 ± 0.9 km.h^{-1}. By fitting a mono-exponential model, it was identified that running at 9.3 ± 1.2 km.h^{-1} resulted in $\dot{V}O_2$ responses of 2091 ± 191 ml.min^{-1} (73 ± 7% of GET) and 2186 ± 172 ml.min^{-1} (76 ± 6% of GET) for tests 1 and 2 respectively. Similarly, it was determined from a double exponential model that running at 15.1 ± 0.9 km.h^{-1} produced $\dot{V}O_2$ values of 3377 ± 324 ml.min^{-1} (48%Δ) and 3400 ± 318 ml.min^{-1} (49.5 %Δ) for the two tests. Such values confirm that the running speeds identified from the incremental exercise test elicited the desired $\dot{V}O_2$ values. As expected, mean HR was lower for the 80%GET than the 50%Δ runs (P=0.023), and only the 50%Δ runs caused an elevation in blood lactate (P=0.013). The physiological measures and $\dot{V}O_2$ kinetics parameters measured at the onset of 80%GET and 50%Δ running during the square-wave protocol are listed below in Table 5.4.

Table 5.4 Test-retest on-transient $\dot{V}O_2$ kinetic parameters (mean ± SD) measured during moderate- and heavy-intensity running (n = 8).

	80%GET		50%Δ	
Measure	**Test 1**	**Test 2**	**Test 1**	**Test 2**
Speed ($km.h^{-1}$)	9.3 ± 1.2	9.3 ± 1.2	15.1 ± 0.9**	15.1 ± 0.9**
HR ($b.min^{-1}$)	148 ± 9	151 ± 7	179 ± 10**	176 ± 9**
Δ[HLa] ($mmol.l^{-1}$)	0.4 ± 0.2	0.6 ± 0.1	2.4 ± 0.6**	2.6 ± 0.8**
T_{D1} (s)	6.6 ± 2.4	6.4 ± 2.1	5.8 ± 3.4	6.0 ± 3.3
T_{D2} (s)			118.3 ± 13.4	121.4 ± 15.3
τ_1 On (s)	23.2 ± 2.9	22.0 ± 2.8	23.7 ± 3.1	24.8 ± 2.6
τ_2 On (s)			177.5 ± 43.9	179.3 ± 40.7
A_1 ($ml.min^{-1}$)	853 ± 354	853 ± 336	2003 ± 218**	2057 ± 178**
A_2 ($ml.min^{-1}$)			289 ± 151	295 ± 118

**Higher for 50%Δ, P<0.01.

5.3.5 Off-transient kinetic characteristics

A typical $\dot{V}O_2$ response during the off-transients of moderate- and heavy-intensity exercise for a representative participant (2) are depicted below in Figure 5.2 (A and B). The phases of the $\dot{V}O_2$ response were similar to those observed during the onset of exercise for both intensities. Therefore, a mono-exponential and double exponential model (see chapter 3.2.5.3) were used to model $\dot{V}O_2$ at the cessation of moderate- and heavy-intensity exercise respectively.

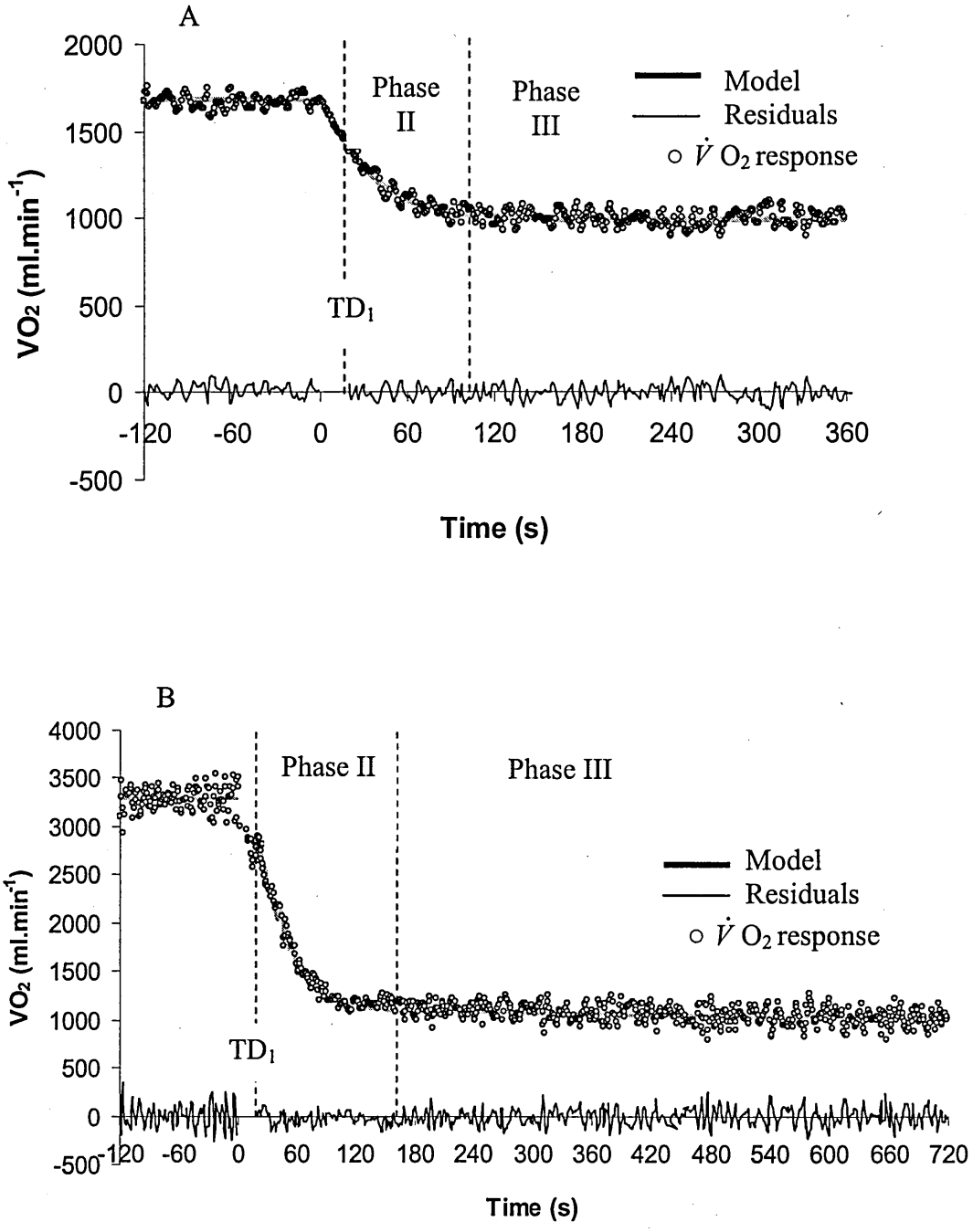


Figure 5.2 A and B. The $\dot{V}O_2$ response from a representative participant (2) during the off-transient of moderate- (A) and heavy- (B) intensity treadmill running. The $\dot{V}O_2$ responses to moderate- and heavy-intensity running are characterised by a mono-exponential and double exponential model respectively. The residuals (Model - $\dot{V}O_2$ response) for phases II and III have been plotted for each exercise intensity to indicate how closely the model fits the $\dot{V}O_2$ data during different phases of the response (n=8).

The physiological measures and kinetic parameters recorded during the off-transient of moderate and heavy-intensity exercise are displayed below in Table 5.5.

Table 5.5 The test-retest (mean ± SD) $\dot{V}O_2$ kinetic parameters and physiological measures recorded during the off-transient of moderate- and heavy-intensity treadmill running (n=8).

	80%GET		50%Δ	
Measure	**Test 1**	**Test 2**	**Test 1**	**Test 2**
Speed (km.h^{-1})	4 ± 0	4 ± 0	4 ± 0	4 ± 0
T_{D1} (s)	6.7 ± 3.7	7.4 ± 4.2	7.8 ± 2.4	7.7 ± 2.6
T_{D2} (s)			124.2 ± 26.3	127.3 ± 25.9
τ_1 (s)	27.4 ± 3.5$^{\#}$	27.6 ± 3.1$^{\#}$	27.1 ± 2.4$^{\#}$	26.0 ± 2.2$^{\#}$
τ_2 (s)			396.1 ± 52.3$^{\#\#}$	403.1 ± 78.2$^{\#\#}$
A_1 (ml.min^{-1})	814 ± 211	865 ± 205	2134 ± 275**	2167 ± 281**
A_2 (ml.min^{-1})			138 ± 59$^{\#\#}$	105 ± 47$^{\#\#}$

** Higher for 50%Δ, P<0.01; $^{\#}$ Different from corresponding on-transient value, P<0.05; $^{\#\#}$ Different from corresponding on-transient value, P<0.01.

5.3.6 Comparison of on- and off-transient kinetics

Two way ANOVA with repeated measures revealed τ_1 for 80%GET and 50%Δ running to be shorter (P=0.033) when measured during the on- than the off-transient of exercise (Figure 5.3 A). However, the analysis found A_1 values to not differ between transients (P=0.312) for both 80%GET and 50%Δ runs, while as expected, A_1 was found to be larger (P=0.029) for the 50%Δ than 80%GET runs, at exercise onset and cessation. Paired sample t-test demonstrated that τ_{2on} was shorter (Figure 5.3 B) than τ_{2off} (P=0.004). The A_{2on} was however found to be larger (P=0.005) than A_{2off}.

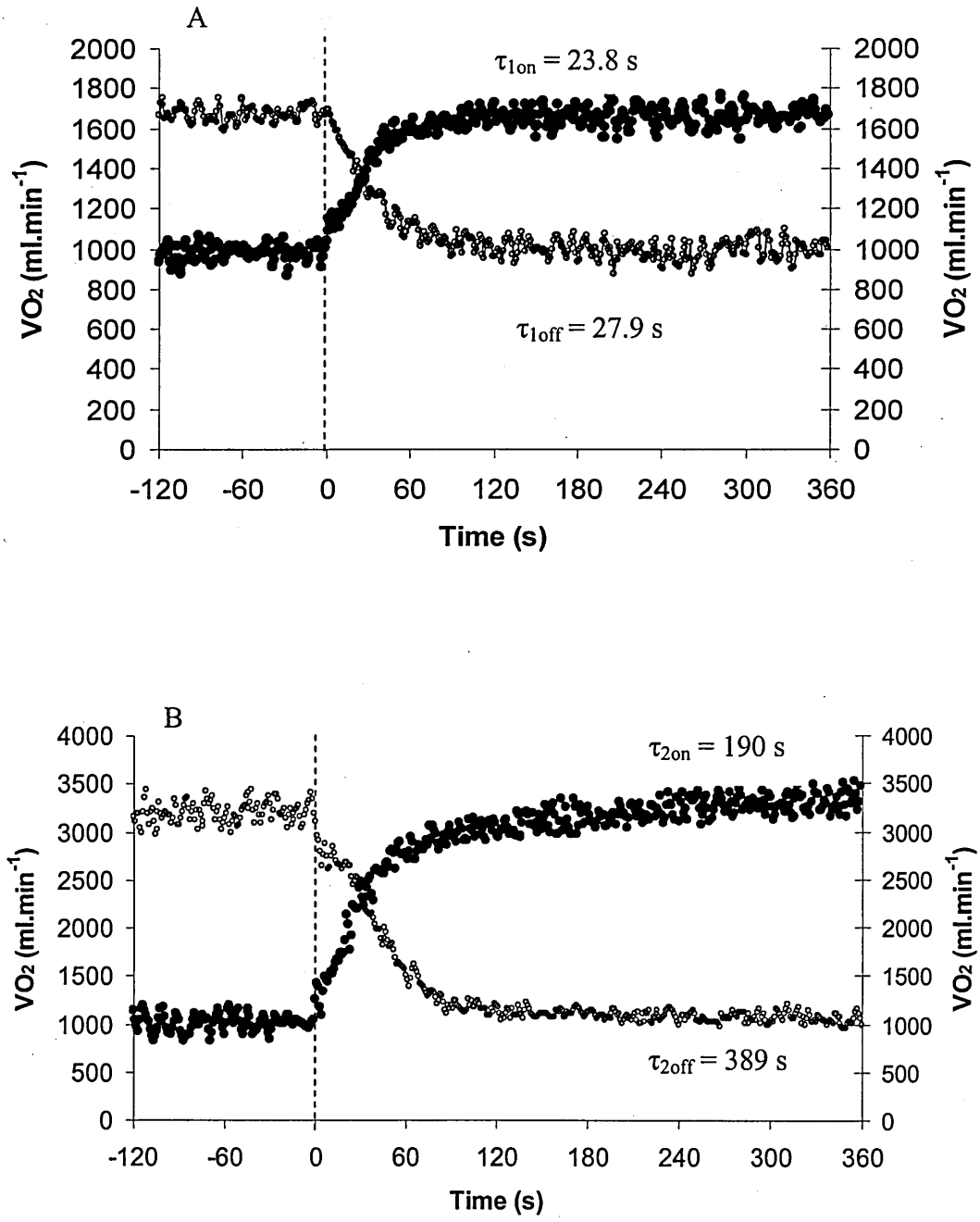


Figure 5.3 A and B. The $\dot{V}O_2$ responses from a representative participant (2) during the on- (•) and off-transients (°) of moderate- (A) and heavy- (B) intensity treadmill running. The τ_{1on} and τ_{2on} are significantly quicker than τ_{1off} and τ_{2off} ($P<0.05$).

5.4 Discussion

5.4.1 The practicality of a repeated exercise-transition treadmill protocol

A repeated square-wave transition treadmill protocol was devised to provide measures of $\dot{V}O_2$ kinetic responses during the on- and off-transitions of moderate- and heavy-intensity running. However, it has not been firmly established if such a protocol can be used to obtain valid and reliable $\dot{V}O_2$ kinetic parameters, as prior exercise bouts have been demonstrated to alter $\dot{V}O_2$ responses during subsequent exercise (Gerbino *et al.*, 1996; Burnley *et al.*, 2000; Koppo and Bouckaert, 2000).

The analysis revealed that 6 min is sufficient time for $\dot{V}O_2$ to return to pre-exercise levels following moderate-intensity treadmill running, ensuring the amplitude response during the subsequent exercise bout was unaffected by an elevated baseline $\dot{V}O_2$ (Burnley *et al.*, 2001). It was also found that no difference existed between the $\dot{V}O_2$ kinetic parameters measured during the onset and cessation of moderate exercise transitions of parts A or B of the protocol.

In contrast, the τ_{2on} and A_{2on} values were found to be shorter and smaller for part B, although the corresponding values for part A were not significantly different. Furthermore, τ_{2off} (P=0.023) and A_{2off} (P=0.028) were significantly slower and larger for part B of the protocol. Such differences in the phase III $\dot{V}O_2$ responses of both transients during part B might indicate that $\dot{V}O_2$ kinetic measures were influenced by a prior bout of heavy-intensity exercise. However, the cause of the distortion to $\dot{V}O_2$ kinetics in this study is unclear, as all physiological measures (HR, $\dot{V}O_2$, blood lactate) were observed to have returned to pre-exercise levels before part B of the protocol commenced. The lack of difference in the blood lactate measured immediately before

parts A (1.23 ± 0.18 mmol.l^{-1}) and B (1.18 ± 0.12 mmol.l^{-1}) indicates that residual lactate acidosis that has been previously associated with a reduction in the slow component during subsequent exercise bouts is not present. Hence, the 42 min (12 min walk + 30 min passive recovery) that separated the heavy-intensity runs was sufficient to remove any lactate acidosis before the next bout of exercise. This might be expected as the recovery of blood lactate has a half-time of 15 to 20 min (Burnley *et al.*, 2005). In addition, if it is accepted that the oxidation of lactate is a contributing factor in the recovery slow component (Özyener *et al.*, 2001), then it is also ambiguous why τ_{2off} and A_{2off} were larger following the heavy-intensity run in part B, as similar blood lactate concentrations were measured after each heavy-intensity run. Although other factors that have been proposed to account for an elevated $\dot{V}O_2$ following heavy-intensity exercise such as increased temperature (Gore and Withers, 1990) were not measured.

An alternative explanation for the differences in phase III kinetic measures between parts A and B of the protocol might be the large variability observed in this study for test-restest measures of the slow component (see chapter 5.3.3). Large day-to-day variability could lead an incorrect assumption that a measure has changed when in fact it has remained stable. Hence, it cannot be firmly concluded that the difference in phase III parameters between parts A and B observed here are attributable to the prior heavy-intensity exercise. An increase in the reproducibility of phase III measures is required if firm conclusions are to be drawn concerning the influence of prior heavy-intensity exercise on $\dot{V}O_2$ kinetic measures during subsequent exercise bouts (see chapter 6).

5.4.2 Reproducibility of $\dot{V}O_2$ kinetics

The findings of this investigation indicate that phase II $\dot{V}O_2$ kinetic responses during the on- and off-transients of moderate and heavy treadmill running are reproducible.

The CV was under 10% for all phase II τ and A, which compares well to the CV of 7.3% reported for $\dot{V}O_2$ max (Katch *et al.*, 1972) which has been frequently used in physiological assessments. Furthermore, LOA (95% confidence) indicated that for both the on- and off-transients of moderate- and heavy-intensity exercise, τ_1 and A_1 measurement error did not exceed 9.8% of the grand mean. Such findings are in agreement with previous research that has reported high reproducibility for $\dot{V}O_2$ kinetic parameters measured during the onset of moderate-intensity cycling (Puente-Maestu *et al.*, 2001).

In contrast, τ_{2on} and τ_{2off} were more variable, with measurement errors of 21% and 24% of their respective grand means. The A_2 during the on- and off-transients expressed the greatest test-retest variability, with measurement errors of 24% and 41% of their grand mean respectively. Although previous research into the variability of phase III $\dot{V}O_2$ kinetic parameters is limited, a substantial variation in τ_2 and A_2 has been reported by Özyener *et al.* (2001) for a range of supra GET cycling intensities.

For the purpose of this thesis, the variability in τ_1 and A_1 found in the current investigation appears to be acceptable as it is not greater than previously observed changes in phase II $\dot{V}O_2$ kinetics (10 to 58%) following a period of training (Berry and Moritani, 1985; Babcock *et al.*, 1994; Phillips *et al.*, 1995). However, the variability of A_{2on} / A_{2off} reported here is similar or greater than the change in slow component amplitude reported by Carter *et al.* (2002) of 32% following six weeks of endurance training. Therefore, an improvement in the test-retest reproducibility of phase III parameters might be necessary if the influence an intermittent training regime has on the slow component is to be assessed in a future study of this thesis.

5.4.2.1 Factors influencing the reproducibility of phase III $\dot{V}O_2$ kinetics

Only two transitions of heavy-intensity treadmill running were ensemble averaged in this study. This might not be a sufficient number of transitions to reduce the influence that the noise inherent in breath-by-breath measurements of $\dot{V}O_2$ (Lamarra *et al.*, 1987) has on parameter estimation. A noisy $\dot{V}O_2$ response would make it difficult to consistently fit an exponential model to the true physiological signal. Visual inspection of the residuals plotted in Figures 5.1 (A+B) and 5.2 (A+B) demonstrate the poor model fit for phase III compared to phase II in both transients. This is supported by the observation that the mean sum of squares for phase II (on-transient, 1647; off-transient, 2059) is smaller than for phase III (on-transient, 10516; off-transient, 7899) during the heavy-intensity exercise transitions.

A contributing explanation for the poor reproducibility is the small signal to noise ratio for phase III $\dot{V}O_2$ measurements. For example, the mean phase III amplitudes in this investigation are 291 ± 134 and 122 ± 73 ml.min^{-1} for the on- and off-transients respectively. Yet the ± S_o of the breath-to-breath noise for these components of the $\dot{V}O_2$ response are 116 ml.min^{-1} during the on- and 47 ml.min^{-1} during the off-transient of exercise. Expressed as a percentage the signal to noise ratio is 39% for τ_{2on} and 38% for τ_{2off}. Based on these data, the ± 95% CI (Lamarra *et al.*, 1987) for τ_2 measurements is estimated to be ± 33.0 s for the on- and ± 120 s for the off-transients, which equates to 18% and 31% of the mean τ_{2on} and τ_{off} values. In comparison, the amplitudes of the phase II response are much larger (On, 853 ± 364 ml.min^{-1}; Off 858 ± 305 ml.min^{-1}) and the ± S_o of breath-to-breath noise relatively smaller (On, ± 61 ml.min^{-1}; Off, ± 47 ml.min^{-1}). Consequently, the signal to noise ratio for phase II of moderate-intensity running is 7.2% for the on-transient and 5.4% for the off-transient. Such an enhanced signal to noise ratios produces smaller 95% CI estimates for τ_1 of ± 1.21 s for the on-

and ± 1.48 s for the off-transient, which correspond to 5.2% and 5.4% of the mean τ_{1on} and τ_{1off} values. Although calculation of 95% CI using the equation of Lamarra *et al.* (1987) was intended for τ estimation during the steady-state response to moderate-intensity exercise, it was used as an estimate of the 95% CI for τ_2 in this study as the phase III $\dot{V}O_2$ response was observed to reach a delayed steady-state before the final 100 s of the heavy exercise transitions for all participants.

The signal to noise ratio during phase III of heavy-intensity running might be improved by measuring the $\dot{V}O_2$ response at a heavier exercise intensity, as the amplitude of the slow component is reported to be larger for very-heavy (80%Δ, Whipp *et al.*, 2005) than heavy-intensity exercise. Although, it must be taken into consideration that exercise in different supra-GET intensity domains can lead to contrasting $\dot{V}O_2$ responses (Özyener *et al.*, 2001). It is also possible that a combination of techniques employed by previous studies such as increasing the number of heavy-intensity exercise transitions (Özyener *et al.*, 2001) and averaging the $\dot{V}O_2$ data (Koga *et al.*, 1999) could help reduce the impact of breath-to-breath noise on parameter estimation.

5.4.3 Characteristics of phase II $\dot{V}O_2$ kinetics

The limited number of studies that have measured the $\dot{V}O_2$ kinetics of recreationally active individuals during treadmill running makes modality specific cross study comparisons difficult. The mean test-retest τ_{1on} for moderate- and heavy-intensity treadmill running in this study were 22.6 ± 2.1 s and 23.7 ± 2.4 s respectively. This is slower than has previously been reported for recreationally active participants following the onset of moderate- (13.9 ± 1.4s, Carter *et al.*, 2000b; 15.0 ± 2.0 s, Carter *et al.*, 2000a; 14.7 ± 2.8 s, Williams *et al.*, 2001) and heavy- (19.4 ± 2.1 s, Carter *et al.*, 2000a; 20.1 ± 2.3 s, Carter *et al.*, 2000b; 19.0 ± 1.8 s, Williams *et al.*, 2001) intensity treadmill

running, but similar to moderate-intensity cycling (23.2 ± 7.0 s, Barstow *et al.*, 1996; 21.2 ± 8.2 s, Koga *et al.*, 1999). In contrast, the τ_{1off} of 27.5 ± 5.5 s and 26.7 ± 5.1 s for moderate- and heavy-intensity treadmill running in this investigation is substantially faster than that previously reported for recreationally active individuals, (39.9 ± 3.0 s, Carter *et al.*, 2000a), and is more comparable to that reported for endurance trained athletes (25.0 ± 1.8 s, Phillips *et al.*, 1995: 27.1 ± 3.0 s, Kilding *et al.*, 2003).

It is unclear why the τ_1 of recreationally active participants in this study should differ to that previously reported for similar participant groups. A possible explanation might be a difference in the aerobic fitness of participants, as $\dot{V}O_2$ max has been shown to be inversely associated with τ_1 (Chilibeck *et al.*, 1996; Fawkner *et al.*, 2002). However, this does not seem to be a causative factor, as the mean $\dot{V}O_2$ max of the participants in the studies of Carter *et al.* (2000a) and Williams *et al.* (2001) were 50.7 ± 13.0 $ml.kg^{-1}.min^{-1}$ and 56.6 ± 3.0 $ml.kg^{-1}.min^{-1}$ respectively, which is similar to 51.3 ± 3.1 $ml.kg^{-1}.min^{-1}$ reported in this study. Furthermore, off-transient $\dot{V}O_2$ kinetics appear to be independent of $\dot{V}O_2$ max, as the more aerobically trained endurance athletes (60.0 ± 4.9 $ml.kg^{-1}.min^{-1}$) in the study of Kilding *et al.* (2003) did not possess faster τ_1 during recovery than the participants of this study. These findings would in fact suggest that $\dot{V}O_2$ max and $\dot{V}O_2$ kinetics are controlled by different mechanisms (Carter *et al.*, 2000a; Kilding *et al.*, 2003). To help clarify the cross study variability in τ_1, further research is warranted to establish the relationship between aerobic fitness and $\dot{V}O_2$ kinetics during the different transients of exercise.

The seemingly fast $\dot{V}O_2$ kinetics observed for recreationally active (Carter *et al.*, 2000a) and untrained (Williams *et al.*, 2001) individuals might be explained by

differences in the methods used to measure $\dot{V}O_2$ kinetic responses. The transition to moderate-intensity running in the current investigation was initiated from walking (4 $km.h^{-1}$), whereas in the studies of Carter *et al.* (2000a) and Williams *et al.* (2001), transitions were initiated from resting conditions. Also, the $\dot{V}O_2$ responses to the onset and cessation of moderate-intensity runs in this study were characterised using a mono-exponential model with a time delay, whereas in the above studies authors used a higher order two component exponential model to take into consideration phase I of the $\dot{V}O_2$ response.

5.4.3.1 Influence of intensity domain on phase II $\dot{V}O_2$ kinetics

An important observation from the analysis of the $\dot{V}O_2$ kinetic responses during the heavy-intensity exercise in this study is that τ_1 is invariant of exercise intensity. Although several cycle based investigations (Özyener *et al.*, 2001; Scheuermann and Barstow, 2003) have also noted τ_1 to be independent of intensity domain, this appears to be the first time it has been reported for square-wave treadmill running. In contrast, a large proportion of the literature has reported τ_1 to lengthen during heavy-intensity exercise, for both treadmill running and cycling. It has been proposed that large inter-individual variability in τ_1 combined with small sample sizes might lead investigators to find non-significant differences in τ_1 between moderate and heavy exercise, even when the mean difference in the values appears to be substantial (Perry *et al.*, 2001). Although the sample size in this study is small (n = 8), further analysis reveals that the invariance of τ_1 across exercise intensities was apparent for all participants (see Figure 5.4). In addition, the standard deviations of the mean τ_1 values for 80%GET (± 2.1 s, 8.5% of mean) and 50%Δ (± 2.3 s, 8.3% of mean) are small. Analysis in this study has also shown τ_1 to be invariant on a test-retest basis, where the variability between tests was

low (see Tables 5.2 and 5.3). So it would appear that the invariant τ_1 in this study are not the consequence of variable data.

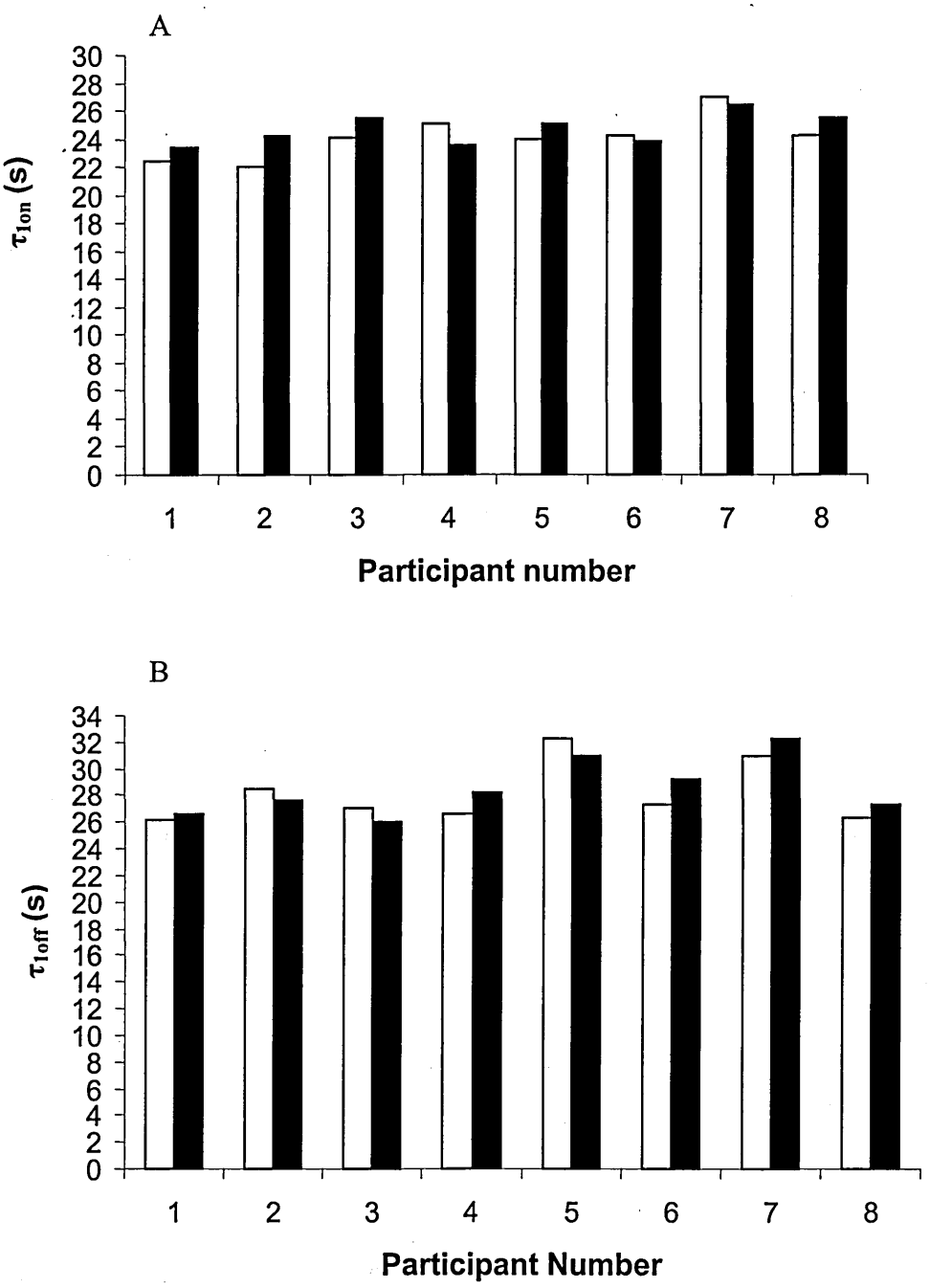


Figure 5.4 Graph A represents τ_{1on} for 80%GET (□) and 50%Δ (■) running from test 1 for each participant. Graph B represents τ_{1off} for 80%GET (□) and 50%Δ (■) running from test 1 for each participant. Such data demonstrate the small inter-participant variability for τ_1 during moderate- and heavy-intensity exercise (n=8).

With regards to future investigations of this thesis, an invariant τ_1 across intensity domains indicates that the primary $\dot{V}O_2$ response reflects a linear system. Consequently, transitions to or from moderate- and heavy-intensity exercise would provide similar information about the speed of O_2 utilisation at the muscle, removing the need to measure τ_1 separately for moderate- and heavy-intensity treadmill running. This would make laboratory based $\dot{V}O_2$ kinetic assessments less time consuming, which could increase accessibility to specialist groups such as elite soccer players whose time can be limited due to training and competition commitments.

5.4.4 Characteristics of phase III $\dot{V}O_2$ kinetics

The values for τ_2 in this study of 178.2 ± 41.3 s and 399.1 ± 86.1 s for exercise onset and cessation are substantially longer than the corresponding τ_1 values, which is in agreement with the findings of previous investigations (Patersson and Whipp, 1991; Barstow *et al.*, 1996; Carter *et al.*, 2000a). Previous investigations have reported the length of τ_2 to range from 32 ± 7 s to 416 ± 406 s at the onset and from 163 ± 46 s to 539 ± 379 s at the cessation of heavy-intensity running and cycling (Carter *et al.*, 2000c; Demarle *et al.*, 2001; Özyener *et al.*, 2001). Clearly, it is not possible from such highly variable observations to make cross-study comparisons, and is difficult to identify what an expected τ_2 should be for a recreationally active participant during both the on- and off-transients of heavy-intensity treadmill running. The cause of such variability in reported τ_2 values is unclear. It could be explained by the poor reproducibility previously observed for phase III $\dot{V}O_2$ kinetics (James and Doust, 1996; Özyener *et al.*, 2001), and which has been identified in the current investigation (see chapter 5.3.3), as any parameter that varies substantially on a day-today basis will lead to inconsistent findings. Alternatively, inconsistent identification of intensity domains in previously published studies could lead to variable phase III $\dot{V}O_2$ measures.

5.4.5 Asymmetry of $\dot{V}O_2$ kinetics

For moderate- and heavy-intensity treadmill running, the τ_1 in this study was quicker for the on- than the off-kinetic response (see Tables 5.4 and 5.5), which is in agreement with previous treadmill (Carter *et al.*, 2000a), cycle (Linnarsson, 1974; Hughson *et al.*, 1988; Scheuermann *et al.*, 1998) and prone leg extension (Rossitter *et al.*, 2002) studies. In contrast, A_1 was found to be similar between transients for both intensities. Asymmetry between phase II parameters is significant, as it implies that the $\dot{V}O_2$ response to moderate and heavy treadmill running does not conform to a dynamic linear model across exercise transients.

The present investigation also found τ_2 for heavy-intensity exercise to be approximately 50% quicker during exercise than in recovery, which is in agreement with earlier cycle investigations (Carter *et al.*, 2000a; Cunningham *et al.*, 2000; Özyener *et al.*, 2001). Asymmetry was also observed to exist in the amplitudes of the $\dot{V}O_2$ kinetic response to heavy-intensity exercise. The A_2 was approximately 3 fold larger for the on- than the off-kinetic response, supporting previous research, (Carter *et al.*, 2000a, Cunningham *et al.*, 2000). Such asymmetry suggests that the slow component during recovery is independent of the slow component during the preceding exercise (Cunningham *et al.*, 2000). This notion is further supported by the findings of Özyener *et al.* (2001) who found no significant differences in the τ of the off-transient slow component for very heavy and severe exercise intensities.

There is currently little evidence explaining the asymmetry between transients. The discrepancy in τ_1 between on and off $\dot{V}O_2$ responses could be indicated from a quantitative analysis of the time course of [PCr] degradation and recovery kinetics. Kushmerick (1998) has shown, via computer modelling, that the full expression of CK

(forward and reverse flux) and differing processes during the imposed increase in ATPase activity in the breakdown and recovery phases, results in asymmetry between [PCr] on and off-transients. As it has been identified that [PCr] and $\dot{V}O_2$ have similar kinetics (Rossiter *et al.*, 1999; Rossiter *et al.*, 2002), this is anticipated to cause an asymmetry between $\dot{V}O_2$ on- and off-transients as was observed in the present study.

It has been hypothesised that the longer τ_2 and smaller A_2 reported during recovery in comparison to exercise could be caused by the different energetics in type I and type II muscle fibres (Barstow *et al.*, 1996; Cunningham *et al.*, 2000). During heavy aerobic exercise, there is an increased recruitment of low efficiency, high oxygen cost, type II fibres, which is consistent with the additional increase in $\dot{V}O_2$ during exercise above GET. In recovery however, the type II recruitment would cease immediately, and without delay mitochondrial oxygen utilisation would contribute to the restoration of PCr in these fibres. This oxidative metabolism in type II fibres will have a slow time course and small oxygen demand, which might be reflected as a prolonged but small A_2 (slow component) during recovery. Alternatively, following heavy-intensity exercise, a slow component might be present during recovery due to lactate serving as a source of glyconeogenesis. Similarly, any lactate reducing equivalents transported into mitochondria as an aerobic source that utilises the α-glycerophosphate shuttle rather than the malate-asparate shuttle would also incur a small and possibly long-lasting additional oxygen cost that would be expressed as a slow component.

5.5 Conclusion

A repeated square-wave transition treadmill protocol can be used to measure $\dot{V}O_2$ kinetic parameters to the onset and cessation of moderate-intensity exercise. It remains to be established however if such a protocol can be used to measure $\dot{V}O_2$ kinetic

parameters during the onset and cessation of heavy-intensity exercise, as the $\dot{V}O_2$ kinetic profile during a second heavy-intensity run was found to differ to that recorded for the first run. The τ_1 was found to be invariant of intensity domain, which suggests $\dot{V}O_2$ kinetic responses to moderate- and heavy-intensity exercise do not need to be measured separately. The implication of such a finding is that the study of $\dot{V}O_2$ kinetics can become less time consuming, potentially increasing access to elite athletes.

The $\dot{V}O_2$ kinetic parameters were found to be reproducible for the on and off phase II transients of the $\dot{V}O_2$ response. The level of reproducibility for the phase II parameters is such that it is concluded they can be used in a future investigation to determine the influence soccer training has on a player's $\dot{V}O_2$ kinetic profile. The level of variability found for $\dot{V}O_2$ kinetic parameters during phase III of the $\dot{V}O_2$ response however was high, limiting their application when investigating the determinants of sports performance. Such variability could also account for the difference observed between phase III parameters measured during parts A and B of the protocol. To address these problems, further research is required to identify whether the reproducibility of phase III parameters can be improved.

The $\dot{V}O_2$ kinetic responses measured during the onset of moderate- and heavy-intensity treadmill running are slower than have previously been reported for recreationally active participants. Conversely, the τ_1 off for moderate and heavy-intensity treadmill running was substantially faster than reported for the same participant group and was similar to that expected for endurance trained athletes. Such findings question observations that $\dot{V}O_2$ kinetics are influenced by $\dot{V}O_2$ max.

CHAPTER 6

Improvement of phase III $\dot{V}O_2$ kinetic parameter reproducibility

6.1 Introduction

The reproducibility of phase III parameters observed in the previous study of this thesis must be improved if the role the slow component plays in soccer performance is to be understood. The cause of the low reproducibility appears to be a poor model fit to the $\dot{V}O_2$ data, due to the phase III response containing a large amount of noise in relation to its amplitude. In an attempt to improve the reproducibility of the phase III response the following modifications to the square-wave protocol will be implemented: 1) an increase in the intensity of the runs from 50%Δ to 80%Δ (80% of the way between GET and $\dot{V}O_2$ max, defined as the very heavy-intensity domain, Whipp *et al.*, 2005) to enlarge the amplitude of the slow component, and 2) an increase in the number of supra-GET exercise transitions from 2 to 4 in an attempt to smooth the noise in the $\dot{V}O_2$ response. It is intended that the product of these two changes will enhance the signal to noise ratio for the phase III $\dot{V}O_2$ response, which should lead to a better model fit to the $\dot{V}O_2$ data and hence less variable parameter estimations.

The influence that prior heavy-intensity exercise has on $\dot{V}O_2$ kinetic parameters during subsequent exercise bouts must be established if such a repeated heavy-intensity transition protocol is to be adopted in future studies of this thesis. An improvement in the reproducibility of phase III parameters will help determine whether any change to the $\dot{V}O_2$ kinetic parameters measured during subsequent heavy-intensity runs are caused by physiological responses to a prior bout of heavy-intensity exercise.

No moderate-intensity transitions have been incorporated into the protocol as the findings of the previous investigation of this thesis showed τ_1 to be invariant across intensity domains, which would suggest no additional information would be gained from measuring phase II $\dot{V}O_2$ kinetics for moderate- and heavy-intensity exercise separately. Furthermore, restricting the measurement of phase II $\dot{V}O_2$ kinetics to the on- and off-transients of very heavy-intensity running will possibly be more relevant to soccer, as the running speeds that elicit a $\dot{V}O_2$ value that corresponds to 80%Δ will be > 15 km.h-^{1}, which is the speed recently used (Mohr *et al.*, 2003) to define the lower boundary of high-intensity running during a competitive game.

6.1.1 Aim

1. To determine if an increase in the number and intensity of square-wave exercise transitions improves the reproducibility of phase III parameters measured at the onset and cessation of treadmill running.

2. To identify whether prior bouts of square-wave 80%Δ running distort $\dot{V}O_2$ kinetic measures during subsequent runs.

6.2 Participants and Methods

6.2.1 Participants

With institutional ethics approval, 10 recreationally active males (mean ± SD): age 25.3 ± 2.1 years, stature 179.6 ± 8.3 cm, body mass 74.7 ± 10.2 participated. Prior to the administration of any test, participants were screened for existing medical conditions that might become aggravated during the testing procedure (Appendix 6, page 238). Pre-test instructions can be seen in chapter 3.2.1.4.

6.2.2 Experimental design

Participants performed three exercise tests, each separated by three days. The first assessment was the incremental exercise test to exhaustion, on the other two occasions participants performed the repeated very heavy-intensity square-wave protocol. All laboratory tests were performed at approximately the same time of day to reduce the effects of diurnal variation. Each time players visited the laboratory stature and body mass were measured and heart rate was recorded on 5 s intervals during each assessment. Temperature in the laboratory was kept within 20°C ± 1°C.

6.2.3 Experimental Protocols

All exercise tests were performed on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany). Pulmonary gas exchange (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA) was measured on a breath-by-breath basis during an incremental exercise test to volitional exhaustion for the identification of $\dot{V}O_2$ values and running speeds that corresponded to $\dot{V}O_2$ max, GET and 80%Δ (see chapter 3.2.4). These running speeds were then used to design a repeated very heavy-intensity square-wave transition protocol that consisted of 4, 6 min runs at 80%Δ. Each run was separated by a 12 min walk and a further 30 min of passive

recovery (see chapter 3.2.5.2). Pulmonary gas exchange was measured through out the test (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA) to determine $\dot{V}O_2$ kinetics.

6.2.4 Data Analysis

Breath-by-breath pulmonary gas exchange data collected during the incremental exercise tests and repeated square-wave transition protocol were analysed following the procedures outlined in chapter 3.2.4.1 and 3.2.5.3 respectively.

6.2.5 Statistical Analyses

Statistical significance was set at $P \leq 0.05$. To determine whether $\dot{V}O_2$ kinetic measures varied across four 80%Δ transitions, the $\dot{V}O_2$ data collected for each transition of test 1 was ensemble averaged with the $\dot{V}O_2$ data collected during the corresponding transition from test 2 (retest). The transitions from the test-retest protocols were combined to reduce noise in the $\dot{V}O_2$ response. These data were then modelled to produce a set of $\dot{V}O_2$ kinetic measures for the onset and cessation of four transitions of 80%Δ running. A two-way ANOVA with repeated measures was then used to identify whether any differences existed between $\dot{V}O_2$ kinetic measures at exercise onset and cessation across the four transitions.

To check a participant's $\dot{V}O_2$ had returned to baseline following a very heavy-intensity exercise transition before the next exercise transition commenced, the actual $\dot{V}O_2$ for the 2 min period preceding each 80%Δ transition was compared using a One-way ANOVA with repeated measures.

Differences in the test-retest data for the repeated heavy-intensity square-wave protocol were assessed using Two way ANOVA with repeated measures. The reproducibility of the test-retest data was analysed using method error, CV and LOA.

6.3 Results

6.3.1 Incremental exercise test performance

The physiological and performance measures recorded during the treadmill based incremental exercise test to exhaustion are listed below in Table 6.1. The aerobic fitness of the participants is comparable to that reported previously for professional soccer players (Reilly, 1996).

Table 6.1 The performance and physiological measures (mean ± SD) recorded from the incremental exercise test to exhaustion (n = 10).

Measure	Mean ± SD
$\dot{V}O_2$ max ($ml.kg^{-1}.min^{-1}$)	55.9 ± 4.3
$\dot{V}O_2$ max ($ml.min^{-1}$)	4175 ± 321
Maximal speed ($km.h^{-1}$)	18.1 ± 0.4
Time to exhaustion (min)	11.2 ± 0.5
Maximal HR ($b.min^{-1}$)	196 ± 12
GET ($ml.kg^{-1}.min^{-1}$)	39.6 ± 3.7
GET ($ml.min^{-1}$)	2978 ± 216
GET as % of $\dot{V}O_2$ max	71 ± 5

6.3.2 Comparison of $\dot{V}O_2$ kinetic measures across four 80%Δ running transitions

The two-way ANOVA repeated measures design revealed no difference to exist for τ_1 (P=0.341) or A_1 (P=0.247) at the onset or cessation of exercise across the four running transitions. As expected, the analysis did show τ_1 to be longer during the off-transient (P=0.036), but no interaction was reported between trial and transient (P=0.196). Although phase III measures are more variable, the two-way ANOVA with repeated measures did not show any difference to exist for τ_2 (P=0.127) or A_2 (P=0.261) for

exercise onset or cessation across the four transitions. The τ_2 was found to be longer (P= 0.025) and A_2 smaller (P= 0.004) during the off-transient, although no interaction was observed between transient and transition (τ_2, P=0.133; A_2, P=0.176). A one-way ANOVA with repeated measures revealed the $\dot{V}O_2$ during the 2 min prior to each 80%Δ transition to not differ (P=0.284).

Table 6.2 The $\dot{V}O_2$ kinetic measures (mean ± SD) during the onset of each of the four 80%Δ running transitions (n = 10).

Measure	Transition 1	Transition 2	Transition 3	Transition 4
τ_1 (s)	24.3 ± 2.3	24.8 ± 1.9	24.4 ± 3.1	25.2 ± 2.7
τ_2 (s)	154.9 ± 28.6	165.7± 30.2	162.1 ± 23.1	196.3 ± 17.5
A_1 (ml.min^{-1})	2534 ± 304	2485 ± 267	2553 ± 253	2562 ± 289
A_2 (ml.min^{-1})	301 ± 54	271 ± 43	265 ± 56	283 ± 47

Table 6.3 The $\dot{V}O_2$ kinetic measures (mean ± SD) during the cessation of each of the four 80%Δ running transitions (n = 10).

Measure	Transition 1	Transition 2	Transition 3	Transition 4
τ_1 (s)	28.1 ±3.8	27.2 ± 5.1	27.8 ± 4.3	27.5 ± 4.1
τ_2 (s)	329 ± 84.6	357 ± 74.1	366 ± 88.5	324 ± 63.8
A_1 (ml.min^{-1})	2593 ± 205	2534 ± 264	2553 ± 278	2522 ± 196
A_2 (ml.min^{-1})	171 ± 57	184 ± 46	192 ± 63	176 ± 41

6.3.3 Reproducibility of time constants

The test-retest $\dot{V}O_2$ kinetic and physiological responses to running at a speed corresponding to 80%Δ are listed below in Table 6.2. Two-way ANOVA with repeated measures found no difference between the test-retest values for τ_1 (P=0.172) or τ_2

(P=0.326) when measured at either the onset or cessation of exercise. The mean τ_1 was shorter for exercise onset than cessation (P=0.034); this was also the case for τ_2 (P=0.001).

Table 6.4 The test-retest $\dot{V}O_2$ kinetic and physiological responses (mean ± SD) to the on-transient of very heavy-intensity treadmill running (n = 10).

Measure	**Test 1**	**Test 2**
Speed ($km.h^{-1}$)	16.2 ± 1.4	16.3 ± 1.3
$\dot{V}O_2$ ($ml.min^{-1}$)	3678± 321	3767 ± 355
HR ($b.min^{-1}$)	178 ± 12	177 ± 11
T_{D1} (s)	8.8 ± 2.3	9.2 ± 3.1
T_{D2} (s)	125.6 ± 12.7	127.4 ± 13.2
τ_1 (s)	24.9 ± 2.6	24.4 ± 2.7
τ_2 (s)	178.7 ± 24.7	156.3 ± 25.4
A_1 ($ml.min^{-1}$)	2508 ± 216	2556 ± 186
A_2 ($ml.min^{-1}$)	282 ± 63	272 ± 48

Table 6.5 The test-retest $\dot{V}O_2$ kinetic and physiological responses (mean ± SD) to the off-transient of very heavy-intensity treadmill running (n = 10).

Measure	Test 1	Test 2
Speed ($km.h^{-1}$)	4.0 ± 0.0	4.0 ± 0.0
T_{D1} (s)	9.3 ± 3.1	9.4 ± 2.7
τ_1 (s)	28.3 ± 4.2$^{\#}$	27.1 ± 4.5$^{\#}$
τ_2 (s)	331.6 ± 41.7$^{\#\#}$	353.8 ± 53.5$^{\#\#}$
A_1 ($ml.min^{-1}$)	2524 ± 106	2573 ± 64
A_2 ($ml.min^{-1}$)	198 ± 43	161 ± 33

$^{\#}$ Different to the corresponding value for the on-transient, $P<0.05$; $^{\#\#}$ Different to the corresponding value for the on-transient, $P<0.01$.

Results from the tests of reproducibility are listed in Table 6.6. The CV and method error did not exceed 3% or ± 1 s for τ_1 during either the on- or off-transients of exercise. The LOA for τ_1 were narrow and suggest there is a small fixed bias of τ_1 being longer during test 1 than test 2 for both transients. The measurement error for τ_1 during the on- and off-transients did not exceed 9% of their grand means, which is comparable to that calculated for heavy-intensity running in the previous chapter.

Greater variance was found for τ_2, as the method error and CV were ± 15.6 s and 14.4% during the on- and ± 32 s and 16.4% at for the off-transients. Although the LOA (95% confidence) were improved for τ_2 during both transients compared to the previous study, they were still wider than those calculated for τ_1. There was a positive fixed bias of τ_2 being longer during test 1 than test 2 for both transients. The measurement error for τ_2 as a proportion of the grand mean approached 20% for both transients.

The test-retest $\dot{V}O_2$ response to the onset and cessation of the very heavy-exercise transitions for a representative participant (4) are listed below in Figure 6.1 (A and B). In Figure 6.2 (A and B) the $\dot{V}O_2$ response (participant 4) to the onset and cessation of very heavy exercise from the first test has been modelled and the residuals plotted to provide an indication of how closely the model fits phases II and III.

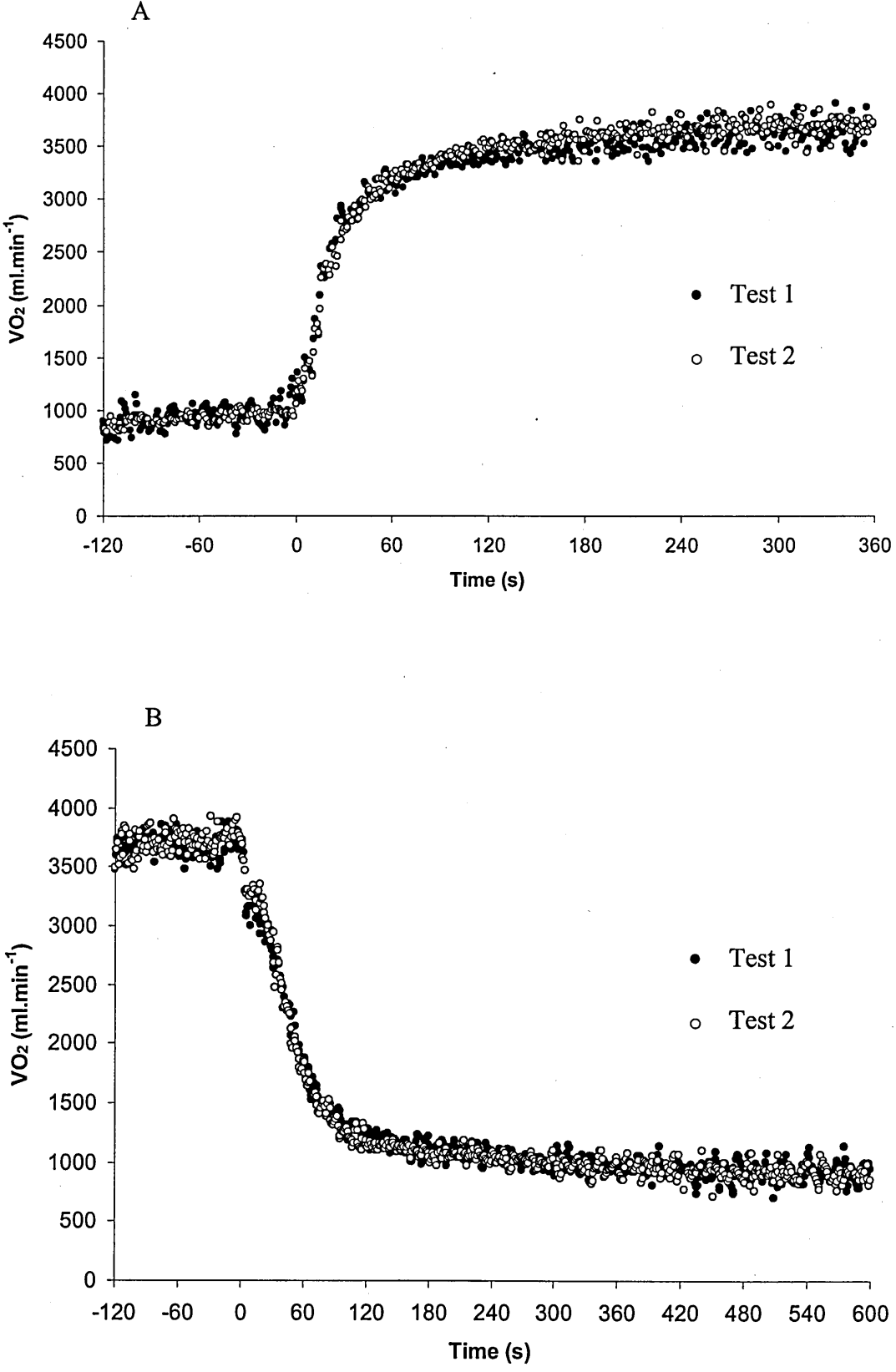


Figure 6.1 A and B. The test-retest $\dot{V}O_2$ response of a typical participant (4) to the onset (A) and cessation (B) of very heavy-intensity treadmill running.

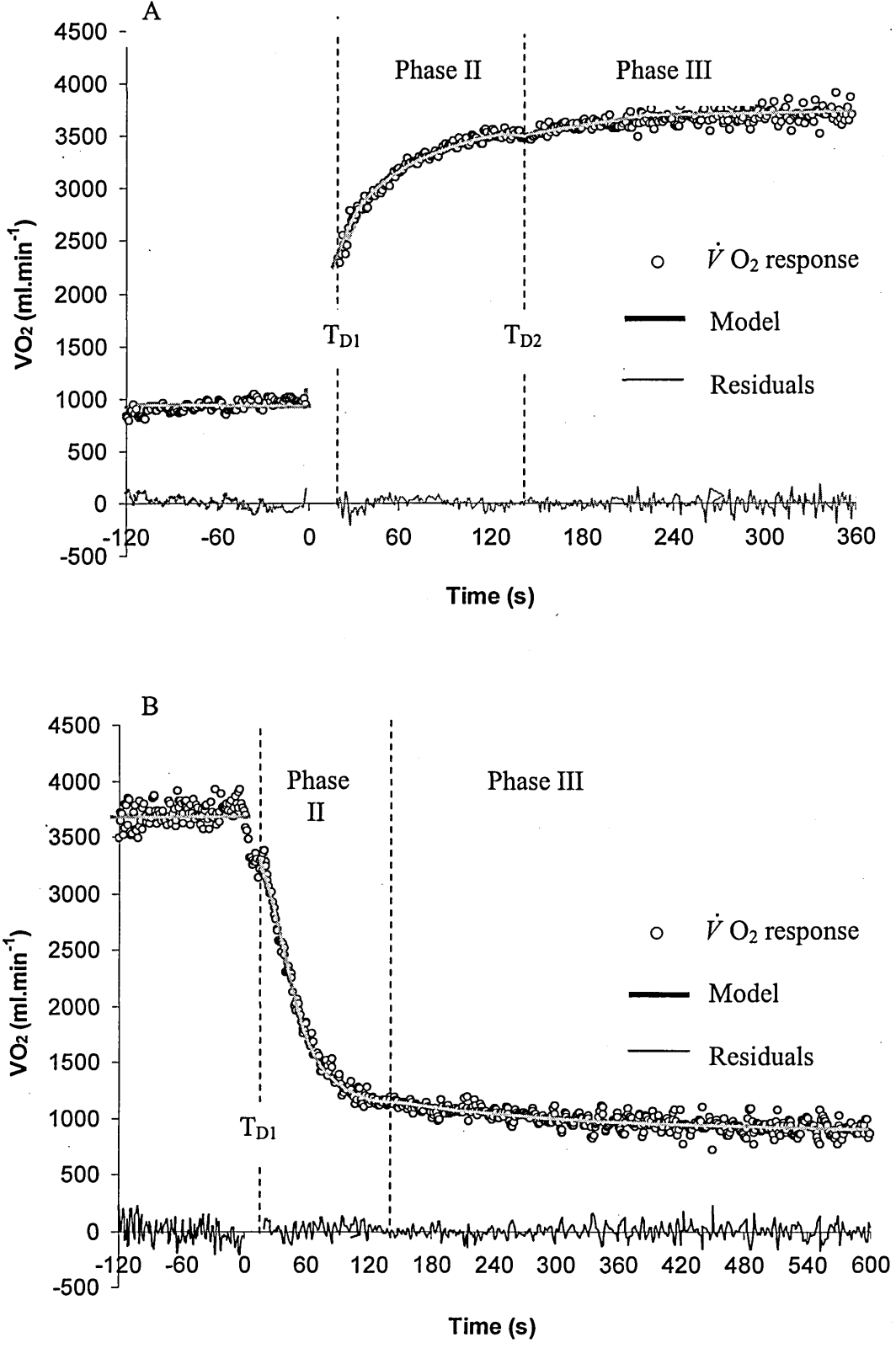


Figure 6.2 A and B. The $\dot{V}O_2$ response from a representative participant (4) during the onset (A) and cessation (B) of very heavy-intensity treadmill running (Test 1). The residuals (Model – $\dot{V}O_2$) have been plotted to demonstrate the closeness of model fit to the $\dot{V}O_2$ data for the phases II and III of the response.

6.3.4 Reproducibility of amplitudes

A two-way ANOVA with repeated measures revealed no difference between test-retest values for A_1 (P=0.115) or A_2 (P=0.134) during either the on- or off-transients of exercise. The mean value for A_1 at exercise onset and cessation did not differ (P=0.168), however A_2 was found to be larger (P=0.032) at exercise onset than cessation. The method error and CV (Table 6.4) for A_1 were low for both transients of exercise. In addition, narrow LOA were calculated for A_1 during both on- and off-transients of exercise, indicating a positive fixed bias of test 1 measuring more than test 2 (Table 6.6). The measurement error as a proportion of the grand mean for A_1 was low, not exceeding 7% for either transient. For A_2 the method error and CV were also low at ± 74 $ml.min^{-1}$ and 1.9% during the on- and ± 22 $ml.min^{-1}$ and 2.3% for the off-transients of exercise. However, LOA analysis revealed A_2 to be highly variable for both transients, with the measurement error exceeding 20% of the grand means for the on- and off-transients respectively.

Table 6.6 The LOA, method error and CV calculations for the $\dot{V}O_2$ kinetic parameters measured during the on- and off-transients of very heavy-intensity treadmill running (n = 10).

	On-transient					Off-transient				
Measure	**Mean ± SD Diff (x1.96)**	**LOA (95%)**	**Measurement Error (%)**	**Method Error**	**CV (%)**	**Mean ± S Difference**	**LOA (95%)**	**Measurement Error (%)**	**Method Error**	**CV (%)**
T_{D1} (s)	1.2 ± 0.8	-0.4 to 2.0	15.1	± 0.76	6.8	1.4 ± 0.8	-0.6 to 2.2	16	± 0.86	9.3
T_{D2} (s)	6.8 ± 1.6	-5.2 to 8.4	4.3	± 3.21	7.3					
τ_1 (s)	0.4 ± 1.6	-1.2 to 2.0	6.5	± 0.55	2.2	1.0 ± 2.4	-1.4 to 3.4	8.6	± 0.8	2.9
τ_2 (s)	15.3 ± 28.4	-13.1 to 43.7	17.2	± 15.6	14.4	20.3 ± 64.9	-44.6 to 85.2	19.2	± 32.1	16.4
A_1 (ml.min^{-1})	38 ± 158	-120 to 196	6.5	± 56.7	1.5	14 ± 121	106 to 135.3	4.7	± 43	3.7
A_2 (ml.min^{-1})	14 ± 55	-41 to 69	20.3	± 74.1	11.9	6 ± 63	56 to 69	35.7	± 22	2.3

6.4 Discussion

No difference was found between the $\dot{V}O_2$ kinetic parameters measured across the four 80%Δ running transitions. Visual inspection of the $\dot{V}O_2$ kinetic parameters (Tables 6.2) also reveals that there is no trend of τ_{2on} and A_{2on} becoming faster and smaller as the transitions are repeated. Similarly, τ_{2off} and A_{2off} do not become longer and larger (Table 6.3). Such findings conflict with those of the previous study of this thesis, where the $\dot{V}O_2$ kinetics measured during a second bout of 50%Δ running differed to those measured in the first run. The cause of such conflicting results might be attributable to the lower level of variability reported for the $\dot{V}O_2$ kinetic parameters measured in the 80%Δ protocol (see chapter 6.3.3) not masking the true kinetic responses for each transition. Furthermore, as exercising at 80%Δ would be expected to result in greater production of lactate and disruption to cell homeostasis than at 50%Δ, these findings strongly suggest that 42 min is an adequate recovery period between very heavy-intensity runs for the reversal of physiological adaptations that might distort $\dot{V}O_2$ kinetic parameters in subsequent runs. Hence, such a repeated transition protocol can be used in future studies to assess $\dot{V}O_2$ kinetics.

The phase II $\dot{V}O_2$ kinetic parameters measured during the on- and off-transients of 80%Δ treadmill running were found to be reproducible. The method error and CV did not exceed 0.8 s and 2.9% for τ_1 and 56.7 ml.min^{-1} and 3.7% for A_1 for either transient. The LOA also indicated a small spread of differences between the measurements (see Table 6.4). Such reproducibility for phase II $\dot{V}O_2$ kinetic responses compares well to that observed in the previous study of this thesis for both moderate- and heavy-intensity running (see chapter 5). It is also in agreement with previous research that has reported reproducible phase II $\dot{V}O_2$ kinetic parameters in response to moderate-intensity cycling

(Puente-Maestu *et al.*, 2001). These findings suggest that day-to-day variability in phase II $\dot{V}O_2$ kinetic parameters derived from very heavy-intensity treadmill running is such that they can be used to investigate the role of $\dot{V}O_2$ kinetics in the performance of high-intensity soccer-specific exercise.

In comparison with the previous study, the test-retest reproducibility for phase III measures was improved. The τ_2 was found to have a method error and CV of ± 15.6 s and 14.4% at the onset and ± 16.4 s and 32.1% at the cessation of exercise. The LOA analysis revealed the test-retest measurement error equated to 17% and 19% of the grand mean τ_2 values for exercise onset and cessation respectively. The A_2 values were found to be more variable, with LOA analysis showing the measurement error between the test-retest data to be proportional to 20% and 35% of the grand means for A_{2on} and A_{2off} respectively.

The improvement in phase III parameter reproducibility was matched by an enhancement in the signal to noise ratio from that observed in the previous study from 39% to 27% for the on-transient and 38% to 23% for the off-transient. Consequently, the model fit to the phase III response appears to have been improved, as the mean sum of squares for the last 100 s of the exercise transient (see chapter 3.2.5.6) is substantially less for this (on-transient, 2947 ± 1036; off-transient, 3010 ± 1056) than the previous study (on-transient, 10516 ± 4416; off-transient, 7899 ± 377). However, it is clear that the enhancement of the signal to noise ratio by 12% for the on-transient and 15% for the off-transient was not sufficient to improve the reproducibility of the phase III parameters to the level observed for phase II parameters. For example, the 95% CI (Lamarra *et al.*, 1987) for τ_2 are still large, ranging from ± 16.7 s during the on-transient to ± 38.9 s for the off-transient. It remains to be established if such variability in phase

III measures will be greater than any change induced to the slow component responses of soccer players through high-intensity intermittent training in future studies of this thesis.

It would appear that greater improvements in the reproducibility of phase III measures were not achieved as the modifications made to the repeated square-wave transition protocol did not increase the amplitude and reduce the noise in the $\dot{V}O_2$ response as expected. For exercise onset, the increase in the number of ensemble averaged transitions from two to four did reduce the noise in the phase III $\dot{V}O_2$ response. This is demonstrated by the smaller ± S_o of the breath-by-breath noise observed in the first assessment of $\dot{V}O_2$ kinetics in this (On-transient, ± 76 ml.min^{-1}) compared to the previous study of this thesis (On-transient, ± 116 ml.min^{-1}). However, this was partly negated by the observation that exercising at a heavier intensity did not increase the signal (A_{2on}) as anticipated. The mean A_{2on} for 80%Δ (277 ± 143 ml.min^{-1}) running was not larger than for 50%Δ (293 ± 135 ml.min^{-1}).

In contrast, for exercise cessation, the increased number of transitions did not considerably reduce the breath-by-breath noise in this (± S_o = 41 ml.min^{-1}) compared to the previous study (± S_o = 47 ml.min^{-1}). It appears this was compensated for to some extent by the A_2 during the off-transient being larger for 80%Δ (179 ± 68 ml.min^{-1}) than 50%Δ (122 ± 64 ml.mn^{-1}) running as expected. Further research is required to identify whether a further increase in the number and intensity of exercise transitions would produce an increase in the amplitude but reduction in the noise of the $\dot{V}O_2$ response for both transients of exercise. This might then allow more reproducible phase III measures to be obtained. However, it is likely that the use of such an intensive protocol in this thesis would exclude elite soccer players from participating, as the time and physical

effort required would impact on their daily training regime during the competitive season.

Despite the above findings, the repeated exercise transition protocol of four square-wave runs at 80%Δ will be used in subsequent studies as it does provide more reproducible measures than two transitions of 50%Δ running. Furthermore, the mean 80%Δ speed of 16.3 ± 1.4 $km.h^{-1}$ is greater than that of ≥15.0 $km.h^{-1}$ used in recent match analysis studies (Mohr *et al.*, 2003) to define the lower boundary of high-intensity running. Therefore, using such a very heavy-intensity protocol will ensure that the measurement of phase II $\dot{V}O_2$ kinetic parameters to the onset and cessation of running will occur at speeds that are classified as high-intensity in relation to soccer performance.

As the overall aim of this thesis is to assess the role of $\dot{V}O_2$ kinetics in soccer performance, ideally, this and the previous reproducibility studies should have been conducted using professional soccer players. However, the recreationally active individuals who participated in the reproducibility studies have $\dot{V}O_2$ max and GET values within the range previously reported for professional soccer players (Reilly, 1996; Edwards *et al.*, 2003). Therefore, the reproducibility of $\dot{V}O_2$ kinetics will have been assessed at exercise intensities similar to those that would be performed by professional soccer players if they were to undertake a 50%Δ or 80%Δ square-wave treadmill protocol. This indicates that the findings of the reproducibility studies are applicable to future investigations examining the role of $\dot{V}O_2$ kinetics in soccer performance.

6.5 Conclusion

The findings from this study show that the kinetic parameters measured during the on- and off-transients of a repeated very heavy-intensity treadmill running protocol do not become distorted after the first transition. Furthermore, $\dot{V}O_2$ kinetic parameters were reproducible for the phase II $\dot{V}O_2$ response. Although the modifications to the square-wave treadmill protocol improved the signal to noise ratio for phase III, they did not lead to a proportional improvement in phase III parameter reproducibility. It is yet to be determined whether the test-retest variability for the phase III parameters will mask any manipulation of the slow component response through training.

CHAPTER 7

Pulmonary $\dot{V}O_2$ kinetics and performance in amateur and professional soccer players

7.1 Introduction

Soccer is an intermittent sport, where a player is required to perform prolonged periods of low-intensity running sporadically interspersed by sprints and high-intensity runs (Mohr *et al.*, 2003). As low-intensity running ($\leq$12 km.h^{-1}) is not physically taxing for elite soccer players (Balsom, 2001), it has been proposed that it is the capability to repeatedly perform the high-intensity runs (>15 km.h^{-1}) that is most important for soccer performance (Bangsbo, 1994). A soccer player's performance could therefore be enhanced through the identification and manipulation of the physiological mechanisms that determine the capability to repeatedly perform high-intensity exercise.

Aerobic metabolism has been shown to play a fundamental role during repeated high-intensity exercise (Spriet, 1995; Bogdanis *et al.*, 1996), and its importance for soccer performance is highlighted by the high GET (Edwards *et al.*, 2003) and $\dot{V}O_2$ max values (Ekblom, 1986; Bangsbo, 1994) observed for elite soccer players. However, recent investigations have reported that $\dot{V}O_2$ max is an insensitive predictor of soccer-specific intermittent high-intensity running capacity. As the ability to utilise O_2 effectively to facilitate a change in exercise intensity, or to assist a rapid recovery between bouts of activity is an aerobic necessity of prolonged intermittent exercise performance, it is possible that the speed and amplitude of a players $\dot{V}O_2$ kinetics rather than $\dot{V}O_2$ max that is more important for successful soccer performance. Rapid phase II kinetics at the onset of high-intensity running would reduce the demand placed on the anaerobic energy pathways, so reducing the oxygen deficit and lowering lactate

accumulation (Hagberg *et al.*, 1980; Demarle *et al.*, 2001). Fast phase II recovery kinetics might indicate that the performer has replenished their phosphate stores, removed metabolic end products and is physiologically ready for another bout of exercise (Gaesser and Brooks, 1984). Furthermore, as the slow component appears to be associated with fatigue processes (Poole *et al.*, 1994), it is possible that a reduced slow component during high-intensity running would increase exercise tolerance and hence performance.

Currently, no research appears to have been conducted to firmly establish the role of on- and off-transient $\dot{V}O_2$ kinetics in high-intensity intermittent exercise performance. A small number of studies have demonstrated however, that elite performers in continuous type sports possess enhanced $\dot{V}O_2$ kinetic profiles compared to non-elite performers. An early investigation by Cerretelli *et al.* (1979) showed that the half-time for the increase in $\dot{V}O_2$ during arm cranking at the same intensity was significantly faster for trained than untrained kayakers. In more recent studies, τ_{1on} has been reported to be quicker for trained than untrained cyclists (Koppo *et al.*, 2004) and for long compared to middle distance runners (Kilding *et al.*, 2003). The amplitude of the slow component has also been found to be smaller for highly trained endurance cyclists than recreationally active individuals (Russell *et al.*, 2002).

It has also been demonstrated that following short periods of endurance training, enhanced continuous high-intensity exercise capacity is matched with a speeding of phase II τ_1 at the onset of running (Demarle *et al.*, 2001) and cycling (Norris and Peterson, 1998) in the absence of a significant change in $\dot{V}O_2$ max. With regards to phase III responses, Carter *et al.* (2000b) reported that following six weeks of training, the slow component was similar or slightly reduced at a faster 50%Δ running speed than

before the training with no change in $\dot{V}O_2$ max. The authors state this would suggest an enhanced exercise tolerance, which would be beneficial for performance.

The purpose of this study is therefore to identify whether $\dot{V}O_2$ kinetics of exercise and recovery play a determining role in high-intensity intermittent running capacity. It will be assessed whether $\dot{V}O_2$ kinetics can discriminate between elite (professional) and non elite (amateur) soccer players, and whether any difference in the $\dot{V}O_2$ kinetic profile between the two groups is associated with an enhanced capacity for soccer-specific running.

7.1.1 Aims

1. To identify if the $\dot{V}O_2$ kinetic profile for an elite soccer player differs from that of a non-elite player.

2. To determine if $\dot{V}O_2$ kinetics are related to the capability to perform soccer-specific high-intensity running.

7.2 Participants and methods

7.2.1 Participants

With institutional ethics approval, 18 professional (Pro) soccer players (mean ± SD): age 23.2 ± 2.4 years, stature 180.3 ± 6.6 cm, body mass 76.4 ± 7.5 kg, and 18 amateur (Am) soccer players (mean ± SD): age 21.1 ± 1.6 years, stature 179.3 ± 8.2 cm, body mass 75.8 ± 11.4 kg participated. All Pro players had been on a full time contract at an English first division professional club for at least 2 years. During the stage of the season that this study was conducted, a typical week for the Pro players consisted five training sessions and one competitive game. Each Am played in a local amateur league and did not play or train more than 3 times per week. Training diaries for the two groups can be seen in appendix 12.1, page 247. Prior to the administration of any test, participants were screened for existing medical conditions that might become aggravated during the testing procedure (appendix 6, page 238). Pre-test instructions can be seen in chapter 3.2.1.4.

7.2.2 Experimental design

All participants performed 4 exercise tests, each separated by 4 days. The first two tests were the incremental exercise test to exhaustion and the repeated very heavy-intensity square-wave protocol. The remaining two tests were field based assessments of soccer-specific fitness, the YoYo Intermittent Recovery Test level 2 (YIRT2) and repeated sprint test (RST). Both laboratory and field tests were performed at approximately the same time of day to reduce the effects of diurnal variation. Each time players visited the laboratory stature and body mass were measured and heart rate was recorded at 5 s intervals during each assessment. Temperature in the laboratory was kept within 20°C ± 1°C.

7.2.3 Experimental protocols

All laboratory based exercise tests were performed on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany). Pulmonary gas exchange (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA) was measured on a breath-by-breath basis during an incremental exercise test to volitional exhaustion for the identification of $\dot{V}O_2$ values and running speeds that corresponded to $\dot{V}O_2$ max, GET and 80%Δ (see chapter 3.2.4). These running speeds were then used to design a repeated very heavy-intensity square-wave transition protocol that consisted of 4, 6 min runs at 80%Δ. Each run was separated by a 12 min walk and a further 30 min of passive recovery (see chapter 3.2.5.2). Pulmonary gas exchange was measured through out the test (MGA 1100 mass spectrometer, Marquette Electronics Inc, Milwaukee, WI, USA) for the determination of $\dot{V}O_2$ kinetics.

The field based tests of soccer-specific fitness were performed outdoors on a dry artificial grass surface. The YIRT2 is an incremental and maximal test that provides a measure of high-intensity intermittent running capacity. The test involves running back and forth along a 25 m track in an intermittent fashion. The running speed was dictated by audible signals generated from a cassette tape. Once participants were unable to maintain the dictated running speed they were withdrawn from the test (see chapter 3.2.7). The test result is the total distance covered. The RST assesses a player's capability to maintain performance over 7 maximal sprints along a 30m course involving a 5 m deviation to the left. Each sprint is separated by 25 s, during which the participant must jog back to the start point (see chapter 3.2.8). The test provides measures of best sprint time, mean sprint time for the 7 sprints and fatigue index.

7.2.4 Data Analysis

Breath-by-breath pulmonary gas exchange data collected during the incremental exercise tests and repeated square-wave transition protocol were analysed following the procedures outlined in chapter 3.2.4.1 and 3.2.5.3 respectively. The equation used to calculate oxygen deficit (DO_2) (Demarle *et al.*, 2001) is presented in chapter 3.2.5.7.

7.2.5 Statistical analyses

To assess if any differences existed between the $\dot{V}O_2$ kinetic parameters for Pro and Am players, a mixed design two way analysis of variance was used. Independent sample t-tests were performed to check for differences in $\dot{V}O_2$ max, GET and field test performance between the Pro and Am players. Pearson's Correlation coefficient was conducted to identify if any relationships existed between the physiological variables measured for Pro and Am players. Statistical significance was set at $P < 0.05$.

7.3 Results

7.3.1 Incremental exercise test performance

The physiological and performance measures recorded during the incremental exercise test to exhaustion for Pro and Am soccer players are listed in Table 7.1. Independent sample t-tests (see appendix 12.2, page 248) revealed no difference (P>0.05) between the physiological and performance measures of Pro and Am players.

Table 7.1 Mean (± SD) physiological and performance measures for Pro (n = 18) and Am (n = 18) players recorded during the incremental exercise test to exhaustion.

Measure	Pro	Am
$\dot{V}O_2$ max (ml.kg^{-1}.min.$^{-1}$)	56.5 ± 2.9	55.7 ± 3.5
$\dot{V}O_2$ max (ml.min^{-1})	4316 ± 221	4272 ± 265
Maximal speed (km.h^{-1})	18.5 ± 0.7	18.6 ± 1.1
Time to exhaustion (min)	11.4 ± 1.2	11.6 ± 0.9
Maximal HR (b.min^{-1})	191 ± 8	193 ± 5
GET (ml.kg^{-1}.min^{-1})	40.6 ± 2.6	38.4 ± 3.2
GET (ml.min^{-1})	3096 ± 188	2925 ± 250
GET as % of $\dot{V}O_2$ max	72 ± 6	69 ± 6

7.3.2 Physiological and $\dot{V}O_2$ kinetic measures

The $\dot{V}O_2$ kinetic responses of Pro and Am players to very heavy-intensity treadmill running were very similar and are presented in Table 7.2. The mixed design two-way ANOVA showed no difference between the two groups at exercise onset or cessation for τ_1 (P=0.923). The τ_2 was however shorter for the Pro players (P=0.034), although independent sample t-tests showed this was not accompanied by a difference in the phase III DO_2 (P=0.086) or total DO_2 (P=0.154) between the two groups. The mean

value for τ_1 was found to be smaller for exercise onset than cessation for Pro and Am (P=0.026), this was also the case for τ_2 (P=0.001).

Table 7.2 The $\dot{V}O_2$ kinetic parameters for Pro (n = 18) and Am (n = 18) soccer players (mean ± SD) measured during the on-transients of very heavy-intensity treadmill running.

Measure	**Professional**	**Amateur**
Speed ($km.h^{-1}$)	16.5 ± 1.2	16.2 ± 1.1
$\dot{V}O_2$ ($ml.min^{-1}$)	3884 ± 377	3864 ± 386
HR ($b.min^{-1}$)	179 ± 8	174 ± 11
T_{D1} (s)	7.7 ± 3.1	8.3 ± 2.8
T_{D2} (s)	126.3 ± 13.4	124.7 ± 106.5
τ_1 (s)	24.5 ± 3.2	24.7 ± 1.8
τ_2 (s)	98.2 ± 36.6	142.4 ± 48.6*
A_1 ($ml.min^{-1}$)	2606 ± 263	2641 ± 159
A_2 ($ml.min^{-1}$)	279 ± 124	301 ± 135
DO_2 for Phase II (ml)	1406 ± 42	1438 ± 96
DO_2 for Phase III (ml)	1173 ± 71	1245 ± 52
DO_2 total (ml)	2579 ± 85	2683 ± 96

* Difference between Pro and Am players, P<0.05.

Table 7.3 The $\dot{V}O_2$ kinetic parameters for Pro (n = 18) and Am (n = 18) soccer players (mean ± SD) measured during the off-transients of very heavy-intensity treadmill running.

Measure	Professional	Amateur
Speed ($km.h^{-1}$)	4.0 ± 0.0	4.0 ± 0.0
T_{D1} (s)	9.1 ± 2.7	8.3 ± 3.5
τ_1 (s)	28.7 ± 2.8	29.3 ± 3.5
τ_2 (s)	261.7 ± 50.2	277.3 ± 41.8
A_1 ($ml.min^{-1}$)	2606 ± 322	2610 ± 248
A_2 ($ml.min^{-1}$)	279 ± 111	291 ± 93

The mixed design two-way ANOVA revealed no difference between Pro and Am for A_1 (P=0.122) at either transient of exercise. The mean value for A_{1on} did not differ from A_{1off} (P=0.092). The A_2 for Am and Pro players did not differ (P=0.183) across transients. The mean A_2 value of both groups was however found to be larger during the on- than off-transient (P=0.022). The $\dot{V}O_2$ response of represenative Pro (9) and Am (4) players to the onset and cessation of very heavy-intensity treadmill running is shown below in Figure 7.1 (A and B).

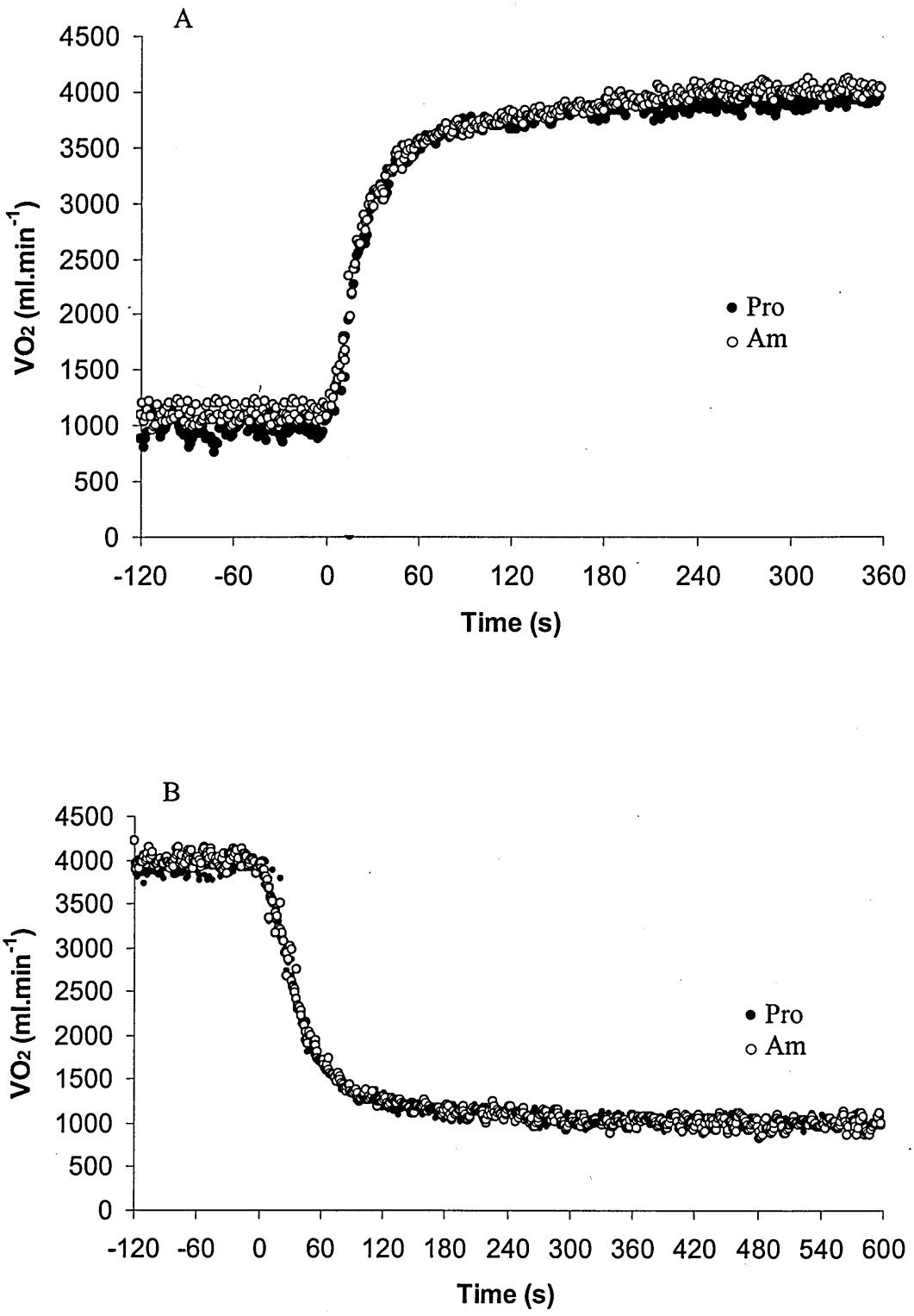


Figure 7.1 A and B. The $\dot{V}O_2$ responses from representative Pro (9) and Am (4) soccer players during the on- (A) and off-transients (B) of very heavy-intensity treadmill running.

7.3.3 Soccer-specific fitness

The performance of the Pro and Am in the field tests of soccer-specific fitness are presented in Table 7.4. The independent sample t-test revealed the Pro group to run further in the YIRT2 than the Am group (P=0.034). The Pro group also out performed the Am group in the RST, performing the course more quickly for one sprint (best time, P=0.012), maintaining a faster speed over the seven sprints (mean time for the seven sprints, P=0.014) and hence experiencing less fatigue during the test (Fatigue Index, fastest time - slowest time, P=0.024).

Table 7.4 Performance of Pro (n=18) and Am (n=18) soccer players in the in the YIRT2 and RST (mean ± SD).

Measure	Professional	Amateur
YIRT2 Distance (m)	966 ± 153	840 ± 156*
RST Best Time (s)	6.46 ± 0.27	6.84 ± 0.24*
RST Mean Time (s)	6.69 ± 0.36	7.02 ± 0.25*
RST Fatigue Index (s)	0.36 ± 0.15	0.51 ± 0.20*

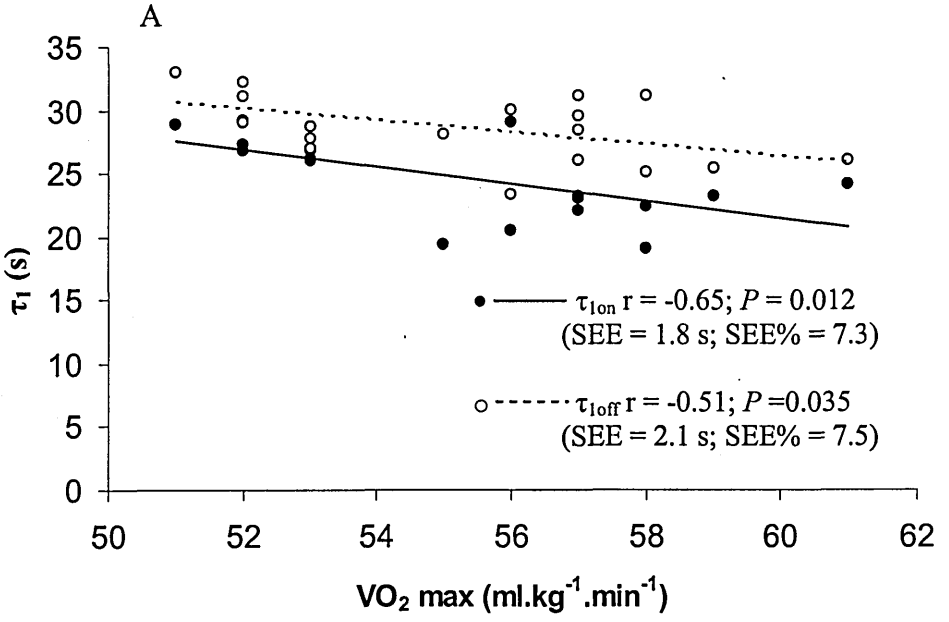
* Difference between Pro and Am players, P<0.05.

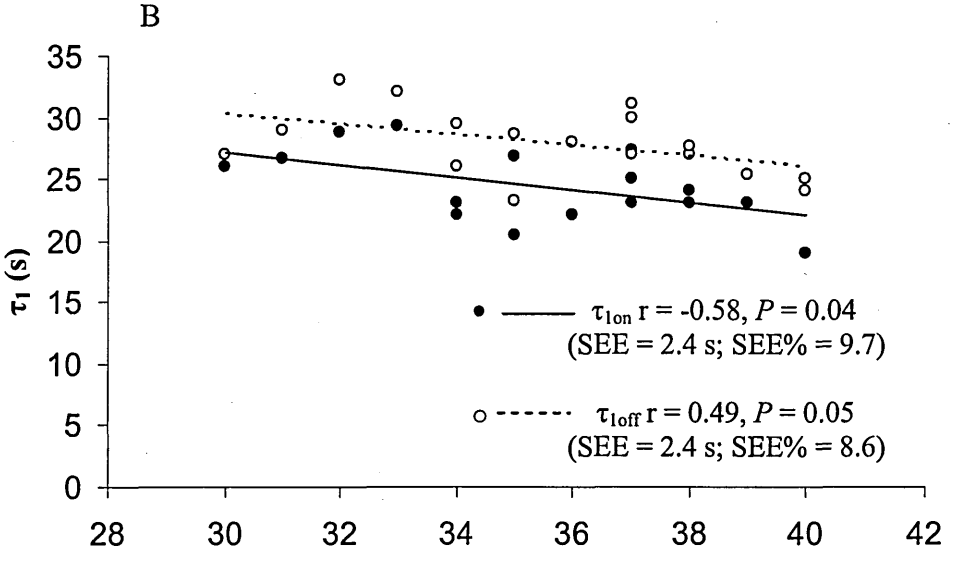
7.3.4 Relationships between physiological and performance measures

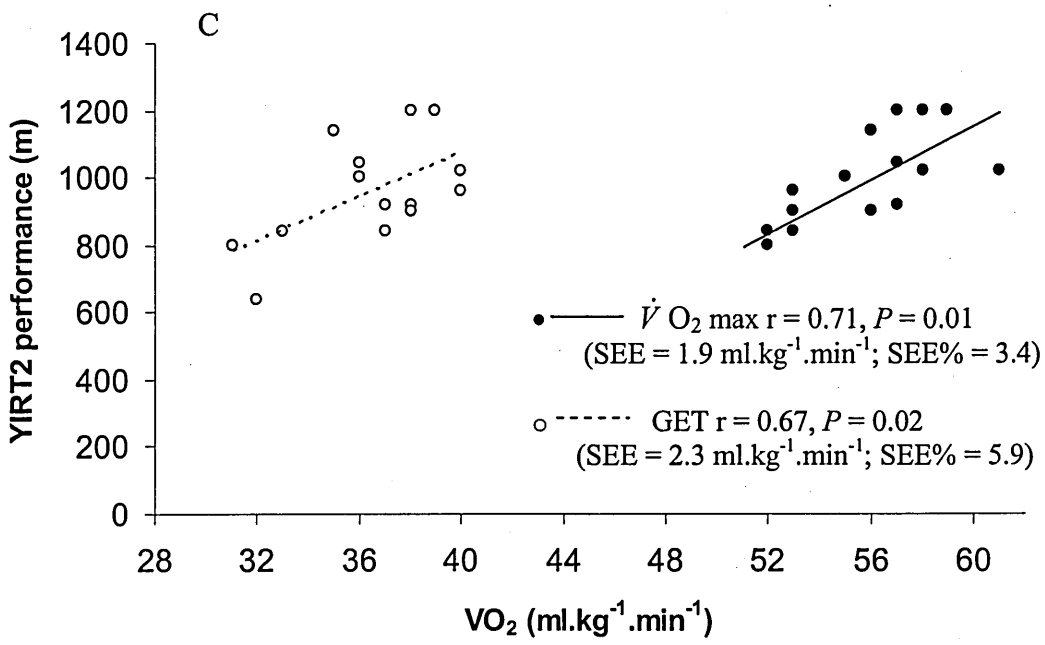
7.3.4.1 Professionals

The relationships between measures of $\dot{V}O_2$ kinetics, aerobic fitness and soccer-specific fitness are illustrated in Figure 7.2 (A to D). The $\dot{V}O_2$ max of Pro players was inversely related with τ_{1on} (r = -0.65; P=0.012) and τ_{1off} (r = -0.51; P=0.035), but positively correlated with the distance run during the YIRT2 (r = 0.71; P=0.011). Similar but less strong associations were found between GET and τ_{1on} (r = -0.58; P=0.048), τ_{1off} (r = -0.49; P=0.052) and YIRT2 distance (r = 0.68; P=0.016). Distance in the YIRT2 was also inversely correlated with τ_{1on} (r = -0.71; P = 0.013) and τ_{1off} (r =

-0.63; P=0.021). A significant correlation was found between τ_{1on} and τ_{1off} (r =0.58; P=0.024) and A_{1on} and A_{1off} (r = 0.55; P=0.034). A full correlation matrix can be seen in appendix 12.2, page 249 and 250.







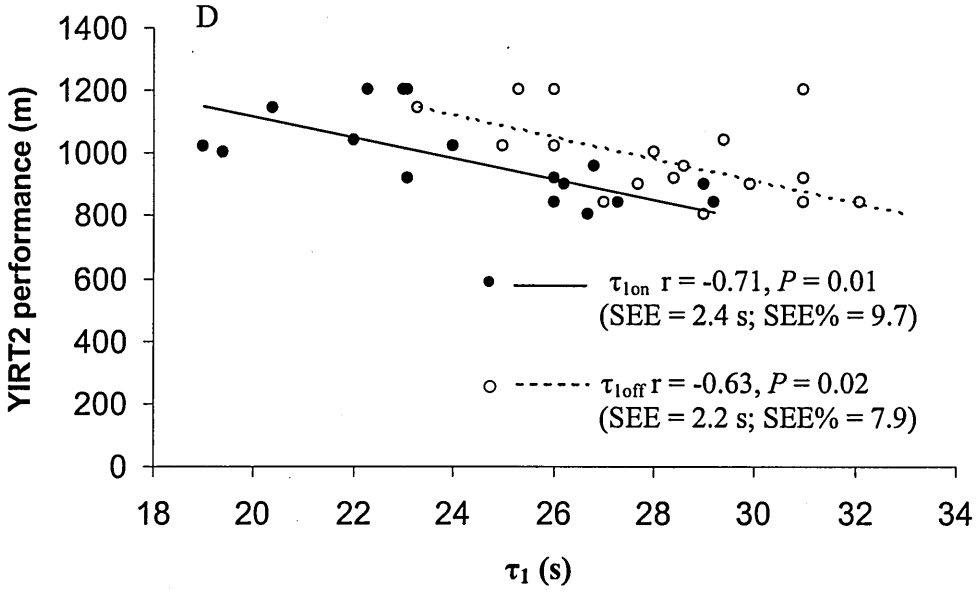
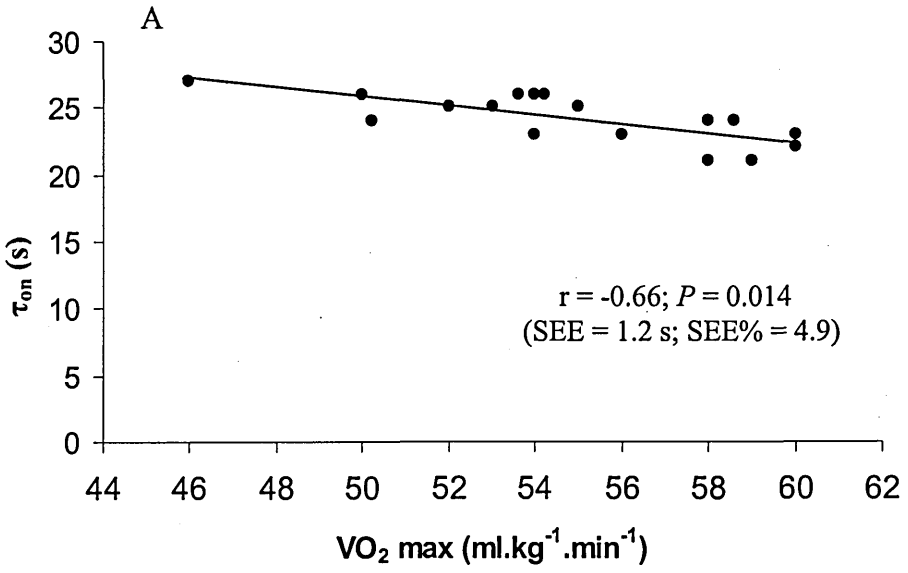
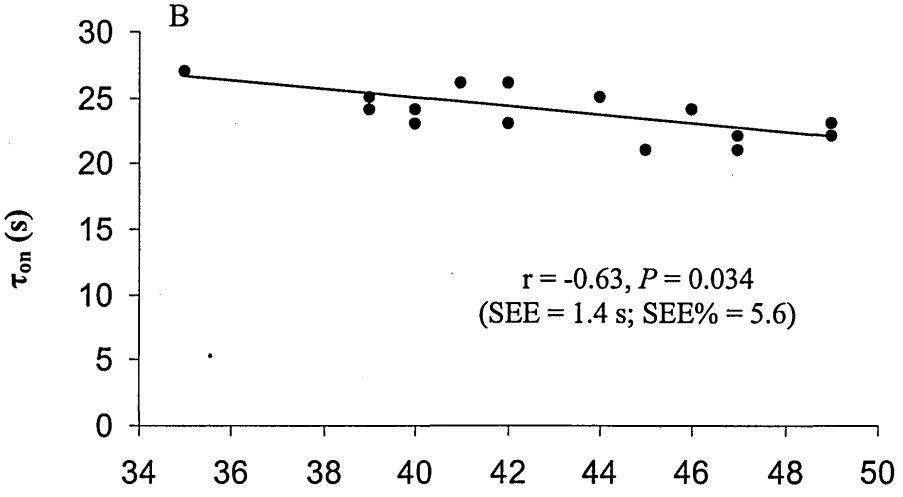


Figure 7.2 A to D. Significant correlations for Pro players (n = 18) between $\dot{V}$ O_2 max and τ_1 (A), GET and τ_1 (B), $\dot{V}$ O_2 max and GET with YIRT2 distance (C) and τ_1 with YIRT2 distance (D).

7.3.4.2 Amateurs

The correlations between measures of $\dot{V}O_2$ kinetics, aerobic fitness and soccer-specific fitness are illustrated in Figure 7.3 (A to D). The $\dot{V}O_2$ max of Am players was inversely related to τ_{1on} (r = -0.66; P=0.012) but unlike Pro players it was not significantly correlated with τ_{1off} (r = -0.34; P=0.14). The $\dot{V}O_2$ max of Am players is strongly correlated with YIRT2 performance (r = 0.78; P=0.001). As for Pro players, GET was also associated with τ_{1on} (r = -0.63; P=0.045) and YIRT2 performance (r = 0.58; P=0.037). The τ_{1on} was correlated with YIRT2 (r = -0.69; P=0.001) but τ_{off} was not (r = -0.34; P=0.091). A significant correlation was also observed between τ_{1on} and τ_{off} (r = 0.53; P=0.045) and A_{1on} and A_{1off} (r = 0.52; P=0.041).


A
30
25
20
15
10
5
0
τ_{on} (s)
r = -0.66; P = 0.014
(SEE = 1.2 s; SEE% = 4.9)
44
46
48
50
52
54
56
58
60
62
VO_2 max (ml.kg^{-1}.min^{-1})


B
30
25
20
15
10
5
0
τ_{on} (s)
r = -0.63, P = 0.034
(SEE = 1.4 s; SEE% = 5.6)
34
36
38
40
42
44
46
48
50

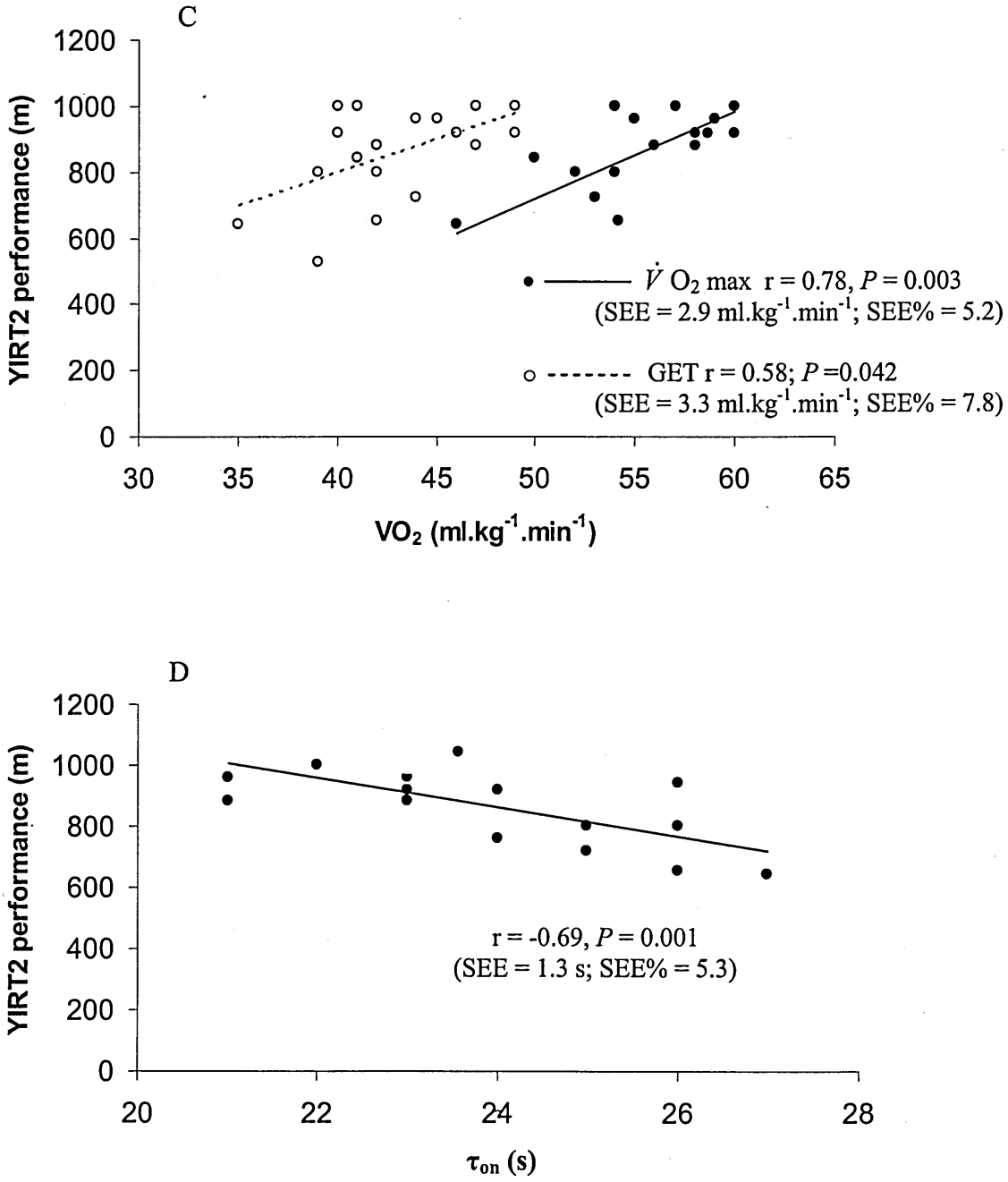


Figure 7.3 A to D. Significant correlations for Am players (n = 18) between $\dot{V}$ O_2 max and τ_{1on} (A), GET and τ_{1on} (B), YIRT2 distance and $\dot{V}$ O_2 max (C) and YIRT2 distance and τ_{1on} (D).

7.4 Discussion

7.4.1 Soccer-specific fitness of Pro and Am soccer players

Pro players out-performed Am players in both the YIRT2 and RST, indicating they have a greater capability to perform soccer-specific high-intensity intermittent exercise. The mean distances of 966 ± 153 m and 840 ± 156 m achieved by the Pro and Am players in the YIRT2 are both within the range of 600 to 1320 m previously observed for elite players (Bangsbo, 1996). In the RST, Pro players were faster over a single sprint (best time: Pro 6.46 ± 0.27 s; Am 6.84 ± 0.24 s), maintained a faster speed over the seven sprints (mean time: Pro 6.69 ± 0.36 s; Am 6.84 ± 0.24 s) and hence experienced less fatigue (fatigue index: Pro 0.36 ± 0.15 s; Am 0.51 ± 0.2 s). However, performance of both groups was comparable or superior to that previously reported by Bangsbo (1994) for elite Danish players (Best time 6.80 s; Mean time 7.10 s; Fatigue index 0.64 s). Although the soccer-specific fitness of the Am in this study is lower than that of the Pro players, it compares well to that previously reported for elite players, indicating the group of Am players used in this study were relatively well trained.

7.4.2 Soccer performance and $\dot{V}O_2$ kinetics

7.4.2.1 Phase II kinetics

There was an inverse relationship between YIRT2 performance and τ_1 at both exercise onset and cessation for Pro players, while the relationship was only found to be significant at exercise onset for Am players. In contrast, no associations were detected between RST performance measures and any phase II $\dot{V}O_2$ kinetic parameters for either group. Such findings suggest that quick $\dot{V}O_2$ kinetics are important for the performance of prolonged high-intensity running. However, $\dot{V}O_2$ kinetics do not account for the greater intermittent high-intensity running capacity of the Pro players as the τ_1 did not differ between player groups (Pro, on-transient, 24.5 ± 3.2 s; off-transient, 28.7 ± 2.8 s:

Am, on-transient, 24.7 ± 1.8 s; off-transient, 29.3 ± 3.5 s). Comparison with previous research also reveals equivalent or quicker phase II τ values at the onset (21.5 ± 8.5 s Barstow *et al.*, 1996; 21.2 ± 8.2 s Koga *et al.*, 1999; 19.1 ± 1.4 s Carter *et al.*, 2000a) and cessation (36.8 ± 1.9 s, Paterson and Whipp, 1991; 33 ± 6.4 s, Koga *et al.*, 1999; 39.9 ± 3 s, Carter *et al.*, 2000a) of exercise for recreationally active and endurance trained individuals. This indicates that the higher frequency of intermittent training undertaken by Pro players does not speed $\dot{V}O_2$ responses to the on- and off-transients of very heavy-intensity running compared to Am players. Consequently, a rapid onset of oxidative metabolism to reduce the oxygen deficit, combined with the quick replenishment of PCr during recovery, do not appear to be the determinants of superior performance in the YIRT2 and RST in this study.

An explanation why phase II $\dot{V}O_2$ kinetics are associated with YIRT2 performance but do not discriminate between players with differing soccer-specific fitness can be estimated from further analysis of the correlation data. A player's $\dot{V}O_2$ max and GET were positively associated with YIRT2 performance, which indicates the importance of aerobic metabolism for the soccer-specific high-intensity exercise. In addition, $\dot{V}O_2$ max and GET were inversely related with τ_{1on} and τ_{1off}, which suggests τ provide similar information about a player's aerobic conditioning as $\dot{V}O_2$ max and GET. Therefore, it seems that the association between τ_1 and YIRT2 performance is a consequence of the importance of aerobic energy production for the performance of soccer-specific exercise. Based on these observations, if it is the case that $\dot{V}O_2$ max is not a determining factor for soccer performance among elite players as has been proposed by Krustrup *et al.* (2003), then it is conceivable that neither will phase II τ. This would explain why the Pro soccer players in the current investigation ran substantially further

than Am players in the YIRT2, even though they were matched for $\dot{V}O_2$ max and phase II $\dot{V}O_2$ kinetic responses.

7.4.2.2 Phase III kinetics

The only observed difference in aerobic measures between the Pro and Am players was the time constant of the slow component response (τ_{2on}), which was longer in Am (P=0.034) despite the similarity in A_2 between the two groups. However, any physiological significance of the quicker $\tau_{2\,on}$ of the Pro is not supported by differences (P=0.086) in the phase III DO_2 of the two groups (Pro 1173 ± 71 ml; Am 1245 ± 62 ml), which was only 6% larger for the Am players. This suggests that disparity in soccer-specific fitness between Pro and Am players cannot be attributed to differences in the speed of the slow component response. Furthermore, previous observations have demonstrated that it is the amplitude of the slow component that is critical for performance (Carter *et al.*, 2000a), with a decreased amplitude following training being associated with enhanced tolerance of high-intensity exercise.

The most plausible rationale for the difference observed for $\tau_{2\,on}$ between Pro and Am players relates to the large inter-participant measurement variability rather than physiological parameters. Large test-retest variability for phase III parameters measured during the on- and off-transients of heavy- and very heavy-intensity running is demonstrated in chapters 5.3.3 and 6.3.2 of this thesis, while large measurement variability has previously been associated with phase III $\dot{V}O_2$ kinetics during cycling (Özyener *et al.* 2001). Therefore, it cannot be concluded that the difference in τ_2 between the two groups is the result of a difference in training status and hence performance potential.

7.4.2.3 Why $\dot{V}O_2$ kinetics do not determine performance in the YIRT2 and RST

Analysis reveals that the maximal running speeds attained by players in the YIRT2 and RST ranged from 17 to 26 $km.h^{-1}$, which are similar or greater than those recorded at $\dot{V}O_2$ max in the treadmill based incremental exercise test. It is also well documented that the lower efficiency of intermittent compared to continuous running results in an increased metabolic load (Christensen *et al.*, 1960). As a consequence of these two factors, it is possible that the intensity of the runs during substantial periods of the YIRT2 and RST will have been above $\dot{V}O_2$ max, which suggests therefore that a player's capability to perform such an intensity and pattern of exercise is heavily influenced by their potential for anaerobic energy provision. Hence, the capacity for soccer-specific high-intensity running might not benefit from a reduced DO_2 at the onset of exercise due to quick $\dot{V}O_2$ kinetics, as the nature of the exercise demands a considerable anaerobic contribution.

The design of the soccer-specific performance tests used in this study make it difficult to establish why off-transient $\dot{V}O_2$ kinetics do not appear to be a determining factor in soccer performance. Previous research suggests that the short periods separating runs in the YIRT2 (10 s) and RST (25 s) will have limited the extent to which recovery processes such as resynthesis of PCr stores (Balsom, 1992) and reversal of muscle pH (Bogdanis *et al.*, 1996) occurred, potentially inhibiting any advantage to be gained from possessing quick recovery kinetics. In contrast, the longer recoveries observed to separate high-intensity runs during competitive games (~90s, Withers *et al.*, 1982) might provide further information about the role off-transient kinetics play in soccer performance, as it would allow any physiological benefits associated with having quicker off-kinetics to become more evident. However, the practical implications of performing match analysis studies to directly assess a player's high-intensity running

capabilities preclude their inclusion from this thesis. With the exception of match analysis data, the YIRT2 and RST are two of the strongest and most practical indirect measures of a soccer player's high-intensity running capability.

The superior performance of the Pro players could be attributable to a larger anaerobic capacity than the Am players, as this would have enabled them to run for longer in the YIRT2 and RST at supra-$\dot{V}O_2$ max intensities. Previous research has demonstrated continuous high-intensity shuttle running capability to be associated with anaerobic capacity (Ramsbottom *et al.*, 2001), although this has not been established for soccer performance. Therefore, further research is warranted to determine whether anaerobic capacity is a key determinant of soccer-specific fitness.

7.4.3 Limitations of correlation analysis

Relationships between $\dot{V}O_2$ kinetics, $\dot{V}O_2$ max and soccer-specific fitness were identified in this study using the Pearson product-moment correlation coefficient (r). However, when interpreting such statistical analyses it is important to realise that a significant relationship between two measures does not prove causation; it only shows that a non chance relationship exists. Furthermore, the strength of a relationship between two variables can be misrepresented. The magnitude of r is influenced by the ranges of the two variables under consideration (Vincent, 1995). Large ranges (heterogeneity) in one or both measures can produce high r values, where as small ranges (homogeneity) can depress r. In addition, the value and significance of r can be influenced by the size of the sample (Vincent, 1995). The number of pairs of scores (n) influences the degrees of freedom (df), which represents the number of values that are free to vary when the sum of variables is set. When n is small, it is possible that artificially high r values can be obtained by chance. In addition, when n is small, r must be high to reach significance and vice versa. The correlation coefficient r can also be

influenced by spurious data that is not representative of the sample. Such data can produce less meaningful high and low r values.

7.5 Conclusion

The Pro players were capable of performing more soccer-specific high-intensity running than Am players, despite the $\dot{V}O_2$ kinetic profiles and aerobic fitness of the two groups being indistinguishable. The findings suggest that although aerobic metabolism is important for intermittent high-intensity running capacity, other physiological mechanisms must account for the difference in soccer-specific fitness between Pro and Am players. This finding should be treated cautiously however, as it is based on a cross section of Pro and Am players, whose training status was only defined by recording the frequency of training sessions they performed in a typical week (appendix 12.1). From such data, it is not possible to precisely discern why both groups of players were matched for $\dot{V}O_2$ max and $\dot{V}O_2$ kinetics but not soccer-specific fitness, as cardiovascular and peripheral adaptations are influenced by the volume (Hickson, 1981), duration (Fox *et al.*, 1975) and intensity (Harms and Hickson, 1983) of training. Therefore, a longitudinal training study is warranted where every aspect of a player's training load can be closely manipulated. It will then be possible to establish whether an enhanced high-intensity running capacity is associated with the speeding of $\dot{V}O_2$ kinetics or other physiological processes such as anaerobic capacity.

CHAPTER 8

A training intervention to identify the physiological determinants of high-intensity soccer-specific running capacity

8.1 Introduction

The physiological determinants of performance during multiple-sprint sports such as soccer remain to be elucidated due to the diverse physical demands of competitive match-play. Elite soccer performance is, in part, dependent on a high level of cardiopulmonary fitness due to the large overall distance a player is required to run. This was demonstrated by Helgerud *et al.* (2001), who reported that an increase in the $\dot{V}O_2$ max of elite adolescent soccer players was associated with a 100% increase in the number of sprints they performed during a competitive game. However, more recent research has demonstrated that $\dot{V}O_2$ max is not correlated to high-intensity exercise during a game (Krustrup *et al.* 2003) and has not consistently been shown to reflect short-term changes in the training condition of elite soccer performers (Edwards *et al.* 2003a). Several researchers have proposed that a $\dot{V}O_2$ max of ~60 ml·kg·min^{-1} is a minimal requirement for elite professional male soccer performance (Bangsbo, 1994; Reilly *et al.* 2000), but beyond the identification of this 'threshold' it is unclear whether cardiopulmonary fitness is of direct value.

Alternative indicators of a player's aerobic status such as $\dot{V}O_2$ kinetics have received little attention. The previous study of this thesis found phase II kinetics to both the onset and cessation of very heavy-intensity running to not discriminate between soccer players who possessed differing capacities for high-intensity soccer-specific running. However, firm conclusions about the role of $\dot{V}O_2$ kinetics in soccer performance cannot be drawn from these findings as they were based on a cross-sectional comparison of

elite and non-elite players, which did not permit the relationship between changes in $\dot{V}O_2$ kinetics and high-intensity running capacity to be assessed.

As a considerable proportion of the runs classed as high-intensity during a game are above those that correspond to GET and $\dot{V}O_2$ max, it is conceivable that it is a player's ability to exercise anaerobically that might be the decisive factor for soccer performance. Although a high level of anaerobic fitness has been alluded to by previous studies (Krustrup and Bangsbo, 2001; Krustrup *et al.*, 2003) as being fundamental for the performance of soccer-specific high-intensity running, none have stated how it would benefit performance. A large anaerobic capacity indicates that an individual has an enhanced ability to derive large amounts of energy from the ATP-PC and glycolytic systems. This would potentially enable prolonged performance of supra-$\dot{V}O_2$ max running speeds.

Evidence for this hypothesis is provided by Ramsbottom *et al.* (2001), who noted that following a period of high-intensity training, improved time to exhaustion in a continuous shuttle run at 120% of $\dot{V}O_2$ max was matched by an increase in MAOD but not $\dot{V}O_2$ max. Similarly, Roberts *et al.* (1982) observed that increased run time to exhaustion during a supra-$\dot{V}O_2$ max run (16 $km.h^{-1}$ at 15% incline) following 5 weeks of high-intensity training was associated with an increase in ATP derived from anaerobic glycolysis rather than aerobic metabolism. Further support is provided by the findings that 400 m runners who are required to run at supra-$\dot{V}O_2$ max speeds for a ~ 45 s have a considerably larger anaerobic capacity as indicated by the MART than middle and long distance runners (Nummela *et al.*, 1996; Vuorimaa *et al.*, 1996). In contrast, Bangsbo and Michalsik (1993) reported similar MAOD values for a range of elite athletes whose sports required distinctly different anaerobic contributions. The

same authors also reported a large variation in MAOD among a group of elite soccer players, leading them to conclude that performance in soccer is actually determined by the rate of aerobic energy turnover or alternatively, anaerobic energy production might not be limiting to performance.

To address the equivocal findings of the above investigations, a longitudinal training study is required that will establish which physiological processes have the strongest association with an improvement in soccer-specific high-intensity running capacity. Although no clear consensus exists as to which model of training is the most effective for improving soccer-specific high-intensity running capacity, it has been reported that if a player performs two or more high-intensity training sessions per week in addition to their normal training regime, significant improvements can be made to their soccer-specific fitness (Bangsbo, 1994). A recent study by Krustrup *et al.* (2005) demonstrated 25% and 10% improvements in YIRT2 performance following eight weeks of repeated 30 s and 10 s sprint training programmes respectively. It has been suggested that the greatest performance gains are achieved if the training undertaken is sport-specific (Bangsbo, 1994). Hence, an intermittent exercise model should be used that incorporates a range of soccer-specific running speeds.

8.1.1 Aims:

1. To identify if performance in the YIRT2 can be increased after 6 weeks of soccer-specific high-intensity training.
2. To identify if it is a change in aerobic ($\dot{V}O_2$ max, GET, on- and off-transient $\dot{V}O_2$ kinetics) or anaerobic (anaerobic capacity) physiological measure that is associated with an increase in YIRT2 performance following the training intervention.

8.2 Participants and methods

8.2.1 Participants

With institutional ethics approval 16 male professional soccer players (mean ± SD): age 21.3 ± 2.1 years, stature 177.4 ± 4.2 cm, body mass 73.1 ± 8.1 kg took part. All the players had been at a professional club for at least two years. Players were randomly allocated to either the training (Tr, n = 8) or control (Cn, n = 8) group. Prior to the administration of any test, participants were screened for existing medical conditions that might become aggravated during the testing procedure (appendix 6, page 238). Pre-test instructions can be seen in chapter 3.2.1.4.

8.2.2 Experimental design

All participants performed four physiological assessments, each separated by four days. The first three assessments were the laboratory based incremental exercise test to exhaustion, repeated very heavy-intensity square-wave protocol and the MART for an indication of anaerobic capacity. The remaining test was the YIRT2. Following completion of the four assessments the Tr group undertook a 6 week high-intensity running programme (see Table 8.1) in addition to the club's normal training regime performed by the Cn group (see appendix 13.1, page 251). After the sixth week the assessments were repeated for both groups to identify if the intervention had influenced any of the physiological and performance measures. On each visit to the laboratory the participants' stature and body mass were measured and heart rate was recorded at 5 s intervals during each assessment. All assessments were performed at the same time of day to reduce the effects of diurnal variation and the temperature of the laboratory was kept within 20°C ± 1°C.

8.2.3 Experimental protocols

All laboratory based exercise tests were performed on a motorised treadmill (Saturn, HP Cosmos, Nussdorf - Traunstein, Germany). Pulmonary gas exchange was measured on a breath-by-breath basis (CPX/D, Medgraphics Corporation, St Paul, MN, USA) during an incremental exercise test to exhaustion for the identification of $\dot{V}O_2$ values and running speeds that corresponded to $\dot{V}O_2$ max, GET and 80%Δ (see chapter 3.2.4). These running speeds were then used to design a repeated very heavy-intensity square-wave transition protocol that consisted of 4, 6 min runs at 80%Δ. Each run was separated by a 12 min walk and a further 30 min of passive recovery (see chapter 3.2.5.2). Pulmonary gas exchange was measured throughout the test (CPX/D, Medgraphics Corporation, St Paul, MN, USA) to determine $\dot{V}O_2$ kinetics during the onset and cessation of very heavy-intensity treadmill running.

The final laboratory test was the MART. Briefly, the test involved 20 s running bouts separated by 100 s of passive recovery. The starting speed was 14.3 $km.h^{-1}$ and increased by 1.2 $km.h^{-1}$ for each subsequent 20 s run. The gradient of the treadmill belt was kept at 10.5%. The participant completed as many 20 s runs as possible until exhaustion (see chapter 3.2.6). Using the equation listed in chapter 3.2.6, performance in the MART provided a measure termed anaerobic power, which due to its strong association (r = 0.81) with MAOD (Maxwell and Nimmo, 1996) is used as an indicator of anaerobic capacity.

The YIRT2 was performed outdoors on a dry artificial grass surface. The YIRT2 is an incremental and maximal test that provides a measure of high-intensity intermittent running capacity. The test involved running back and forth along a 25 m track in an intermittent fashion. The running speed was dictated by audible signals generated from

a cassette tape. Once participants were unable to maintain the dictated running speed they were withdrawn from the test (see chapter 3.2.7). The test result is the total distance covered.

8.2.4 High-intensity training programme

The training programme ran for 6 weeks and consisted of three running sessions per week, all were performed outside on a soccer pitch with a natural grass surface. The running involved performing pre-determined courses (see appendix 13.2, page 252) of different lengths that incorporated changes in direction to make them more applicable to soccer. The volume of the training was gradually increased over the 6 weeks and its structure is listed below in Table 8.1. Sets of repetitions were split into sub sets, with 2 min of active recovery separating each sub set. Based on match analysis data (Van Gool *et al.*, 1988; Mohr *et al.*, 2003) each running course was performed at speeds that spanned the high-intensity spectrum ($\geq$18 km.h^{-1} to maximum sprint speed) of soccer performance as follows: session 1) ~ 19 km.h^{-1} , session 2) ~ 24 km.h^{-1} and session 3) maximal sprint (~ 30 km.h^{-1}). The duration of the runs were set at 60 s, 35 s and 10 s for sessions 1, 2 and 3 respectively to ensure that the players were capable of maintaining the desired speed for each run of each session. Players were instructed to complete each running course within a predetermined time to ensure they were running at the correct speed. The exercise to recovery ratio was 1:3, as this has previously been used in a training study that reported an increase in high-intensity shuttle running capacity (Ramsbottom *et al.*, 2001). The recovery periods consisted of low-intensity jogs back and forth along a 10-metre track. Heart rate was recorded for each session as it was intended that the participants would be exercising at $\geq$95% of HRmax. Before and after each running session participants performed an appropriate 10 min warm-up and cool down.

Table 8.1 The 6-week high-intensity intermittent training schedule followed by the training group.

Week	Session sets and repetitions		
	1	2	3
1	4 x 60 s (2 + 2)	6 x 35 s (3 + 3)	10 x 10 s (5 + 5)
2	4 x 60 s (2 + 2)	6 x 35 s (3 + 3)	10 x 10 s (5 + 5)
3	6 x 60 s (3 + 3)	8 x 35 s (4 + 4)	12 x 10 s (6 + 6)
4	6 x 60 s (3 + 3)	8 x 35 s (4 + 4)	12 x 10 s (6 + 6)
5	8 x 60 s (4 + 4)	10 x 35 s (5 + 5)	14 x 10 s (7 + 7)
6	8 x 60 s (4 + 4)	10 x 35 s (5 + 5)	14 x 10 s (7 + 7)

8.2.5 Data Analysis

Breath-by-breath pulmonary gas exchange data collected during the incremental exercise tests and repeated square-wave transition protocol were analysed following the procedures outlined in chapter 3.2.4.1 and 3.2.5.3 respectively.

8.2.6 Statistical Analyses

To determine if any differences existed for measures between and within the Tr and Cn groups, pre and post the training programme, a mixed design two-way analysis of variance analysis was performed. Pearson's correlation was conducted to assess the strength of association between measures pre and post the training intervention. Significance was set at $P<0.05$.

8.3 Results

8.3.1 Incremental exercise test performance

A two-way ANOVA mixed design found measures of aerobic fitness and exercise performance (Table 8.2) recorded during the incremental exercise test to exhaustion not to differ (P>0.05) between or within the Tr and Cn groups before or after the training intervention (see appendix 13.3, page 254).

Table 8.2 Values (mean ± SD) for aerobic and performance recorded from the incremental exercise test before and after the training intervention for the Tr (n = 8) and Cn (n = 8) groups.

	Cn		Tr	
Measure	**Before**	**After**	**Before**	**After**
$\dot{V}O_2$ max (ml.kg^{-1}.min^{-1})	57.1 ± 3.6	57.6 ± 3.1	57.6 ± 5.4	58.9 ± 4.7
GET (ml.kg^{-1}.min^{-1})	41.8 ± 1.7	40.3 ± 1.7	42.5 ± 3.6	42.8 ± 4.4
GET % of $\dot{V}O_2$ max	71 ± 4.3	72 ± 3.4	70 ± 4.2	69 ± 3.2
HR max (b.min^{-1})	192 ± 8	193 ± 7	191 ± 6	193 ± 6
Max speed (km.h^{-1})	19.0 ± 0.9	18.6 ± 0.6	19.2 ± 1.2	19.1 ± 0.9
Time to exhaustion (s)	708 ± 54	702 ± 30	705 ± 73	709 ± 74

8.3.2 Measures of $\dot{V}O_2$ kinetics

The mean $\dot{V}O_2$ kinetic parameters for the Tr and Cn groups before and after the training intervention are listed below in Table 8.3. The mixed design two-way ANOVA revealed no difference for τ_1 between (τ_{1on}, P=0.475; τ_{1off}, P=0.832) or within (τ_{1on}, P=0.568; τ_{1off}, P=0.736) Tr and Cn before or after the training intervention for either transient of exercise. Before the intervention the Cn had a quicker τ_{2on} (P=0.039) than that of the Tr (Cn τ_{2on}, 96.4 ± 38.7 s vs. Tr τ_{2on}, 133.8 ± 77.5 s). After the intervention however, τ_{2on}

for the Cn increased (P=0.012) to a value similar to that of the Tr group (Cn τ_2, 139.5 ± 55.7 s vs. Tr τ_2, 131.3 vs. 107.1 s). As a consequence, the phase III DO_2 was found to differ before and after the intervention within the Cn group (P=0.041) and between the Cn and Tr groups (P=0.038). This is further supported by a significant interaction between player group and time of measurement for phase III DO_2 (P=0.036). However, this difference in phase III DO_2 was not large enough to cause a difference in the total DO_2 (phase II DO_2 + phase III DO_2) for the onset of exercise, with no difference observed within (P=0.176) or between (P=0.218) groups before or after the intervention for total DO_2. There was also no interaction between time of measurement and player group for total DO_2 (P=0.071). This is attributable to the similar phase II DO_2 values between (P=0.195) and within (P=0.276) groups before and after the intervention. There was no interaction between player group and time of measurement for Phase II DO_2 (P=0.572). No difference was found for τ_{2off} before or after the intervention within (P=0.319) or between (P=0.461) groups. There was also no interaction between time of τ_{2off} measurement and player group (P=0.368).

Table 8.3 The physiological and $\dot{V}O_2$ kinetic parameters (mean ± SD) measured during the on-transient of very heavy-intensity treadmill running for the Cn (n = 8) and Tr (n = 8) groups before and after the training intervention.

	Cn		Tr	
Measure	**Before**	**After**	**Before**	**After**
HR ($b.min^{-1}$)	174 ± 7	176 ± 5	178 ± 9	176 ± 4
T_{D1} (s)	7.8 ± 3.5	8.6 ± 2.2	8.9 ± 3.8	8.2 ± 3.1
T_{D2} (s)	125.4 ± 11.7	128.3 ± 9.4	124.2 ± 13.2	126.6 ± 12.8
τ_1 (s)	25.7 ± 1.9	24.3 ± 2.9	24.6 ± 4.2	24.1 ± 2.3
τ_2 (s)	96.4 ± 38.7*	139.5 ± 55.7	133.8 ± 77.5	131.3 ± 107.1
A_1 ($ml.min^{-1}$)	2859 ± 114	2774 ± 142	2802 ± 226	2673 ± 185
A_2 ($ml.min^{-1}$)	363 ± 23	379 ± 54	323 ± 71	355 ± 155
DO_2 for Phase II (ml)	1478 ± 39	1437 ± 47	1458 ± 52	1423 ± 36
DO_2 for Phase III (ml)	916 ± 55*#	1045 ± 61	1009 ± 78	1028 ± 57
DO_2 total (ml)	2394 ± 62	2482 ± 47	2467 ± 89	2451 ± 75

* Difference before and after the intervention for corresponding values within the same group $P<0.05$. # Difference before the intervention for corresponding values between the different groups $P<0.05$.

Table 8.4 The $\dot{V}O_2$ kinetic parameters (mean ± SD) measured during the off-transient of very heavy-intensity treadmill running for the Cn (n = 8) and Tr (n = 8) groups before and after the training intervention.

	Cn		Tr	
Measure	**Before**	**After**	**Before**	**After**
T_{D1} (s)	8.9 ± 3.2	9.4 ± 3.4	8.4 ± 4.1	8.7 ± 3.7
τ_1 (s)	29.1 ± 1.6	28.3 ± 1.8	30.3 ± 1.2	29.8 ± 1.1
τ_2 (s)	314.6 ± 56.2	295.3 ± 42.5	300.4 ± 65.6	275.4 ± 43.6
A_1 (ml.min^{-1})	2604 ± 122	2634 ± 171	2797 ± 100	2892 ± 105
A_2 (ml.min^{-1})	131 ± 32	124 ± 41	118 ± 48	129 ± 39

A two-way mixed design ANOVA indicated that A_1 did not differ between (A_{1on}, P= 0.746; A_{1off}, P=0.474) or within (A_{1on}, P=0.274; A_{1off}, P=0.216) groups before or after the training intervention for either exercise transient. These findings were replicated for A_{2on} and A_{2off} between (A_{2on}, P=0.328; A_{2off}, P=0.104) and within (A_{2on}, P=0.735; A_{2off}, P=0.093) groups. There was no interaction for A_{2off} between time of measurement and player group (P=0.142).

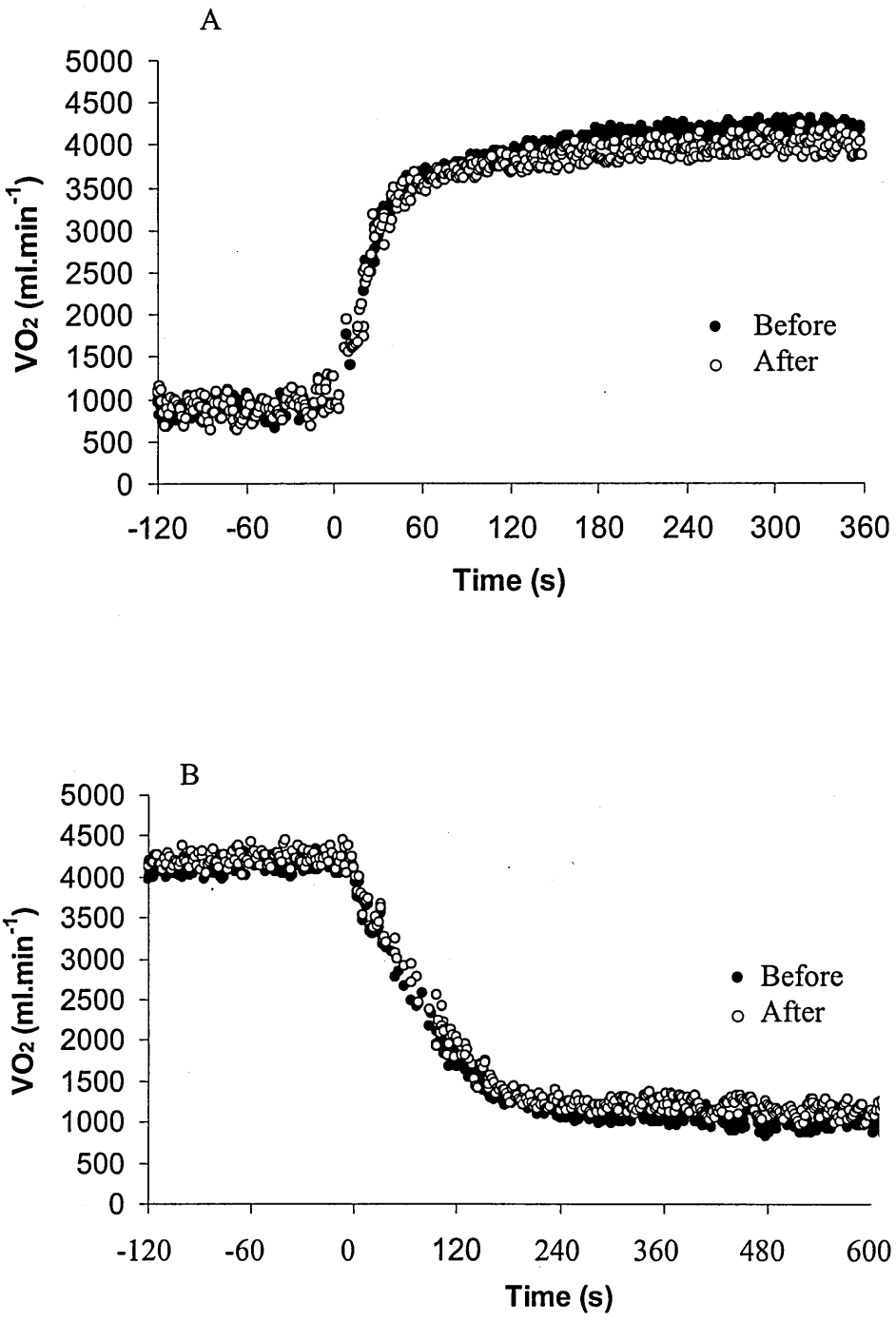


Figure 8.1 The $\dot{V}O_2$ response to the on- (A) and off-transients (B) of very heavy-intensity treadmill running for a representative participant (2) from the Tr group before (•) and after (○) the training intervention.

8.3.3 MART performance

Measures obtained from the MART are listed in Table 8.5. The two-way ANOVA mixed design revealed the anaerobic power (P=0.021), time to exhaustion (P=0.019) and maximal running speed (P=0.023) to only increase for the Tr group following the training intervention, and as a result were greater for the Tr than the Cn group (anaerobic power, P=0.024; time to exhaustion, P=0.012; maximal running speed, P=0.037). Blood lactate did not differ between (P=1.217) or within groups (P=1.246) before and after the training intervention.

8.3.4 YIRT2 performance

A two-way mixed ANOVA showed that following the training intervention, distance run increased for the Tr (P=0.015), consequently the Tr group were capable of running further in the YIRT2 after the intervention that the Cn group (P=0.011).

Table 8.5 Performance and physiological measures recorded from the YIRT2 and MART for the Cn (n = 8) and Tr (n = 8) groups before and after the training intervention (mean ± SD).

	Cn		Tr	
Measure	**Before**	**After**	**Before**	**After**
YIRT2 Distance (m)	891 ± 46	888 ± 42	896 ± 37	987 ± 44**$^{\#\#}$
YIRT2 HR max (b.min^{-1})	190 ± 5	191 ± 4	193 ± 6	192 ± 7
MART Power (ml.kg^{-1}.min^{-1})	113.1 ± 5.1	112.6 ± 5.7	115.2 ± 4.0	124.2 ± 5.2*$^{\#\#}$
MART Speed (km.h^{-1})	22.3 ± 1.0	22.6 ± 1.4	22.4 ± 1.2	24.6 ± 1.1*$^{\#}$
MART Time (s)	170 ± 16	172 ± 16	167 ± 18	184 ± 16*$^{\#\#}$
MART [Hla] mmol.l^{-1})	17.6 ± 1.3	16.7 ± 1.7	17.2 ± 1.2	17.4 ± 1.4

* Difference after the intervention within a group, P<0.05; ** Difference after the intervention within a group, P<0.01; $^{\#}$ Difference after the intervention for corresponding values between groups, P<0.05; $^{\#\#}$ Difference after the intervention for corresponding values between groups, P<0.01.

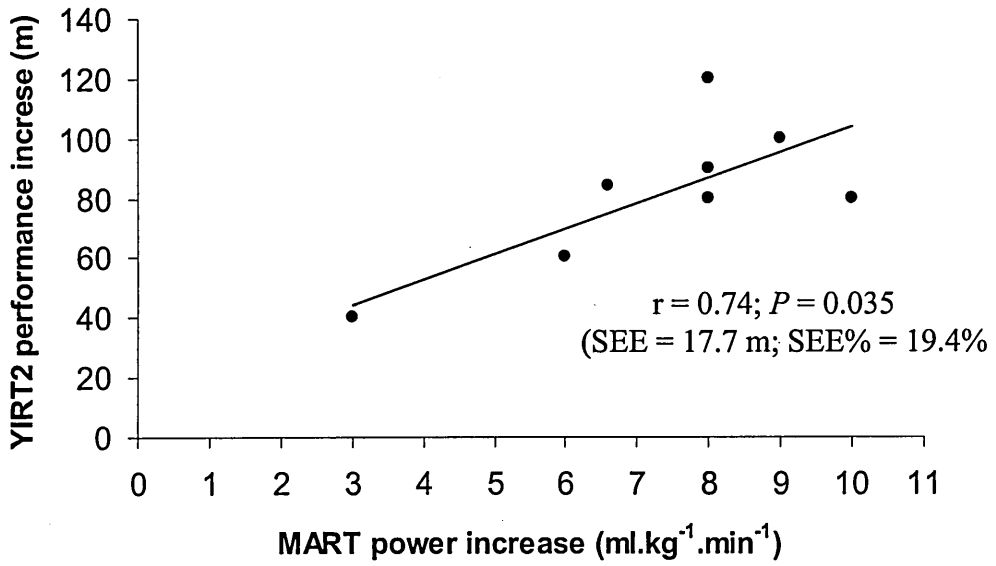
8.3.5 Relationship between YIRT2 performance and physiological measures

A full correlational matrix is listed in appendix 13.3, page 256. The association detected between YIRT2 performance and $\dot{V}O_2$ max was not found to change before or after the training intervention for the Cn (Pre: r = 0.71; P=0.041; Post: r = 0.73; P=0.033) or Tr groups (Pre: r = 0.69; P=0.031; Post r = 0.70; P=0.030). In comparison, τ_{1on} was inversley related with YIRT2 performance before and after the intervention for the Tr (Pre r = -0.65; P=0.032: Post r = -0.66; P=0.034) and Cn (Pre r = -0.75; P=0.029: Post r = -0.71; n = 8; P=0.030) groups, although no change in the strength of the association between measures is apparent for either group. The τ_{1off} was not related to YIRT2 performance before or after the intervention for either Tr (Pre r = -032; n = 8; P=0.068:

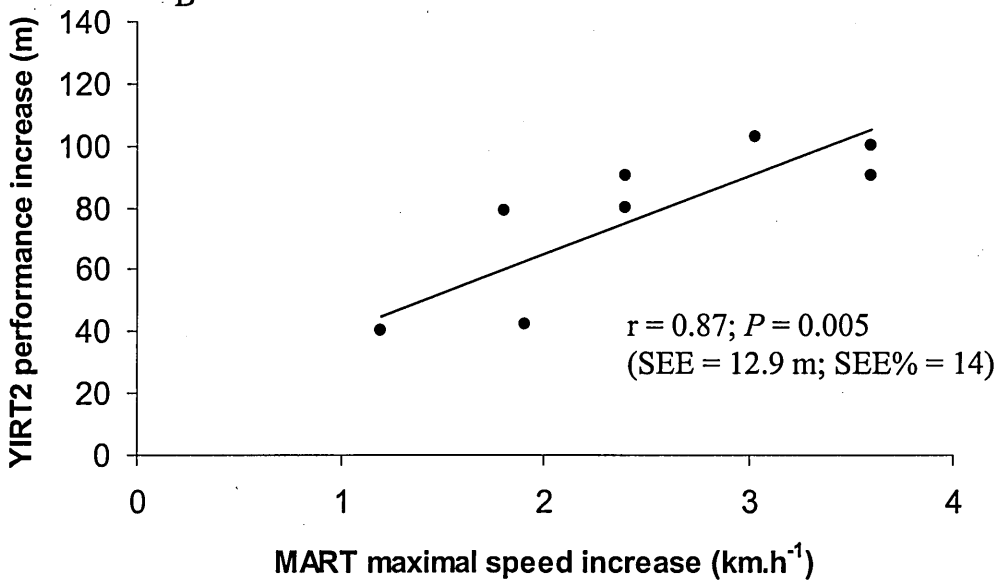
Post r = -0.28; n = 8; *P*=0.067) or Cn (Pre r = -0.39; n = 8; *P*=0.061: Post r = -0.34; n = 8; *P*=0.066) groups.

After the training intervention, stronger relationships existed between YIRT2 performance and the MART measures of power (Pre = 0.81; *P*=0.023: Post r = 0.89; *P*=0.014:), maximal speed (Pre r = 0.74; *P*=0032: Post r = 0.84; *P*=0.016) and time to exhaustion (Pre r = 0.81; *P*=0.021: Post r = 0.85; *P*=0.015) for the Tr group. However, there was no noticable change in these associations after the intervention for the Cn group (power, Pre r = 0.78; *P*=0.028: Post r = 0.75: *P*=0.029; speed, Pre r = 0.72; *P*= 0.033: Post r = 0.72; *P*=0.033; time to exhaustion, Pre r = 0.76; *P*=0.029: Post r = 0.77; *P*=0.024). Relationships between the change in variables were only found to exist for the Tr group. Associations were observed (Figure 7.2 A to D) between the increases in YIRT2 performance and MART power (r = 0.89; *P*=0.013), time to exhaustion (r = 0.90; *P*=0.011) and maximal speed (r = 0.87; *P*= 0.015), with no association being revealed between YIRT2 performance improvement and any other physiological measure (see appendix 13.3, page 256).

A


YIRT2 performance increse (m)
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11
MART power increase (ml.kg-1.min-1)
r = 0.74; P = 0.035
(SEE = 17.7 m; SEE% = 19.4%

B


YIRT2 performance increase (m)
140
120
100
80
60
40
20
0
0 1 2 3 4
MART maximal speed increase (km.h-1)
r = 0.87; P = 0.005
(SEE = 12.9 m; SEE% = 14)

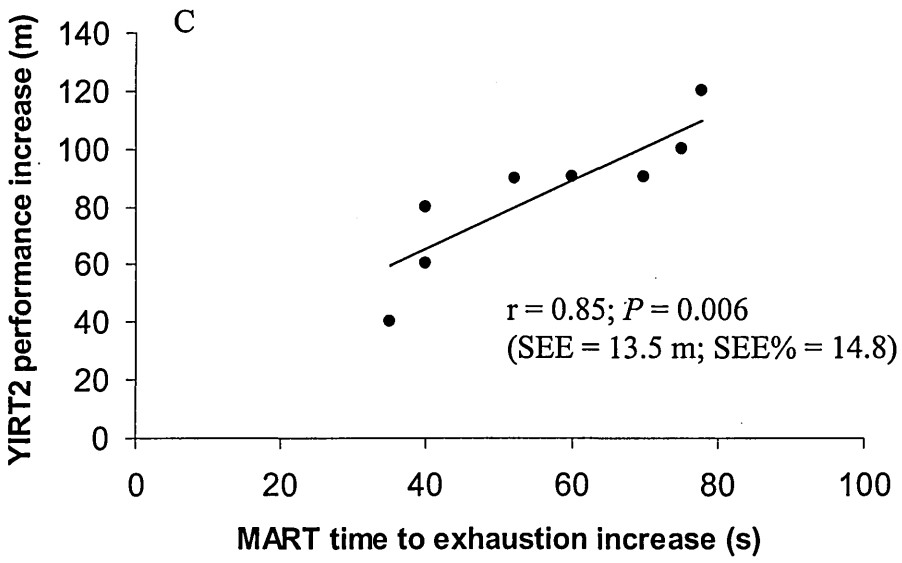


Figure 8.2 A to D. Graphs A to D represent the significant correlations between improvements in YIRT2 performance and power (A), maximal running speed (B) and time to exhaustion for the Tr group (C) (n = 8).

8.4 Discussion

8.4.1 Training induced changes to the physiological and fitness status of the players

The findings of this study show that six weeks of high-intensity intermittent training in addition to an elite soccer player's normal training regime can increase their soccer-specific high-intensity running capacity. The Tr group improvement in YIRT2 performance of 13.1% was matched by an 8.7% increase in anaerobic power derived from the MART. The only aerobic measure that changed after the intervention was τ_2 of the Cn group, which lengthened considerably to match that of the Tr group.

The change in YIRT2 for the Tr group is in accordance with improvements of 26 % and 31% reported for elite soccer referees (Krustrup and Bangsbo, 2001) and players (Krustrup *et al.*, 2003) performing level one of the test. The improvement in MART performance is greater than that of 3.4% previously observed for elite sprinters following 10 weeks of training (Nummela *et al.*, 1996), and was sufficient to give the players an anaerobic power (124.2 ± 5.2 ml.kg.min^{-1}) comparable to that previously reported (Nummela *et al.*, 1996) for elite 400 m runners (122.6 ± 4.9 ml.kg.min^{-1}).

Significant relationships were observed before and after the training intervention between YIRT2 performance and $\dot{V}O_2$ max, GET, τ_{1on} and the anaerobic performance parameters derived from the MART, which supports previous suggestions that both the aerobic and anaerobic energy systems are important for the performance of soccer-specific high-intensity intermittent exercise (Bangsbo, 1993). However, the post training improvement in YIRT2 performance was only associated with the increase in the player's anaerobic fitness when expressed as anaerobic power, maximal speed attained and time to exhaustion in the MART.

8.4.2 The role of aerobic and anaerobic metabolism in YIRT2 performance

During the sub-maximal stages of the YIRT2, a high level of aerobic fitness will prevent reliance on anaerobic energy production and so potentially delay the onset of fatigue. However, as the intensity of the shuttle runs increase and exceed a player's $\dot{V}O_2$ max, a large capacity for anaerobic energy production will be beneficial as it will enable a player to exercise for a sustained period at these supra-$\dot{V}O_2$ max running speeds. Therefore, when soccer players are matched for aerobic fitness and $\dot{V}O_2$ kinetics, it is the players who possess a larger anaerobic capacity that will be capable of performing more high-intensity soccer-specific running. Analysis of HR measures taken during the YIRT2 supports this hypothesis. As the max HR values recorded during the YIRT2 (Table 8.5) were similar to those recorded at $\dot{V}O_2$ max during the incremental exercise test to exhaustion (Table 8.2), it can be assumed that $\dot{V}O_2$ max was attained in the YIRT2. Based on this association, HR data would indicate that although the Tr group reached $\dot{V}O_2$ max at the same stage of the YIRT2 before (720 m) and after (728 m) training, they were capable of running further at speeds above $\dot{V}O_2$ max after (259 m) than before (176 m) the intervention.

These findings support Ramsbottom *et al.* (2001) who reported improved continuous high-intensity running capacity was associated with an increased anaerobic capacity without a change to $\dot{V}O_2$ max. The decisive contribution made by the anaerobic energy system during high-intensity exercise could also help to explain the results of Krustrup and Bangsbo (2001) and Krustrup *et al.* (2003) who reported increases in YIRT1 performance with no or only small changes in $\dot{V}O_2$ max after periods of high-intensity soccer-specific training.

The τ_{2on} of the Cn group was significantly shorter than that of the Tr before the intervention (P=0.031). Consequently, analysis revealed the Cn group (916 ± 55 ml) to have a smaller (P=0.039) phase III DO_2 than the Tr group (1009 ± 61 ml), although there was no difference in phase II (P=0.195) or total (P=0.218) DO_2 between groups. It is also arguable whether a difference of 93 ml in DO_2 between groups (10.2%) would be of physiological significance for soccer performance, as no difference in YIRT2 performance was observed between groups before the intervention. Following the intervention, the phase III DO_2 of the groups was similar as the τ_{2on} of the Cn group lengthened considerably to a value similar to that of the Tr group. Such a change in a kinetic measure for the Cn group was not expected and is possibly a result of the poor test-retest reproducibility that seems inherent in phase III measures. Furthermore, the lengthening in τ_2 of the Cn group did not lead to a decrease in their YIRT2 performance. This supports the findings of the previous study of this thesis that the speed of the slow component and hence DO_2 does not influence soccer-specific high-intensity running capacity in players matched for aerobic fitness.

8.4.3 Physiological adaptations associated with an improved anaerobic capacity

Following a period of either run (Neville *et al.*, 1989; Medbo and Burgers, 1989) or cycle (Boobis, 1987) sprint training, improvements in high-intensity exercise performance have been mirrored by increased glycolytic contribution to anaerobic energy production. Therefore, any increase in anaerobic capacity and hence performance is largely attributable to adaptations that will enhance a skeletal muscle's glycolytic capacity (Sahlin *et al.*, 1979; Soderland *et al.*, 1991; Bangsbo *et al.*, 1992; Bogdanis *et al.*, 1996).

Several adaptations within skeletal muscle have been associated with an increase in glycolytic capacity following a period of training. The metabolic enzymes that drive and regulate glycolysis such as Phos, PFK, Hx and LDH have been reported to increase between 10 and 56% along with a concomitant improvement in high-intensity exercise performance following periods of high-intensity training (Roberts *et al.*, 1982; Simoneau *et al.*, 1985; MacDougall *et al.*, 1998). The increase in glycolytic enzymes following a period of high-intensity training causes a change in the characteristics of the different muscle fibre types. It has been reported that high-intensity interval training similar to that performed in this study increases the glcolytic capabilities of type I fibres, while instigating type IIa to take on the characteristics of type IIx fibres and promotes greater type II fibre recruitment during exercise (Boobis, 1987; Jannsson *et al.*, 1990). The high force generation and rapid contraction time of type II fibres would also be beneficial for the large forces required in the performance of high- and maximal-intensity runs. In addition, type II fibres possess greater levels of the substrates required for anaerobic energy production. Soderlund and Hultman (1991) and Greenhaff *et al.* (1991) reported that PCr content could be 15% and 25% higher in type II than type I fibres.

8.5 Conclusion

The findings of this study indicate that performance of elite soccer players in the YIRT2 significantly improved after 6 weeks of high-intensity intermittent training designed to increase soccer-specific high-intensity running capacity. The increase in YIRT2 was matched by an increase in anaerobic capacity as indicated by the MART and the change in the two measures was positively correlated. Although measures of aerobic fitness and $\dot{V}O_2$ kinetics were correlated with YIRT2, they were not observed to increase

following the training. Consequently they were not correlated with the change in YIRT2.

These findings show that when soccer players are matched for aerobic fitness, it is the ones who possess the largest anaerobic capacity that will be capable of performing the most high-intensity intermittent running. The high-intensity and sporadic nature of YIRT2, where a player performs a high-intensity run every 10 s, would appear to be such that the $\dot{V}O_2$ kinetic responses of elite players do not substantially reduce anaerobic contributions to the onset of exercise or enhance recovery processes at exercise cessation. Further research is required to determine which of the physiological adaptations that accompany an increase in anaerobic capacity are responsible for the improvement in YIRT2 test performance. These findings could potentially have important implications for the way in which the fitness of soccer players is trained in the future.

CHAPTER 9

Overall Discussion

9.1 Overall Aim

The pulmonary $\dot{V}O_2$ kinetics of elite soccer players during the onset and cessation of moderate- and heavy-intensity running has not previously been investigated. Therefore, the overall aim of this thesis was to establish if on- and off-transient $\dot{V}O_2$ kinetics were a determinant of soccer-specific high-intensity running capacity.

9.2 Methodological investigations

The initial studies of this thesis investigated key methodological issues involved in the study of $\dot{V}O_2$ kinetics that had not previously been addressed in the scientific literature. It was first established that the physiological markers of 80%GET and 50%Δ commonly used to define moderate- and heavy-intensity exercise demonstrated low levels of day-to-day variability, and could be accurately determined from a rapidly incrementing treadmill test to exhaustion.

The accurate and reliable identification of intensity domains allowed for the design of a multiple square-wave protocol for the measurement of $\dot{V}O_2$ kinetics during the onset and cessation of moderate- and heavy-intensity treadmill running. The use of such a protocol would enable $\dot{V}O_2$ kinetics to be measured during a pattern of running that was similar to that performed by soccer players during a game. A reproducibility study was conducted using the protocol to establish the day-to-day variability in $\dot{V}O_2$ kinetics during both the on- and of-transients of moderate- and heavy-intensity treadmill running. Statistical analysis of test-retest data showed the reproducibility of phase II parameters in the moderate- and heavy-intensity domains to be satisfactory, as the

variability in the measures was smaller than the change in phase II kinetics previously reported after some form of training intervention (Berry and Moritani, 1985). In comparison, previous studies reported poor reproducibility of their measures of phase II on- (Özyener *et al.*, 2001; Puente-Maestu *et al.*, 2001) and off-transient (Özyener *et al.*, 2001) $\dot{V}O_2$ kinetics.

The phase III parameters measured during the heavy-intensity runs however demonstrated large test-retest variability for the on- and off-transients, which is in agreement with previous cycle based research (Özyener *et al.*, 2001). The variable phase III response appeared to be caused by a poor signal to noise ratio, as the amplitude of phase III for both transients was small in comparison to the inherent breath-by-breath noise. In a subsequent study, although an increase in the number (4) and intensity (80%Δ) of the supra-GET transitions (very heavy-intensity treadmill protocol) improved the signal to noise ratio by 12% and 15% for the phase III on- and off-transients respectively, they were still highly variable in comparison to the phase II measures. Although a further increase in the number and intensity of transitions might have further improved phase III parameter reproducibility, it would not have been possible to use such a demanding protocol with elite soccer players due to their training and competition commitments. Therefore, the very-heavy intensity protocol was used in future studies, as it produced more reproducible kinetic measures than two 50%Δ transitions. Furthermore, the running speeds involved would ensure $\dot{V}O_2$ kinetic parameters would be measured at running speeds that were within the high-intensity spectrum identified for competitive match-play. However, it is not clear if the variability in phase III parameters generated from such a protocol would mask any training induced changes to the slow component response.

9.3 Characteristics of $\dot{V}O_2$ kinetics during treadmill running

The characteristics of $\dot{V}O_2$ kinetic responses measured during the on- and off-transients of moderate- and heavy-intensity exercise were investigated so that their role in soccer-performance could be fully understood. The $\dot{V}O_2$ data collected during the repeated square-wave transition protocol in this thesis showed phase II τ to be invariant across moderate- and heavy-intensity domains during the onset and cessation of exercise. Such a finding is significant as it suggests the control of $\dot{V}O_2$ kinetics follows a simple linear model, which is in disagreement with previous research that has reported τ to become slowed in the heavy-intensity domain (Williams *et al.*, 2001; Carter *et al.*, 2002).

It has been proposed that during moderate-intensity exercise, where it is thought mainly mitochondria rich type I fibres are recruited, kinetics are expected to be faster than during heavy-intensity exercise when less oxidative type IIa fibres become increasingly involved in muscle contraction (Carter *et al.*, 2002). This hypothesis is supported by the observations that the speed of τ is inversely related to the percentage of type I fibres in humans (Pringle *et al.*, 2003), and that τ is longer in mouse muscle that predominantly consists type II fibres. However, the arbitrary classification of human muscle fibres into aerobic type I and glycolytic type II is not always appropriate. It has been suggested that type IIa fibres can exhibit considerable variability in their oxidative potential across individuals (Kushmerick *et al.*, 1992) and that a continuum of oxidative potential of fibres from type I to type IIx probably exists. It is therefore feasible that the exercising muscle groups of the recreational athletes and soccer players (both Pro and Am) who participated in this thesis contained type I and type II fibres that possessed similar aerobic properties, which would account for why the speed of τ was not slowed in the heavy- compared to the moderate-intensity domain. In contrast, in studies where τ is

observed to lengthen between intensity domains, the participants might display a clearer distinction in the aerobic properties of their type I and type II fibres.

The cause of such muscle fibre characteristics among participants in this thesis might be attributable to some high-intensity aspect of their training routine increasing the aerobic potential of type II fibres. For example, Henricksson and Reitman (1976) found that high-intensity interval training carried out at maximal intensity increased SDH activity in type II fibres by ~50% with no increase in SDH activity in type I fibres. Although training diaries were not acquired for the recreational athletes in this thesis, they were for the Am and Pro soccer players. Both groups of players played soccer at least three times per week, which will have involved the obligatory performance of high-intensity exercise.

9.4 Role of $\dot{V}O_2$ kinetics in soccer performance

Phase II τ at the onset of very heavy-intensity running was found to be inversely associated with YIRT2 performance among both Am and Pro soccer players. Yet Pros ran significantly further than the Ams in the YIRT2, despite the phase II $\dot{V}O_2$ kinetic profiles of the two groups being indistinguishable. Rather than quick $\dot{V}O_2$ kinetics at the onset of exercise enabling superior performance through a reduction in the oxygen deficit, the association appears to be a consequence of the importance of aerobic metabolism for performance of soccer-specific exercise, as $\dot{V}O_2$ max and GET were also positively related with YIRT2 performance. The slow component also appears to be of little importance during soccer performance, as although the τ_{2on} was longer in Am than Pro, despite a similar amplitude, no difference was observed in the phase III or total DO_2 of the two groups. This suggests that disparity in soccer-specific fitness

between Pro and Am players cannot be attributed to differences in the speed or amplitude of the slow component response.

It is conceivable that performance in soccer-specific tests such as the YIRT2 and RST will not benefit from quick $\dot{V}O_2$ kinetics due to the high-intensity and short duration of the activities involving a substantial anaerobic contribution that would negate a decrease in DO_2 caused by quick $\dot{V}O_2$ kinetics. Even though performance in the YIRT2 has been associated with high-intensity running during a game, the progressive and maximal pattern of running incorporated in the test is not truly representative of competitive match-play, where high-intensity runs can be separated by 90 s (Withers *et al.*, 1982). Therefore, any conclusions made about the physiological determinants of soccer-specific running capacity based on YIRT performance should be treated cautiously. It is possible that the physiological processes associated with performance in this thesis would be different if the measure of high-intensity running capacity was obtained from a competitive game, where the intermittent exercise pattern differs markedly from that of the YIRT2.

9.5 The physiological determinants of soccer performance?

It is clear from the findings of this thesis that both aerobic and anaerobic energy systems interact in the performance of high-intensity soccer-specific exercise. A novel finding of this thesis is however, that if elite soccer players are matched for aerobic fitness and $\dot{V}O_2$ kinetics, their soccer-specific fitness can be enhanced by increasing their anaerobic capacity via a high-intensity intermittent training programme in addition to their normal training regime. Although it cannot be concluded from this observation that the largest gains in soccer-specific fitness among elite soccer players are attained through an increase in anaerobic rather than aerobic fitness, such a finding could have

significant implications for the training of elite soccer players. The duration and volume of training required to increase anaerobic capacity is smaller than that for aerobic capacity. Therefore, during the competitive season, when players are required to play in excess of 40 games, plus technical and tactical training sessions, it might be more practical to increase soccer-specific fitness via an increase in anaerobic rather than aerobic capacity.

The absence of change in the $\dot{V}O_2$ kinetics of the Tr group could be attributed to the structure of the training programme not being suitable to induce adaptations in a muscle's aerobic potential that are associated with a speeding of $\dot{V}O_2$ kinetics. The intensity (≥95 % max HR; including high intensity runs and maximal sprints) and duration (12, 30 or 60 s) of the runs incorporated in the intervention were such that for aerobically trained soccer players ($\dot{V}O_2$ max = 57.8 ± 4.2 $ml.kg^{-1}.min^{-1}$), it was only the anaerobic energy systems that were sufficiently stimulated by the high-intensity running to demonstrate an enhancement through training. For example, during 10 and 30 s maximal sprints, aerobic metabolism has been reported to only provide ~ 9% (Serresse *et al.*, 1991) and 30% (Bogdanis *et al.*, 1996) of the energy for muscle contraction respectively. Although the contribution from aerobic metabolism towards energy provision can exceed 50% as sprints are repeated, previous research (Tabata *et al.*, 1996; MacDougall *et al.*, 1998) has reported that for less aerobically trained individuals (51 to 53 $ml.kg^{-1}.min^{-1}$), repeated sprint training only induces a small increase in aerobic compared to anaerobic fitness.

In contrast, it has been shown that if the training undertaken is of a more moderate intensity, an increase in the aerobic potential of muscle occurs through increased levels of myoglobin (Hickson, 1981), mitochondrial size and number (Kiessling *et al.*, 1971),

oxidative enzyme activity (Gollnick *et al.*, 1972) and altered muscle fibre composition (Henricksson and Reitman, 1977). Such an increase in muscle aerobic potential results in tighter coupling between ATP supply and demand (Dudley *et al.*, 1987), characterised by a smaller increase in ADP, AMP, IMP, Cr and Pi and a lesser decrease in PCr. It is these improvements that are thought to influence the speed of $\dot{V}O_2$ kinetic responses. Also, a high volume ($\geq$ 5 times per week) of endurance training is thought to be a stimulus for faster $\dot{V}O_2$ kinetics (Kilding, 2003) as oxidative adaptations to training in muscle fibres have been shown to be proportional to the volume of training (Fitts *et al.*, 1975; Sjoldin *et al.*, 1976; Terung, 1976; Hickson, 1981). Consequently, the time spent training at the high-intensities in this study will reduce the opportunity for aerobic adaptations to take place, resulting in less speeding of $\dot{V}O_2$ kinetics. This is supported by Edwards *et al.* (1999) who found slower kinetics for sprinters than endurance runners.

The observation that following a high intensity training programme, it is solely an increase in anaerobic capacity that improves YIRT2 performance however is perhaps too simplistic. It is plausible that the adaptations to skeletal muscle which contribute to an increased anaerobic capacity would slow an individual's $\dot{V}O_2$ kinetics as the skeletal muscle has become more anaerobic than aerobic in nature (Crow and Kushmerick, 1982; Pringle *et al.*, 2003). For example, an increases in [PCr] and [Cr] observed with high-intensity training (Parra *et al.*, 2000) has been proposed to result in a slower turn-on of mitochondria, while studies involving rats reported that CS activity increased after a reduction in Cr (Sweeney, 1994). In support, Meyer and Folery (1994) demonstrated that the rate of oxidative phosphorylation is linearly dependent upon total Cr([PCr]+[Cr]). Collectively, these findings suggest that $\dot{V}O_2$ kinetics might be slowed

in soccer players who experience an increase in anaerobic capacity in response to the high-intensity training.

However, the observation of this study that τ remained unaltered after the training intervention suggests that changes to the aerobic capacity of muscle might have also occurred to prevent a slowing of $\dot{V}O_2$ kinetics. This is supported by several investigations where an increase in mitochondrial enzyme activity has been observed following a period of high-intensity or maximal sprint interval training (Henriksson and Reitman, 1976; MacDougall *et al.*, 1998). Therefore, it is conceivable that changes to aerobic metabolism at the muscular level might have contributed to the increase in soccer-specific fitness following the high-intensity training programme, but were masked by the changes that also occurred to anaerobic metabolism.

9.6 Limitations

In chapters seven and eight, the role of aerobic and anaerobic physiological mechanisms in soccer was investigated by assessing their relationship with performance in YIRT2, which is an indirect measure of soccer-specific high-intensity running capacity. Firmer conclusions might have been drawn if these physiological mechanisms were investigated in relation to the actual amount of high-intensity running the players performed over several competitive games. The recovery between shuttles in the YIRT2 was fixed at 10 s, whereas during a competitive game it has been observed that a high-intensity run is performed every 25 to 90s (Withers *et al.*, 1982; Bangsbo, 1994). It is conceivable that longer periods between runs i.e. > 10 s, would make it possible to distinguish between players who possessed different recovery capabilities associated with aerobic fitness. This might explain the conflicting results of Helgerud *et al.* (2001)

who reported an increase in $\dot{V}O_2$ max was associated with an increase in sprints performed during competitive games.

In chapter eight, anaerobic capacity was measured indirectly from the MART. Although direct estimations from muscle biopsy also have their limitations, such a technique might have provided a greater insight into the role of anaerobic metabolism during soccer performance. Furthermore, no direct determinations of the mechanisms proposed as being responsible for an increase in anaerobic capacity and hence soccer-specific high-intensity running capacity were taken. The addition of these measures to the analysis might have allowed firmer conclusions to be drawn regarding which physiological mechanism determined soccer performance.

9.7 Future directions

Due to methodological restrictions imposed upon the studies of this thesis, it has only been possible to hypothesise as to the physiological processes and adaptations following a period of high-intensity training that account for an improvement in soccer-specific high-intensity running capacity. Furthermore, a high-intensity training model as used in chapter 8 of this thesis might not have stressed aerobic metabolism sufficiently to bring about improvements in aerobic fitness or $\dot{V}O_2$ kinetics of aerobically trained soccer players. Hence, a future longitudinal training study is required where direct assessments of the physiological processes that are involved in soccer performance can be assessed in conjunction with improvements in soccer-specific fitness. To identify which physiological processes are most important for increasing soccer-specific fitness, the training study must incorporate a range of exercise intensities to induce training adaptations to both aerobic and anaerobic energy systems. From such research it would then be possible to determine which physiological process should be targeted through

training to bring about optimal increases in soccer-specific fitness. The aims of a future study are:

1. To directly determine the physiological processes and adaptations responsible for improvements in soccer-specific fitness.

2. To determine if an increase in aerobic, anaerobic or both energy systems brings about the largest increase in soccer-specific fitness.

The proposed study would involve a training intervention lasting 6 to 8 weeks (Saltin and Gollnick, 1983), comprising three groups acting as their own controls who would undertake different intensities and volumes of intermittent running: Group 1) high-intensity, Group 2) moderate-intensity and group 3) a combination of high and moderate-intensity running. The training of the high-intensity group would comprise repeated maximal sprints for durations ranging from 10 to 30 s to primarily tax the high-energy phosphagen and glycolytic systems to cause an increase in anaerobic capacity. In contrast, the moderate-intensity training group would run at speeds that correspond to ~90% velocity $\dot{V}O_2$ max for 4 min to predominantly tax the aerobic system in order to increase $\dot{V}O_2$ max and induce peripheral adaptations to enhance the aerobic potential of skeletal muscle. The combination group would perform a mixture of moderate- and high-intensity runs to stress both the aerobic and anaerobic energy systems.

To ensure an adequate and continuous overload, the volume of the training regime would be progressively increased. The study would involve elite soccer players, as less trained players would be more likely to experience an increase in soccer-specific fitness

as a result of any type of training stimulus, regardless of which energy system was being targeted.

Prior to the training intervention, physiological and performance measures for each participant would be performed. This would allow the intensity of training to be made specific for each participant. The physiological and performance measures would comprise the following:

1. Quantification of the amount of high-intensity running a player performs over three consecutive competitive games. Direct assessment of the amount of high-intensity running a player performs will be a more accurate and valid measure of high-intensity running capability than that provided by the YIRT2. It has been suggested (Bangsbo, 1994) that to ensure representative measures of a player's activity profile are obtained, match analysis should be performed over three consecutive games, as performance in an individual game might be artificially limited due the playing tactics employed.

2. Graded exercise test to volitional exhaustion. This will allow for measures of $\dot{V}O_2$ max and GET, plus the identification of the running speed required to elicit a $\dot{V}O_2$ value corresponding to 80%Δ in future assessments of $\dot{V}O_2$ kinetics.

3. Very heavy-intensity square-wave treadmill protocol. This would be performed so that $\dot{V}O_2$ kinetic data could be obtained for phases II and III at the on- and off-transients of high-intensity running. Combined with the above measures of $\dot{V}O_2$ max and GET, it will be possible to determine which components of a player's aerobic profile are responsible for changes in soccer-specific fitness following a period of training.

3. Direct assessment of anaerobic capacity. Although the direct assessment of anaerobic capacity through muscle biopsy does have limitations, it will provide direct evidence of adaptations within skeletal muscle following the training interventions. The taking of muscle biopsies will also allow any enzymatic (aerobic and anaerobic) and/or muscle fibre changes that have occurred as the result of the three interventions to be assessed. Such data would make it possible to draw firmer conclusions about the processes that determine soccer-performance.

Following the intervention this range of measures would be repeated. Statistical analysis would consist of a mixed factorial ANOVA to identify if any differences existed in the repeated measures for the three groups. Any difference could be identified from post-hoc analysis of the data. Pearson's correlation coefficient could also be used to determine the strength of association between physiological and performance measures.

CHAPTER 10

Conclusions

The findings of this thesis suggest that a player's soccer-specific intermittent high-intensity running capacity is not determined by the speed of their $\dot{V}O_2$ kinetics during either the onset or cessation of exercise. However, further research is required to determine the role of $\dot{V}O_2$ kinetics in the performance of high-intensity running during competitive games, and whether the speeding of $\dot{V}O_2$ kinetics through an appropriate training programme will lead to an improvement in soccer-specific fitness. The specific conclusions drawn from this thesis are:

1. The physiological markers 80%GET and 50%Δ can be reliably and accurately determined from a rapidly incrementing treadmill protocol to exhaustion. Consequently they can be used to set moderate- and heavy-intensity running speeds.

2. Phase II τ and A are invariant of intensity domain but not exercise transient for treadmill running.

3. Phase II $\dot{V}O_2$ kinetics are reproducible at the onset and cessation of moderate-, heavy- and very heavy-intensity treadmill running. Phase III $\dot{V}O_2$ kinetics however are highly variable during both the onset and cessation of heavy- and very heavy-intensity treadmill running.

4. Performance in the YIRT2 and τ_1 at both exercise onset and cessation were inversely related for elite soccer players. However, this association appears to be an artefact of

the importance of aerobic metabolism in YIRT2 performance, as $\dot{V}O_2$ max and GET were related to both τ_1 and YIRT2 performance.

5. Aerobic and anaerobic fitness are both associated with soccer-specific high-intensity running capacity. However, when elite players are matched for aerobic fitness, it is the ones who possess the largest anaerobic capacity that can perform the most soccer-specific high intensity running.

6. Three high-intensity intermittent running sessions per week in addition to a professional player's normal training regime can increase a player's soccer-specific fitness during the competitive season by enhancing their anaerobic capacity.

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Appendix 1

Conference Communications

1.1 BASES Abstract 2002

ASYMMETRY OF OXYGEN UPTAKE KINETIC RESPONSES TO THE ONSET AND OFFSET OF TREADMILL RUNNING

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Oxygen uptake ($\dot{V}O_2$) kinetic responses to the onset and offset of running could be important measures for athletes who compete in intermittent type sports. Fast $\dot{V}O_2$ kinetics at the onset of exercise would result in less reliance on fatigue-inducing anaerobic pathways to meet energy demands. Also, the $\dot{V}O_2$ kinetic response at the offset of exercise might indicate how quickly an athlete can recover. However, whether or not these responses are symmetrical is unclear as they seem to depend on the intensity (Özyener *et al.*, 2001) and mode of exercise (Carter *et al.*, 2000). The aim of this study was to investigate whether there was symmetry between the fast and slow components of the $\dot{V}O_2$ kinetic responses to the onset and offset of treadmill running.

With institutional ethics approval eight men mean ± s: age 23.5 ± 1.3 years, stature 179 ± 7 cm, body mass 77.1 ± 12.2 kg provided written informed consent and participated. Participants performed a graded exercise test (GXT) in which running speed was increased 1 $km \cdot h^{-1}$ every minute until volitional exhaustion. During the GXT respiratory gases were measured breath-by-breath using a mass spectrometer (Marquette 1100 ME, Milwaukee, USA) so that a running speed / $\dot{V}O_2$ relationship could be established. On a later occasion, participants performed a treadmill protocol for the measurement of $\dot{V}O_2$ kinetic responses to the onset and offset of exercise, above and below the gas exchange threshold (GET). The protocol was continuous and consisted of 4 square-wave walk-to-run transitions (6 min walking, 6 min running); 3 at a running speed equivalent to 80% of $\dot{V}O_2$ at GET (subGET) and 1 equivalent to half way between GET and maximal oxygen consumption (supraGET). A 12 min walk ended the protocol. Following 1 hour of recovery the protocol was repeated, producing 6 subGET and 2 supraGET transitions.

Using two way analysis of variance with repeated measures, the phase II time constants (τ_1) were shorter ($P = 0.017$) at the onset (24 ± 5) than the offset (27 ± 6) of exercise, with no interaction between exercise intensity and exercise transition ($P = 0.803$). The τ_1 for sub and supra GET exercise at onset and offset did not differ ($P = 0.861$). The amplitude of the phase II response (A_1) was less ($P = 0.041$) for exercise onset than offset (1441 ± 232 vs. 1541 ± 298 $ml.min^{-1}$). No interaction was found for A_1 between exercise intensity and the exercise transition ($P = 0.431$). The A_1 for sub and supra GET exercise at onset and offset was also different ($P = 0.021$). Paired sample t tests showed that the phase III time constant (τ_2) was less at the onset than the offset of exercise (177 ± 44 vs. 396 ± 72 s; $P = 0.001$). The amplitude of the phase III response (A_2) was greater for exercise onset than offset (295 ± 118 vs. 138 ± 89 $ml.min^{-1}$; $P = 0.013$).

The results suggest that there is asymmetry between $\dot{V}O_2$ kinetic responses to the onset and offset of treadmill running, both above and below GET and that τ_1 is independent of exercise intensity for exercise onset and offset. These findings support the work of Carter *et al.*, (2000) and Özyener *et al.*, (2001) for the fast and slow components respectively. Identification of mechanisms that account for this asymmetry and the implications for intermittent-type sports performance requires further investigation.

Carter, H., Jones, A.M., Barstow, T.J., Burnley, M., Williams, C.A. and Doust, J.H. (2000). Oxygen uptake kinetics in treadmill running and cycle ergometry: a comparison. Journal of Applied Physiology, 89, 899-907.

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Characteristics of the phase II oxygen uptake response to the onset and offset of exercise in different intensity domains

It is accepted (Özyener *et al.*, 2001 *Journal of Physiology*, **533.3**, 891-902) that the characteristics of oxygen uptake ($\dot{V}O_2$) kinetics at the onset and offset of exercise depend upon whether exercise is performed above or below the gas exchange threshold (GET). However it is unclear if the time constant (τ_1) of the phase II response is independent of the exercise intensity domain, and the transient of exercise in which it is measured. The aim of this study was to determine if the phase II response was constant at the onset and offset of sub and supra GET exercise.

With institutional ethics approval ten men (mean ± *s*): age 23.5 ± 1.3 years, stature 179 ± 7 cm, body mass 77.1 ± 12.2 kg, $\dot{V}O_2$ max 3859 ± 512 ml.min^{-1}, provided written informed consent and participated. Participants performed a graded exercise test (GXT) in which running speed was increased 1 km·h^{-1} every minute until volitional exhaustion. During the GXT respiratory gases were analysed breath-by-breath using a mass spectrometer system (Marquette 1100 ME, Milwaukee, USA) so that a running speed / $\dot{V}O_2$ relationship could be established. On a later occasion, participants performed a treadmill protocol for the measurement of $\dot{V}O_2$ kinetic responses to the onset and offset of exercise above and below the gas exchange threshold (GET). The protocol was continuous and consisted of 4 square-wave walk-to-run transitions (6 min walking, 6 min running), 3 at a running speed equivalent to 80% of $\dot{V}O_2$ at GET (subGET) and 1 equivalent to mid way between GET and maximal oxygen consumption (supraGET). The walking speed in between each run was 4 km.h^{-1}. A 12 min walk ended the protocol. Following 1 hour of recovery the protocol was repeated so producing 6 sub GET and 2 supra GET transitions.

After verifying underlying assumptions such as the normality of distribution and sphericity of the data, phase II $\dot{V}O_2$ kinetic parameters were compared using two way analysis of variance with repeated measures. The τ_1 for sub did not differ from supra GET exercise, at onset (23.2 ± 5.5 vs. 23.7 ± 4 s) or offset (27.1 ± 4.9 vs. 27.4 ± 6.9 s) of exercise (P = 0.86). The mean τ_1 of the sub and supra GET exercise was less at the onset (23.5 ± 4.5 s) than the offset (27.2 ± 5 s) of exercise (P = 0.02), with no interaction between exercise intensity and exercise transition (P = 0.80). The A_1 was less for sub than supra GET exercise, at both the onset (853 ± 124 vs. 2003 ± 218 ml.min^{-1}) and offset (878 ± 133 vs. 2134 ± 275 ml.min^{-1}) of exercise (P = 0.02). The mean A_1 for sub and supra GET exercise was less (P = 0.04) for the onset of exercise than offset (1441 ± 232 vs. 1541 ± 298 ml.min^{-1}). There was no interaction for A_1 between exercise intensity and the exercise transition (P = 0.43).

As anticipated, there was a difference between A_1 at sub and supra GET exercise. However, τ_1 was independent of the exercise intensity domain for both the onset and offset of exercise. The autonomy of τ_1 in relation to intensity domain in this study supports previous research where square-wave exercise has been performed on a cycle ergometer (Özyener *et al.*, 2001 *Journal of Physiology*, **533.3**, 891-902). Conversely a recent study by Carter *et al.* (2002 *Journal of Applied Physiology*, **86**, 347-354) observed a marked increase in τ_1 during supra compared to sub GET exercise performed on a treadmill. Differences in τ_1 above GET might be attributable to the different techniques used to model the $\dot{V}O_2$ data or the design of the protocols used to generate square-wave changes in $\dot{V}O_2$. The results suggest that there is an asymmetry in $\dot{V}O_2$ kinetics between the onset and offset of exercise, further research is required to determine the precise mechanisms responsible.

Performance of professional and amateur soccer players in the YoYo Recovery test level 2

Performance in the YoYo recovery test level 1 (YRT1) is strongly related to the high-intensity running performed during soccer and distinguishes between international and standard level players (Mohr *et al.*, 2003: *Journal of Sport Sciences,* **21**, 519-528). It is therefore seen as a valid measure of a player's ability to perform soccer-specific exercise (Krustrup *et al.*, 2003: *Medicine and science in sport and exercise*, **35**, 697-705). In contrast there is little research into the validity of the YoYo recovery test level 2 (YRT2) as a measure of soccer-specific fitness, even though it might be a more appropriate test for elite level players as it incorporates faster running speeds than YRT1. Therefore the purpose of this investigation was to see if performance in the YRT2 was more sensitive to differences in the fitness of soccer players of different standards during the competitive season than a laboratory based assessment of aerobic fitness.

With institutional ethics approval 18 professional soccer players (mean ± s): age 23.2 ± 2.4 years, stature 180.3 ± 6.6 cm, body mass 78.4 ± 7.5 kg, $\dot{V}O_2$ max 58.2 ± 2.8 ml.kg^{-1}.min^{-1} and 18 amateur soccer players (mean ± s): age 21.1 ± 1.6 years, stature 179 ± 8.2 cm, body mass 75.8 ± 11.4 kg and $\dot{V}O_2$ max 57.1 ± 3.9 ml.kg^{-1}.min^{-1} provided written informed consent and participated. Participants performed a graded exercise test in which running speed was increased 1 km·h^{-1} every minute until volitional exhaustion. During the graded exercise test respiratory gases were analysed breath-by-breath using a mass spectrometer system (Marquette 1100 ME, Milwaukee, USA) so that gas exchange threshold (GET) and maximal oxygen consumption ($\dot{V}O_2$ max) could be determined. Time to exhaustion and maximal running speed were also recorded. Seven days after the graded exercise test participants performed the YRT2. The test was performed outside on Astroturf in dry weather conditions. Participants were required to run back and forth along a 25 m track, keeping in time to audible signals from a cassette tape that sounded intermittently. Participants stopped when they could no longer keep in time with the signals.

The mean physiological and performance measures for both groups of players are illustrated in Table 1. After verifying underlying assumptions such as normal distribution and homogeneity of variances, a two sample t-test was used to compare the values recorded for the two groups in the different tests. Statistical significance was set at $P \leq 0.05$. No difference was observed between professional and amateur soccer players for $\dot{V}O_2$ max (T (34) = 1.61; P = 0.117), GET (T (34) = 1.12; P = 0.211), time to exhaustion (T (34) = 1.64; P = 0.124) and maximal running speed (T (34) = 2.01; P = 0.21). Professional players ran further during the YRT2 (T (34) = 2.419; P = 0.021).

Table A1. Physiological and performance measures for professional and amateur players (mean ± s).

Group	$\dot{V}O_2$ max (ml.kg^{-1}.min^{-1})	GET (l.min^{-1})	Time to exhaustion (min)	Max Speed (km.h^{-1})	YoYo Test (m)
Professional (n = 18)	58.2 ± 2.8	3.32 ± 0.25	10.55 ± 0.84	18.6 ± 0.9	840 ± 153
Amateur (n = 18)	57.1 ± 3.9	3.10 ± 0.39	10.36 ± 1.02	18.3 ± 1.1	716 ± 123

Measures of aerobic fitness did not differentiate the two groups. However professionals out performed amateurs in the YRT2. This indicates that the YRT2 is more appropriate for measuring differences in the fitness of soccer players than $\dot{V}O_2$ max or GET during the competitive season. These findings support the use of the YRT2 as a measure of fitness among soccer players. Future research should be conducted to determine mechanisms that explain professional players' enhanced performance in the YRT2, as this could have implications for the fitness training of soccer players in the future. It would also be beneficial to know the strength of the relationship between performance in the YRT2 and the amount of high-intensity running performed during a game. It would then be possible to determine which level of the YoYo Recovery test was the most appropriate for measuring the soccer-specific fitness of elite players.

1.4 Appendix ACSM Abstract

Title: Pulmonary kinetics of oxygen uptake and sport specific fitness in soccer players.

Elite soccer performance is, in part, dependent on a high level of cardiopulmonary fitness, which can either be assessed maximally (VO_2max), or as a dynamic response to a change in work (VO_2 kinetics). The kinetics of oxygen uptake (VO_2) are enhanced following endurance training, and are also differentially fastest in elite endurance athletes; it is therefore surprising that VO_2 kinetics have not been reported in intermittent sports such as soccer, where the ability to rapidly respond to a change in work is of importance. **PURPOSE:** To compare laboratory (VO_2 kinetics & VO_2 max) and field based (Yo-Yo intermittent test) assessments of cardiopulmonary fitness in professional and amateur soccer players. **METHODS:** A group of professional (Pro) (n=18) and amateur (Am) (n=18) soccer players agreed to participate in the study. Both groups performed 3 tests: 1) a graded exercise test to exhaustion for the determination of VO_2 max 2) four repeats of a single exercise transient from walking to 80%Δ (80% of the difference between GET and VO_2 max) for the assessment of VO_2 kinetics, and 3) a soccer-specific test (Yo-Yo Intermittent Recovery Test level 2). Gas exchange was measured breath-by-breath and a two component exponential model was used to characterise the kinetics of the VO_2 response. Statistical analyses were made using ANOVA and 't' tests as appropriate. **RESULTS:** There was no difference in the cardiopulmonary fitness of the professional and amateur players when expressed as VO_2 max (Pro 56.5 ±2.9ml·kg·min^{-1} vs. Am 55.7 ±3.5ml·kg·min^{-1}) or the VO_2 kinetic fundamental (τ_1 onset, Pro 24.5 ± 3.2 s vs. Am 24.0 ± 1.8 s; τ_1 offset, Pro 28.7 ± 2.8 s vs. Am 29.3 ± 3.5 s) and slow components (τ_2 onset, Pro 98.2 ± 56.6 s vs. Am 142.2 ± 58.6 s; τ_2 offset, Pro 261.1 ± 60.7 s vs. 277.8 ± 71.7 s). However, professionals (966 ±153m) achieved a greater total distance covered than amateurs (840 ±156m) in the Yo-Yo test ($P<0.05$).
CONCLUSION: The intermittent demands of soccer make the determination of specific fitness difficult to quantify. Nevertheless, the findings of this study suggest that sport specific assessment has greater relevance to the identification of fitness in performers participating at different levels of the sport. Whilst cardiopulmonary fitness is important for soccer performance, the ability to rapidly respond to a change in work rate does not contribute to the difference in soccer-specific performance observed between the professional and amateur players. Other factors, such as anaerobic fitness, may be of more importance.

Appendix 2

Verification of treadmill speed and gradient

2.1 Treadmill speed

The speed of the treadmill belt was verified over a range of speeds applicable to investigations within this thesis (Table A.1). A participant with a body mass of 72.4 kg ran on the treadmill while these speeds were manually calculated.

Table A.2 Treadmill belt speeds that were verified for accuracy.

$m.s^{-1}$	1.0	1.2	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
$km.h^{-1}$	3.6	4.3	7.2	9	10.8	12.6	14.4	16.2	18	19.8	21.6

To manually calculate treadmill belt speed the following formula was used:

Speed = d/t

where d is the distance (m) that would be travelled in 20 revolutions of the treadmill belt and t is the mean of 3 measures of the time (s) taken for 20 revolutions. A marker was placed on the treadmill belt to allow identification of one full revolution of the belt. Time taken for twenty revolutions of the belt at each pre-selected speed was recorded with a stopwatch (C200sport, Casio, UK) to the nearest 0.1 s.

It can be seen from Figure A.1 that a close relationship ($r^2 = 0.997$) exists between the belt speeds displayed on the console of the treadmill and the manually calculated belt speeds. The linear modelling technique used was least squares regression (x on y).

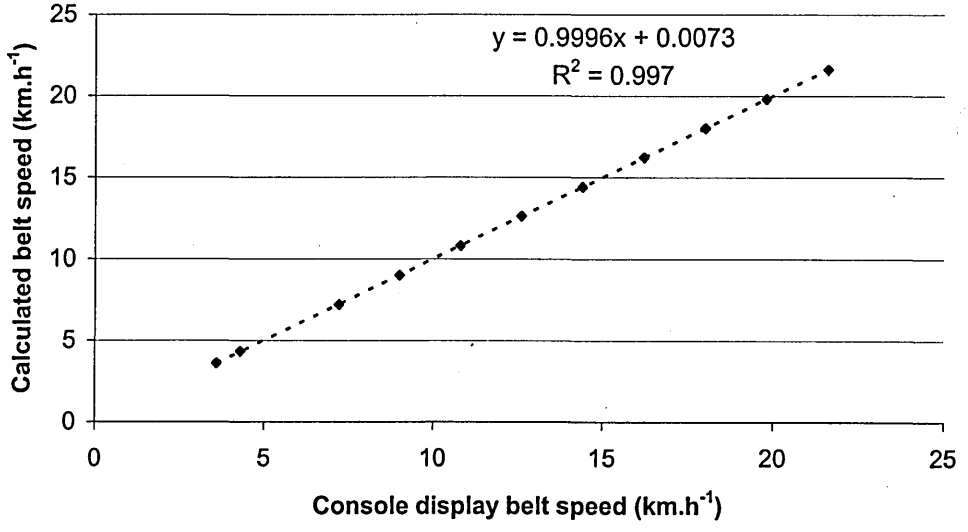


Fig A.1 Verification of displayed treadmill belt speed vs. calculated treadmill belt speed in a loaded condition. Dashed line represents line of identity.

2.2 Verification of treadmill incline

To verify zero incline the elevation meter of the treadmill was set to zero and a spirit level was used to ensure the treadmill belt was level in the horizontal plane. Vertical incline of the treadmill belt was expressed as the sine of the angle, in which sine equals the vertical rise over the hypotenuse:

Treadmill Incline (sine) = (rise / hypotenuse) * 100

The treadmill used in this investigation has moveable front and rear axles. The vertical rise is equal to the sum of the rise of the front axle and the drop of the rear axle. When this total is divided by the axle to axle length, the grade is expressed as a fraction. Linear regression analysis demonstrated a close relationship between the displayed an actual treadmill incline (Figure A.2) which remained linear through out the range of inclines measured ($r^2 = 0.99$).

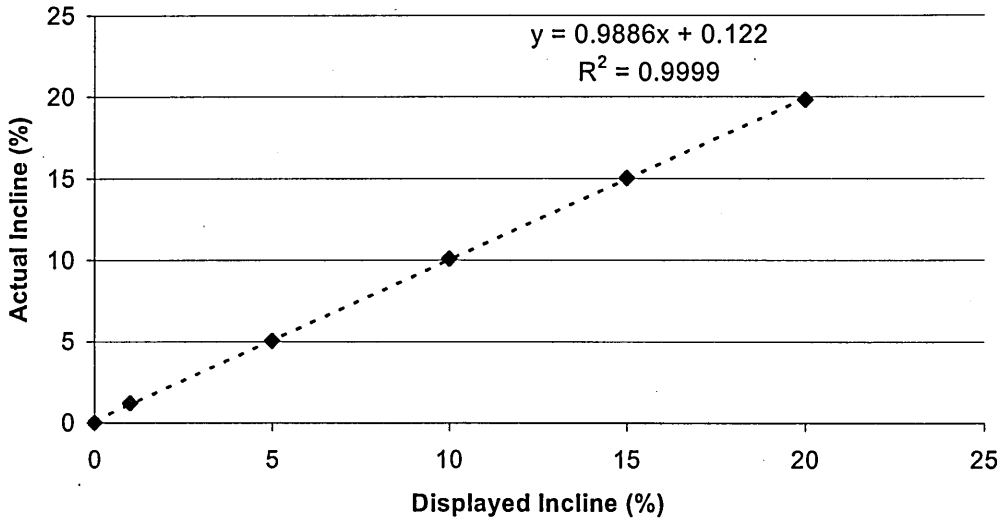


Fig A.2 Displayed treadmill gradient vs. actual gradient. Dashed line represents line of identity.

Appendix 3

A comparison of two systems of pulmonary gas analysis

For the first three studies of this thesis, pulmonary gas analysis was performed using a respiratory mass spectrometer, the MGA 1100 (Marquette Electronics Inc, Milwaukee, WI, USA). Unfortunately after the third study the MGA 1100 became unusable and beyond repair. To allow data collection for the thesis to be completed, during the fourth study pulmonary gas analysis was performed using the Medgraphics CPX/D (St Paul, Mn, USA) which consists of rapid response zirconian O_2 and infrared CO_2 analysers. To determine whether the Medgraphics CPX/D could be used as a suitable replacement, pulmonary gas exchange data from the two systems was compared for accuracy.

3.1 Accuracy

A pilot study was conducted to compare the accuracy of the two systems of pulmonary gas analysis over a range of intensities that ranged from sub-maximal to $\dot{V}O_2$ max.
Four participants (3 male, 1 female) mean ± s: age 24.3 ± 4.2 years, stature 175.3 ± 12.1 cm; body mass 65.3 ± 8.2 kg took part. All participants were healthy and performed physical activity on a regular basis. Prior to the administration of any test, participants were screened for existing medical conditions that might become aggravated during the testing procedure.
Participants undertook the same exercise test on two occasions, separated by seven days. Pulmonary gas analysis was performed using the CPX/D during the first test and the MGA 1100 for the second. The exercise tests involved cycling on an electronically braked cycle ergometer (Excalibur Sport, Lode, Netherlands) for four incrementing six min bouts at 50 W, 100 W, 150 W and 175 W to obtain steady-state pulmonary gas exchange data. On completion of the last six min bout, the exercise intensity was increased by 25 W per min until exhaustion for a measure of $\dot{V}O_2$ max. Each assessment was carried out at the same time of day to reduce the effects of diurnal variation and heart rate was measured every 5 s.
The test data for the two systems is presented below in Table A.3. Although the MGA 1100 appears to constantly measure higher than the CPX/D, a paired sample t-test revealed that the mean $\dot{V}O_2$ data from the two systems did not differ for any of the exercise intensities: 50 W, ($t(5) = -2.17$; $P = 0.072$); 100 W, ($t(5) = -2.11$; $P = 0.081$); 150 W, ($t(8) = -1.89$; $P = 0.094$); 175 W, ($t(5) = -1.81$; $P = 0.112$), $\dot{V}O_2$ max, ($t(8) = -1.32$; $P = 0.162$).

Table A.3 Test-retest data (mean ± s) for sub-maximal and maximal $\dot{V}O_2$ data measured by the CPX/D and MGA 1100.

Intensity	CPX/D Test 1	MGA 110 Test 2
50 W ($L.min^{-1}$)	0.93 ± 0.03	1.03 ± 0.02
100 W ($L.min^{-1}$)	1.39 ± 0.04	1.46 ± 0.04
150 W ($L.min^{-1}$)	2.02 ± 0.06	2.07 ± 0.04
175 W ($L.min^{-1}$)	2.31 ± 0.06	2.36 ± 0.03
$\dot{V}O_2$ max ($L.min^{-1}$)	3.89 ± 0.12	3.86 ± 1.1

Such pilot data indicates that the CPX/D pulmonary gas analysis system provides measures of $\dot{V}O_2$ that are comparable to those of the MGA 1100 across a range of exercise intensities for participants with similar levels of aerobic fitness as soccer players.

3.2 $\dot{V}O_2$ kinetic parameter estimation

The S_o of the noise for the steady-state $\dot{V}O_2$ response to the 100 W cycling was determined for each system (CPX/D, 0.07 L.min^{-1}; MGA 1100, 0.10 L.min^{-1}) so that the 95% CI for 80%Δ τ_1 estimation could be calculated (Lamarra *et al.*, 1987). It was revealed that to estimate 80%Δ τ_1 to within ± 2 s, 1 exercise transition was required for both the CPX/D and the MGA 1100. The analysis also revealed that if four 80%Δ exercise transitions were performed, τ_1 estimation would be within ± 0.35 s for the CPX/D, which is smaller than the ± 0.5 s calculated for the MGA 1100. So it would appear that parameter estimation is comparable if not superior for the CPX/D.

Appendix 4

Reproducibility of the Yo-Yo Intermittent Recovery Test Level 2 (YIRT2)

The reproducibility of the performance scores obtained from the YIRT2 has not previously been investigated. As the test is to be used in the thesis as the indicator of soccer-specific high-intensity running capacity this was a paucity in the knowledge that needed to be addressed.

With institutional ethical approval eight professional soccer players (mean ± s) age 21.2 ± 3.2 years, stature 178.4 ± 6.2 cm, body mass 76.7 ± 4.3 and $\dot{V}O_2$max 58.4 ± 2.8 $ml.kg^{-1}.min^{-1}$ took part. All players had been at a professional club for at least two years. Prior to the administration of any test, participants were screened for existing medical conditions that might become aggravated during the testing procedure.

Players performed the YIRT2 on two occasions, separated by seven days. The test was performed outdoors on a dry artificial grass surface. Players were withdrawn from the test when they could no longer perform 25m shuttles in time with audible signals generated from a cassette tape. Test performance was recorded as number of shuttles and distance covered (see chapter 3.2.7).

The players' performance over the two tests is listed below in Table A.6. Paired sample t-tests revealed that the test-retest data for shuttles ($t(8) = -1.12$; $P = 0.18$) and distance ($t(8) = -1.01$; $P = 0.21$) covered did not differ.

Table A.4. Test-retest data (mean ± s) for the number of shuttles and distance covered by professional soccer players in the YIRT2.

Measure	Test 1	Test 2
Shuttle number	21.24 ± 0.41	21.12 ± 0.43
Distance run (m)	864 ± 87	851 ± 91

The CV for the test-retest data was low at 3%, as was the method error ± 15.5 m. These findings compare well to those of Krustrup *et al.* (2003) who reported a corresponding CV value of 5% for level 1 of the test. The LOA calculation indicated a narrow range of differences between test-retest measures with a range of –24 ± 43 m. This 95% spread of differences equates to 3.8% of the grand mean YIRT2 distance of 857 m.

Such data indicates that performance measures from the YIRT2 are reproducible on a test-retest basis and compare well to reproducibility data previously published for level 1 of the test.

Appendix 5

Lactate Analyser Reproducibility

The CV of the lactate analyser was established. Five 25 µl samples of 5 mmol.l^{-1} were analysed. The pippetting sequence and technique were standardised to reduce any inter-sample variation that might influence reproducibility.

Table A.5. The CV data for the measures of lactate standard.

5 mmol.l^{-1} Sample	Reading
1	5.01
2	5.10
3	5.02
4	4.98
5	4.96
Mean	5.06
s	0.05
CV%	1.1

Appendix 6

Pre-test Medical Questionnaire

Name:

Date of Birth: _____ Age: _____ Sex: _____

Please answer the following questions by putting a circle round the appropriate response or filling in the blank.

1.	How would you describe your present level of activity?				
	Sedentary	moderately active	Active	Highly active	
2.	How would you describe your present level of fitness?				
	Low level of fitness	Moderately fit	Fit	Very fit	
3.	How would you consider your present body weight?				
	Underweight	Ideal	Slightly overweight	Very overweight	
4.	Smoking Habits:	Do you currently smoke?		Yes	No
		Are you a previous smoker?		Yes	No
		How long is it since you stopped			Years
		Were you an occasional smoker		Yes	No
					Per day
		Were you a regular smoker		Yes	No
					Per day
5.	Do you drink alcohol?			Yes	No
	If you answered Yes, do you have:				
	An occasional drink	A drink every day	More than one drink a day		
6.	Have you had to consult your doctor within the last six months?			Yes	No
	If you answered Yes, please give details to the tester.				
7.	Are you presently taking any form of medication?			Yes	No
	If you answered Yes, please give details to the tester.				

8.	As far as you are aware, do you suffer or have you ever suffered from:							
	a.	Diabetes?	Yes	No	b.	Asthma?	Yes	No
	c.	Epilepsy?	Yes	No	d.	Bronchitis?	Yes	No
	e.	Any form of heart complaint?	Yes	No	f.	Raynaud's Disease?	Yes	No
	f.	Marfan's Syndrome?	Yes	No	h.	Aneurysm or embolism?	Yes	No

9.	Is there a history of heart disease in your family	Yes	No

10.	Do you currently have any form of muscle or joint injury?	Yes	No

11.	Have you had to suspend your normal training in the last two weeks	Yes	No

12.	As far as you are aware, is there anything that might prevent you from successfully completing the tests that have been outlined to you?	Yes	No

13. Please read the following questions.

As far as you are aware:

a. Are you suffering from any known active serious infection?

b. Have you had jaundice within the previous year?

c. Have you ever had any form of hepatitis?

d. Are you HIV antibody positive?

e. Have you had unprotected sexual intercourse with any person from an HIV high risk population.

f. Have you ever been involved in intravenous drug use?

g. Are you a haemophiliac?

If you can answer yes to any of questions a – g, please sign here _____

If you have answered no to all of question a – g, please sign here _____

Thank you

Appendix 7

Informed Consent

Sheffield Hallam University

Faculty of Health and Wellbeing
Sport and Exercise Research Ethics Committee

INFORMED CONSENT FORM	
TITLE OF PROJECT:	
The participant should complete the whole of this sheet himself/herself	
Have you read the Participant Information Sheet?	YES/NO
Have you had an opportunity to ask questions and discuss this study?	YES/NO
Have you received satisfactory answers to all of your questions?	YES/NO
Have you received enough information about the study?	YES/NO
To whom have you spoken? ..... Do you understand that you are free to withdraw from the study: • at any time • without having to give a reason for withdrawing • and without affecting your future medical care	YES/NO
Have you had sufficient time to consider the nature of this project?	YES/NO
Do you agree to take part in this study?	YES/NO
Signed ..... Date ..... (NAME IN BLOCK LETTERS).....	
Signature of Parent / Guardian in the case of a minor	

Appendix 8

Participant Information Sheet

Sheffield Hallam University

School of Sport and Leisure Management

Research Ethics Committee
Participant Information Sheet

Project Title	Oxygen uptake kinetics of football players
Name of Participant	
Supervisor/Director of Studies	Dr Mary Fysh
Principal Investigator	Carl Wells

Purpose of Study and Brief Description of Procedures
(Not a legal explanation but a simple statement)

The purpose of the investigation is to identify if oxygen uptake ($\dot{V}O_2$) kinetics determine the ability to perform football specific exercise. $\dot{V}O_2$ kinetics refers to the rate at which oxygen is used at the muscle in response to changes in exercise intensity. In the current study this will be achieved by measuring the air a person breathes out when exercising. The procedure for this is explained below. When participants visit the laboratory they are expected to behave in a sensible and orderly manner.

The testing procedures involved are:

1. Graded exercise test.
This will involve running on a motorised treadmill. The speed of the treadmill will start at 8 $km.h^{-1}$ (a slow jog) and will be increased by 1 $km.h^{-1}$ (a small increase) every minute. You will be required to run for as long as possible until you are exhausted. The test is maximal and will cause feelings of fatigue that will last for a few minutes and are similar to those experienced at the end of a hard training session. During the test you will have to breathe through a mouthpiece that is connected to a gas analyser, this is so the amount of oxygen you are using to produce energy can be measured.

2. Intermittent treadmill test.
You will be required to walk slowly for 2 minutes, run at a 3/4 pace for 6 minutes and then walk for 12 minutes at a slow pace, followed by a 20-minute passive recovery period. This protocol is performed four times. The test is sub-maximal. During the protocol, you will have to breathe through a mouthpiece as for the graded exercise test.

3. Maximal anaerobic run test (MART)
You will be required to perform a series of runs on a motorised treadmill. Each run will last for 20 seconds followed by 100 seconds of passive recovery. The starting speed is 14.3 $km.h^{-1}$ (a fast jog) with the gradient at 10.5% (steep hill), thereafter the speed is increased by 1.2 $km.h^{-1}$ for each run and the gradient is kept constant. This process is repeated until you reach voluntary exhaustion.

4. YoYo Intermittent endurance test.
You will be instructed to run back and forth along a twenty-meter course, keeping in time with audible bleeps generated from an audiocassette tape. The time between the bleeps will gradually decrease so that you have to run faster to keep in time with the tape. You must try to keep in time with the bleeps until you are exhausted. The test is maximal and will cause fatigue.

Risk of injury or cardiovascular complication during these testing procedures is very low.
If necessary continue overleaf

Purpose of Study and Brief Description of Procedures
(Not a legal explanation but a simple statement)

Study design

The study is structured in the following way:

Weeks 1 - 2: perform the physiological assessments detailed above

Weeks 3 - 8: perform 6 weeks of soccer-specific training. This will involve high-intensity exercise consisting of small sided games and running drills. It is intended that the exercise performed is at an intensity equal to or greater than 90% of your maximal heart rate. You will be required to perform the exercise 3 times per week. The timetabling of this will be arranged subsequently.

Weeks 9 - 10: repeat the physiological assessments to identify any changes in fitness.

It has been made clear to me that, should I feel that these Regulations are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform Professor Edward Winter, Chair of the School of Sport and Leisure Management Research Ethics Committee (Tel: 0114 225 4333) who will undertake to investigate my complaint.

Appendix 9

Chapter 4 Statistical analyses

9.1 Statistical analyses

Table A.6 Paired sample –t-tests: Pairs 1 to 6 are the test-retest values for measures taken from the incremental exercise test to exhaustion. Pairs 7 to 8 are the actual and predicted values for 80%GET and 50%Δ. Pair 9 is the blood lactate measured after the 80%GET and 50%Δ runs.

Paired Sample t-test						
		95% confidence interval of differences		t	df	sig. 2 tailed
		Lower	Upper			
Pair 1	Maxspeed	-0.244	2.136	1.18	8	0.242
Pair 2	tiexh	-0.196	2.443	1.09	8	0.273
Pair 3	VO2 1 - *VO2 2*	-0.471	2.032	1.14	8	0.271
Pair 4	TVEN1 - TVEN 2	-0.342	2.113	1.32	8	0.214
Pair 5	80%1-80%2	-0.733	3.143	0.89	8	0.296
Pair 6	50%1-50%2	-0.436	2.043	1.25	8	0.232
Pair 7	80%P-80%A	-0.455	3.121	1.46	8	0.194
Pair 8	50%P-50%A	-0.212	1.675	1.13	8	0.257
Pair 9	80%la-50%la	-0.547	4.087	3.85	8	0.002

Appendix 10

Chapter 5 Statistical Analyses

10.1 Statistical Analyses

Table A.7 Paired sample t-tests: Pairs 1 to 14, comparison of measures from parts A and B of the square-wave treadmill protocol: Pairs 15 to 18, comparison of phase III test-retest kinetic parameters measured during the heavy-intensity square-wave protocol: Pairs 19 to 20, comparison of on- and off-transient phase III kinetic parameters.

Paired Sample t-test						
		95% confidence interval of differences		t	df	sig. 2 tailed
		Lower	Upper			
Pair 1	LaA-LaB	-0.341	2.447	1.45	7	0.141
Pair 2	LapostA-LapostB	-0.277	2.342	1.05	7	0.253
Pair 2	*vo2A-vo2B*	-0.457	2.324	1.26	7	0.162
Pair 3	HRA-HRB	-0.565	3.326	1.51	7	0.125
Pair 4	HR80%-HR50%	-0.322	3.05	2.68	7	0.023
Pair 5	50%la1-50%la2	-0.466	2.875	3.23	7	0.013
Pair 6	t_1A-tBon	-0.287	1.032	1.07	7	0.297
Pair 7	tA-tBoff	-0.219	1.045	1.02	7	0.311
Pair 8	A_1A-A_1Bon	-0.436	2.899	1.34	7	0.156
Pair 9	A_1A-ABoff	0.154	1.654	1.17	7	0.183
Pair 10	td1A-td1B	-0.644	4.323	1.11	7	0.216
Pair 11	t2A-t2Bon	-0.366	2.073	1.15	7	0.192
Pair 12	t2A-t2Boff	-0.287	2.194	2.68	7	0.023
Pair 13	A_2A-A_2Bon	-0.433	2.561	1.09	7	0.231
Pair 14	A_2A-A_2Boff	-0.336	2.675	2.56	7	0.028
Pair 15	t21on-t22on	-0.287	1.032	1.28	7	0.168
Pair 16	t21off-t22off	-0.426	2.563	1.01	7	0.291
Pair 17	A21on-A22on	-0.575	4.332	1.47	7	0.115
Pair 18	A21off-A22off	-0.453	2.376	2.26	7	0.083
Pair 19	t2on-t2off	-0.211	1.126	3.78	7	0.004
Pair 20	A_2on-A_2off	-0.371	2.878	3.51	7	0.005

Table A.8 Two-way ANOVA repeated measures: Comparison of test-retest phase II kinetic parameters measured during for moderate- and heavy-intensity exercise.

Parameter	Source		F	Sig
$\tau_1$on	Sphericity assumed	Intensity	1.46	0.184
		Test	1.43	0.191
		Intensity*Test	1.21	0.373
$\tau_1$off	Sphericity assumed	Intensity	1.78	0.152
		Test	1.35	0.216
		Intensity*Test	1.29	0.285
A_1on	Sphericity assumed	Intensity	5.43	0.029
		Test	1.52	0.175
		Intensity*Test	1.45	0.201
A_1off	Sphericity assumed	Intensity	6.76	0.004
		Test	1.33	0.267
		Intensity*Test	1.58	0.142
td1 on	Sphericity assumed	Intensity	1.04	0.437
		Test	1.72	0.168
		Intensity*Test	1.13	0.351
td1off	Sphericity assumed	Intensity	1.69	0.171
		Test	1.44	0.234
		Intensity*Test	1.06	0.436

Table A.9 Two-way ANOVA repeated measures: comparison of on- and off-transient phase II kinetic parameters for moderate- and heavy-intensity running.

Parameter	Source		F	Sig
τ_1	Sphericity assumed	Intensity	1.78	0.137
		Transient	4.32	0.033
		Intensity*transient	1.86	0.116
A_1	Sphericity assumed	Intensity	6.52	0.029
		Transient	1.12	0.312
		Intensity*transient	1.33	0.228
td1	Sphericity assumed	Intensity	1.08	0.324
		Transient	1.02	0.368
		Intensity*transient	1.76	0.146

Appendix 11

Chapter 6 Statistical Analyses

11.1 Statistical analyses

Table A.10 Paired sample t-tests: Pair 1, comparison of phase II time delays for the on- and off-transients: Pair 2, comparison of phase II and phase III time delays during exercise onset.

Paired Sample t-test						
		95% confidence Interval of differences		t	df	sig. 2 tailed
		Lower	Upper			
Pair 1	td1on-td1off	-0.451	1.027	1.87	9	0.343
Pair 2	td1on-td2-on	-0.238	2.772	4.34	9	0.021

Table A.11 Two-way ANOVA repeated measures: comparison of test-retest phase II and III parameters measured during the onset and cessation of very heavy-intensity running

Parameter	Source		F	Sig
t1 test-retest	Sphericity assumed	Transient	5.47	0.034
		Test	2.56	0.172
		Transient*Test	1.36	0.295
t2 test-retest	Sphericity assumed	Transient	78.91	0.000
		Test	1.12	0.326
		Transient*Test	2.41	0.193
t1 and t2 comparison	Sphericity assumed	Phase	43.54	0.000
		Transient	17.85	0.006
		Phase*Transient	1.32	0.312
A1on test-retest	Sphericity assumed	Transient	2.66	0.168
		Test	3.78	0.115
		Transient*Test	0.87	0.479
A2 off test-retest	Sphericity assumed	Transient	5.41	0.032
		Test	2.89	0.134
		Transient*Test	1.77	0.227
A1 and A2 comparison	Sphericity assumed	Phase	63.87	0.000
		Transient	12.32	0.014
		Phase*Transient	2.32	0.211

Table A.12 Two-way ANOVA repeated measures: comparison of phase II and phase III parameters at the onset and cessation of exercise across the four 80%Δ transitions.

Parameter	Source		F	Sig
t1 1-4	Sphericity assumed	Transition	1.06	0.341
		Transient	5.19	0.036
		Transition*Transient	2.28	0.196
t2 1-4	Sphericity assumed	Transition	3.83	0.127
		Transient	5.79	0.025
		Transition*Transient	3.63	0.133
A1 1-4	Sphericity assumed	Transition	1.77	0.247
		Transient	1.64	0.271
		Transition*Transient	2.37	0.194
A2 1-4	Sphericity assumed	Transition	1.61	0.261
		Transient	15.65	0.004
		Transition*Transient	2.32	0.176

Appendix 12

Chapter 7 Training Diaries and Statistical Analyses

12.1 Training Diaries

A record of the training sessions and games performed by players during the eight weeks prior to the investigation.

Table A.13 Professional

Week	*No. of training sessions*	*No. of matches*
1	5	1
2	6	1
3	3	2
4	5	1
5	6	1
6	5	1
7	3	2
8	5	1
Mean	**4.8**	**1.3**

Table A.14 Amateur

Week	**No. of training sessions**	**No. of matches**
1	1	1
2	1	2
3	1	2
4	1	2
5	1	1
6	1	1
7	1	1
8	1	1
Mean	**1**	**1.4**

12.2 Statistical Analyses

Table A.15 Independent sample t-tests: Pairs 1 to 5, comparison of physiological and performance measures recorded for the Pro and Am soccer players during the incremental treadmill test to exhaustion: Pairs 6 to 9, comparison of performance measures recorded for Pro and Am soccer players recorded from the soccer-specific field tests. Pairs 10 to 12, comparison of DO_2 between the two groups during the onset of 80%Δ running.

Independent Sample t-test						
		95% confidence interval of differences		t	df	sig. 2 tailed
		Lower	Upper			
Pair 1	VO2maxAm-VO2maxPro	-2.143	1.966	-0.11	35	0.484
Pair 2	GETAm-GETPro	-1.531	1.695	1.26	35	0.291
Pair 3	TimeexAm-TimeexPro	-2.448	2.176	-0.16	35	0.426
Pair 4	MxspAm-MxspPro	-3.187	2.664	-0.14	35	0.463
Pair 5	MxHrAm-MxHrPro	-1.388	1.684	0.89	35	0.326
Pair 6	YoYoAm-YoYoPro	-2.432	1.674	2.54	35	0.034
Pair 7	RSTbesAm-RSTbesPro	-1.887	1.455	-4.41	35	0.012
Pair 8	RSTAvAm-RSTAvPro	-3.322	2.564	-3.06	35	0.014
Pair 9	RSTfiAm-RSTfiPro	-2.318	1.986	-2.42	35	0.024
Pair 10	DO2IIAm-Pro	-3.216	1.984	0.69	35	0.368
Pair 11	DO2IIIAm-Pro	-1.659	2.032	1.97	35	0.086
Pair 12	DO2totAm-Pro	-2.547	2.196	1.68	35	0.154

Two-way mixed ANOVA: Comparison of phase II and III kinetic parameters between and within the Pro and Am soccer players measured during the on- and off-transients of very heavy-intensity treadmill running.

Table A.16 Tests of Within subject effects

Parameter	Source		F	Sig
t1	Sphericity assumed	Transient	8.79	0.026
		Transient*Player	1.51	0.283
t2	Sphericity assumed	Transient	112.46	0.000
		Transient*Player	5.05	0.037
A1	Sphericity assumed	Transient	3.12	0.092
		Transient*Player	2.72	0.118
A2	Sphericity assumed	Transient	9.89	0.022
		Transient*Player	1.89	0.265

Table A.17 Tests of Between subjects effects

Parameter	F	Sig
t1	0.10	0.923
t2	4.03	0.034
A1	2.76	0.122
A2	2.52	0.183

Table A.18 Correlations Amateur

		VO2KG	TVENT	T1ON	T1OFF	T2ON	T2OFF	A1ON	A1OFF	A2ON	A2OFF	YOYO	FATIGUE	AV	BEST
VO2KG	Pearson Corr	1.00	0.79	-0.66	-0.34	0.26	-0.12	-0.48	-0.18	-0.53	0.31	0.78	0.23	0.21	0.16
	Sig. (2-tailed)	.	0.00	0.01	0.14	0.57	0.76	0.05	0.47	0.02	0.54	0.00	0.68	0.62	0.70
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
TVENT	Pearson Corr	0.79	1.00	0.63	0.18	0.05	-0.30	-0.58	0.08	0.22	0.11	-0.58	0.14	0.08	0.31
	Sig. (2-tailed)	0.00	.	0.05	0.49	0.89	0.22	0.05	0.83	0.64	0.75	0.03	0.83	0.78	0.24
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
T1ON	Pearson Corr	-0.66	-0.63	1.00	0.53	-0.10	0.20	0.61	0.31	0.41	0.10	-0.69	0.16	0.12	0.26
	Sig. (2-tailed)	0.01	0.04	.	0.04	0.70	0.61	0.00	0.18	0.09	0.75	0.00	0.86	0.88	0.70
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
T1OFF	Pearson Corr	-0.34	0.18	0.53	1.00	-0.39	0.17	0.35	0.32	0.07	-0.26	-0.34	0.12	0.17	0.18
	Sig. (2-tailed)	0.14	0.49	0.04	.	0.11	0.49	0.11	0.13	0.86	0.43	0.09	0.55	0.92	0.86
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
T2ON	Pearson Corr	0.26	0.05	-0.10	-0.39	1.00	-0.07	0.28	0.31	-0.11	0.29	0.22	0.28	-0.06	-0.22
	Sig. (2-tailed)	0.57	0.89	0.70	0.11	.	0.78	0.68	0.22	0.81	0.69	0.38	0.66	0.92	0.64
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
T2OFF	Pearson Corr	-0.12	-0.30	0.20	0.17	-0.07	1.00	0.11	0.11	-0.11	-0.55	-0.36	-0.02	0.10	0.01
	Sig. (2-tailed)	0.67	0.22	0.42	0.49	0.78	.	0.80	0.81	0.65	0.02	0.14	0.94	0.69	0.96
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A1ON	Pearson Corr	-0.48	-0.58	0.61	0.35	0.28	0.11	1.00	0.52	0.32	0.00	0.19	0.38	0.07	-0.13
	Sig. (2-tailed)	0.05	0.04	0.00	0.11	0.68	0.80	.	0.04	0.45	0.99	0.62	0.09	0.89	0.72
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A1OFF	Pearson Corr	-0.18	0.08	0.31	0.32	0.31	0.11	0.52	1.00	0.22	0.34	-0.31	0.21	0.09	0.28
	Sig. (2-tailed)	0.47	0.83	0.18	0.13	0.22	0.81	0.04	.	0.65	0.17	0.22	0.54	0.82	0.43
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A2ON	Pearson Corr	-0.53	0.22	0.41	0.07	-0.11	-0.11	0.32	0.22	1.00	0.08	0.19	0.24	0.31	-0.12
	Sig. (2-tailed)	0.02	0.64	0.09	0.86	0.81	0.85	0.45	0.65	.	0.81	0.69	0.23	0.29	0.64
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A2OFF	Pearson Corr	0.31	0.11	0.10	-0.26	0.29	-0.55	0.00	0.34	0.08	1.00	0.29	0.06	0.09	-0.04
	Sig. (2-tailed)	0.54	0.75	0.75	0.43	0.69	0.02	0.99	0.17	0.81	.	0.24	0.87	0.85	0.91
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
YOYO	Pearson Corr	0.78	-0.58	-0.69	-0.34	0.22	-0.36	0.19	-0.31	0.19	0.29	1.00	-0.23	-0.37	-0.41
	Sig. (2-tailed)	0.00	0.04	0.00	0.09	0.38	0.14	0.65	0.22	0.69	0.24	.	0.36	0.13	0.09
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
FATIGUE	Pearson Corr	0.23	0.14	0.16	0.12	0.28	-0.02	0.38	0.21	0.24	0.06	-0.23	1.00	0.71	0.74
	Sig. (2-tailed)	0.68	0.83	0.97	0.55	0.66	0.94	0.26	0.54	0.23	0.87	0.36	.	0.00	0.00
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
AVERAGE	Pearson Corr	0.21	0.08	0.12	0.17	-0.06	0.10	0.07	0.09	0.31	0.09	-0.37	0.71	1.00	0.84
	Sig. (2-tailed)	0.92	0.78	0.88	0.73	0.92	0.69	0.89	0.85	0.29	0.85	0.13	0.00	.	0.00
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
BEST	Pearson Corr	0.16	0.31	0.26	0.18	-0.22	0.01	-0.23	0.28	-0.12	-0.04	-0.41	0.74	0.84	1.00
	Sig. (2-tailed)	0.70	0.24	0.70	0.86	0.64	0.96	0.72	0.43	0.64	0.91	0.09	0.00	0.00	.
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00

Table A.19 Correlations Professional

		VO2MLKG	TVENT	T1ON	T1OFF	T2ON	T2OFF	A1ONNN	A1OFFF	A2ONN	A2OFFF	YOYOO	RSFATI	RSMEAN	RSBEST
VO2MLKG	Pearson Corr	1.00	0.62	-0.65	-0.51	0.17	0.13	0.65	0.38	0.22	0.02	0.71	0.15	0.28	0.21
	Sig. (2-tailed)	.	0.02	0.01	0.03	0.51	0.71	0.00	0.10	0.62	0.83	0.01	0.68	0.92	0.66
	N	18.00	16.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	17.00	17.00	17.00
TVENT	Pearson Corr	0.62	1.00	-0.58	-0.49	-0.18	-0.27	0.15	0.14	0.12	-0.08	0.67	0.31	0.31	0.29
	Sig. (2-tailed)	0.02	.	0.04	0.05	0.48	0.29	0.54	0.58	0.64	0.75	0.02	0.21	0.21	0.24
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
T1ON	Pearson Corr	-0.65	-0.58	1.00	0.58	0.18	0.24	0.73	0.40	0.75	-0.12	-0.71	0.25	0.23	0.10
	Sig. (2-tailed)	0.01	0.04	.	0.02	0.73	0.34	0.00	0.10	0.00	0.63	0.01	0.97	0.88	0.70
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	17.00	17.00	17.00
T1OFF	Pearson Corr	-0.51	-0.49	0.58	1.00	0.24	0.24	0.23	0.12	0.23	-0.34	-0.63	0.15	0.22	0.31
	Sig. (2-tailed)	0.03	0.05	0.02	.	0.11	0.65	0.35	0.63	0.36	0.17	0.02	0.55	0.92	0.86
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	17.00	17.00	17.00
T2ON	Pearson Corr	0.17	-0.18	0.18	-0.39	1.00	-0.33	-0.01	-0.14	0.20	0.02	-0.26	-0.37	-0.43	-0.23
	Sig. (2-tailed)	0.51	0.48	0.70	0.11	.	0.21	0.96	0.59	0.44	0.94	0.30	0.13	0.08	0.37
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
T2OFF	Pearson Corr	0.13	-0.27	0.24	0.24	-0.09	1.00	-0.12	-0.50	-0.08	-0.48	-0.47	0.04	0.17	0.07
	Sig. (2-tailed)	0.71	0.29	0.34	0.65	0.71	.	0.63	0.03	0.76	0.04	0.05	0.87	0.51	0.78
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A1ONNN	Pearson Corr	0.65	0.15	0.73	0.23	-0.01	-0.12	1.00	0.55	0.11	0.00	0.11	-0.28	-0.24	-0.11
	Sig. (2-tailed)	0.00	0.54	0.00	0.35	0.96	0.63	.	0.03	0.76	0.99	0.76	0.26	0.34	0.65
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A1OFFF	Pearson Corr	0.38	0.14	0.40	0.12	-0.14	-0.50	0.55	1.00	0.56	0.28	-0.07	0.10	0.06	0.21
	Sig. (2-tailed)	0.01	0.58	0.10	0.63	0.59	0.03	0.03	.	0.02	0.25	0.77	0.68	0.80	0.40
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A2ONN	Pearson Corr	0.22	0.12	0.75	0.23	0.20	-0.08	0.11	0.56	1.00	-0.03	-0.22	-0.28	-0.27	-0.12
	Sig. (2-tailed)	0.62	0.64	0.00	0.36	0.44	0.76	0.76	0.02	.	0.90	0.57	0.26	0.28	0.64
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
A2OFFF	Pearson Corr	0.45	-0.08	-0.12	-0.34	0.02	-0.48	0.00	0.28	-0.03	1.00	0.44	0.00	0.03	-0.02
	Sig. (2-tailed)	0.06	0.75	0.63	0.17	0.94	0.04	0.99	0.25	0.90	.	0.07	0.99	0.91	0.94
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
YOYOO	Pearson Corr	0.71	-0.68	-0.71	-0.63	-0.26	-0.47	-0.50	-0.07	-0.62	0.44	1.00	0.19	0.17	0.03
	Sig. (2-tailed)	0.00	0.45	0.01	0.33	0.30	0.05	0.04	0.77	0.01	0.07	.	0.44	0.51	0.90
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
RSFATI	Pearson Corr	0.15	0.31	-0.01	0.15	-0.37	0.04	-0.28	0.10	-0.28	0.00	0.19	1.00	0.85	0.88
	Sig. (2-tailed)	0.68	0.21	0.97	0.55	0.13	0.87	0.26	0.68	0.26	0.99	0.44	.	0.00	0.00
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
RSMEAN	Pearson Corr	0.28	0.31	-0.04	0.02	-0.43	0.17	-0.24	0.06	-0.27	0.03	0.17	0.85	1.00	0.89
	Sig. (2-tailed)	0.92	0.21	0.88	0.92	0.08	0.51	0.34	0.80	0.28	0.91	0.51	0.00	.	0.00
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
RSBEST	Pearson Corr	0.21	0.29	0.10	0.04	-0.23	0.07	-0.11	0.21	-0.12	-0.02	0.03	0.88	0.89	1.00
	Sig. (2-tailed)	0.66	0.24	0.70	0.86	0.37	0.78	0.65	0.40	0.64	0.94	0.90	0.00	0.00	.
	N	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00

Appendix 13

Chapter 8: Training Diary, Running Courses and Statistical Analyses

13.1 Training Diary

A record of the soccer clubs normal training regime performed by the control group during the six week training intervention. This regime was also performed by the training group in addition to the running program listed in chapter 7.2.4.

Table A.20 Training diary

Week	No. of training sessions	No. of matches
1	6	1
2	4	1
3	3	2
4	5	1
5	5	1
6	5	1
Mean	**4.7**	**1.2**

13.2 Running Courses (not to scale)

10 s runs

Distance: 84 m, Time Target: 10 s, Estimated running speed: 30 km.h^{-1}

Figure A.3

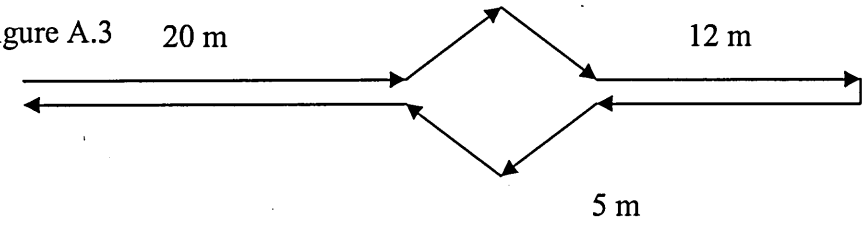
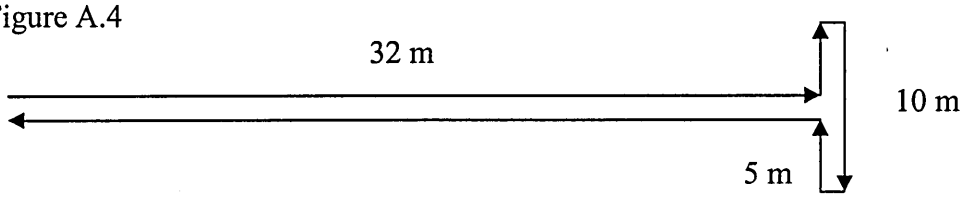


Figure A.4



35 s runs

Distance: 235 m, Time Target: 35 s. Estimated running speed: 24 km.h^{-1}

Figure A.6

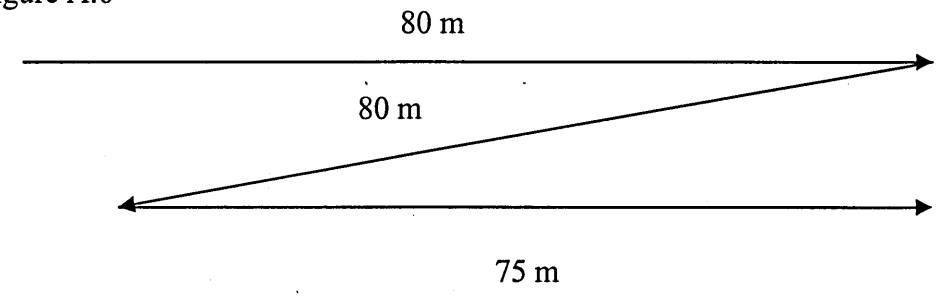
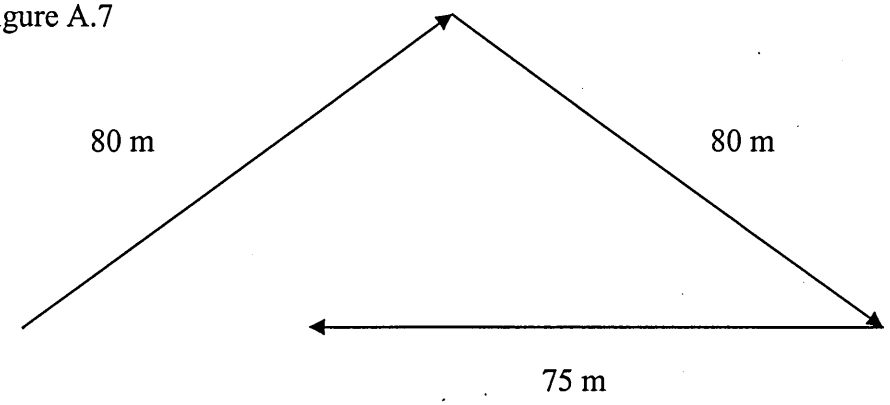


Figure A.7



60s runs

Distance: 320 m, Time target: 60 s, Estimated running speed: 19 $km.h^{-1}$

Figure A.8

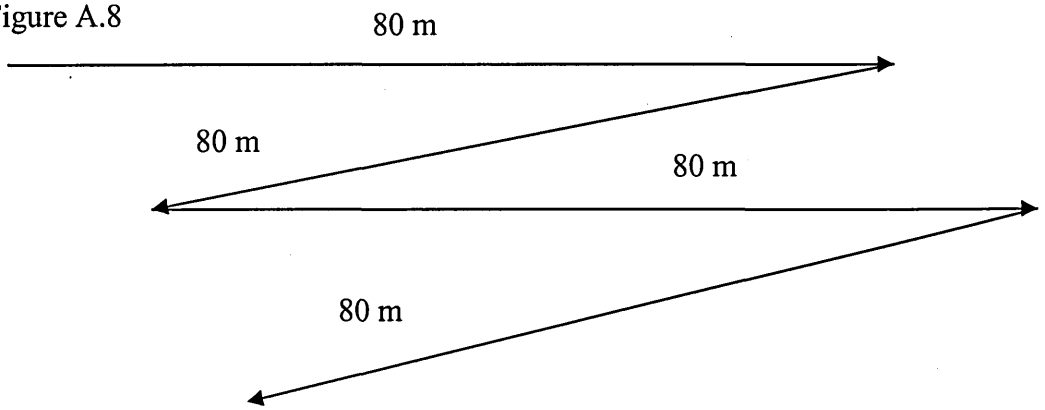
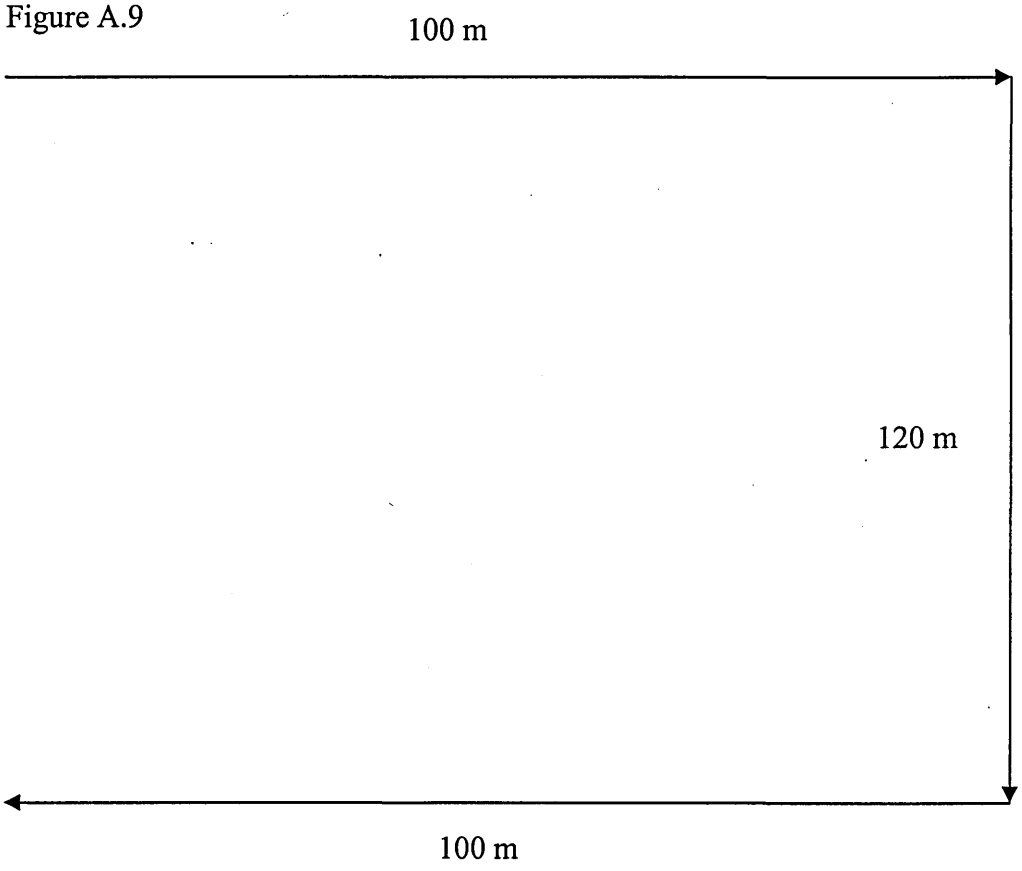


Figure A.9



13.3 Statistical Analyses

Two-way ANOVA mixed design: comparison of physiological, kinetic and performance measures within and between the Tr and Cn groups before and after the six week training intervention.

Table A.21 Tests of Within subject effects

Parameter	Source		F	Sig
VO2 max	Sphericity assumed	Time	3.46	0.081
		Time*Group	0.11	0.822
GET	Sphericity assumed	Time	2.03	0.175
		Time*Group	0.59	0.464
Exhtime	Sphericity assumed	Time	1.41	0.216
		Time*Group	0.85	0.344
Maxspeed	Sphericity assumed	Time	0.37	0.517
		Time*Group	0.59	0.433
t1on	Sphericity assumed	Time	0.34	0.568
		Time*Group	0.43	0.532
t1off	Sphericity assumed	Time*Group	0.17	0.736
		Time	2.01	0.191
t2 on	Sphericity assumed	Time	10.21	0.012
		Time*Group	6.28	0.038
t2 off	Sphericity assumed	Time	1.16	0.319
		Time*Group	0.84	0.368
A1on	Sphericity assumed	Time	1.22	0.274
		Time*Group	0.13	0.742
A1off	Sphericity assumed	Time	1.99	0.216
		Time*Group	0.58	0.472
A2on	Sphericity assumed	Time	0.15	0.735
		Time*Group	2.58	0.637
A2off	Sphericity assumed	Time	3.18	0.093
		Time*Group	1.87	0.142
Td1on	Sphericity assumed	Time	0.39	0.522
		Time*Group	2.06	0.185
Td1off	Sphericity assumed	Time	2.18	0.114
		Time*Group	0.24	0.738
Td2on	Sphericity assumed	Time	0.46	0.577
		Time*Group	0.16	0.763
YoYo	Sphericity assumed	Time	10.12	0.015
		Time*Group	9.56	0.022
MARTpower	Sphericity assumed	Time	9.58	0.021
		Time*Group	10.56	0.012
MARTspeed	Sphericity assumed	Time	9.45	0.023
		Time*Group	9.57	0.020
MARTtime	Sphericity assumed	Time	10.32	0.019
		Time*Group	10.54	0.012
MARTla	Sphericity assumed	Time	2.95	1.246
		Time*Group	0.35	0.865
DO2 Total	Sphericity assumed	Time	2.34	0.176
		Time*Group	4.67	0.071
DO2 II	Sphericity assumed	Time	1.87	0.276
		Time*Group	0.49	0.572
DO2 III	Sphericity assumed	Time	5.81	0.041
		Time*Group	6.97	0.036

Table A.22Tests of Between subjects effects

Parameter	F	Sig
VO2 max	0.23	0.737
GET	0.63	0.467
Exhtime	0.18	0.765
Maxspeed	0.39	0.646
t1on	0.58	0.475
t1off	0.12	0.832
t2on	6.98	0.039
t2off	0.72	0.461
A1on	0.19	0.746
A1off	0.57	0.474
A2on	1.18	0.328
A2off	2.93	0.104
Td1on	1.21	0.288
Td1off	1.56	0.222
Td2on	1.41	0.254
YoYo	12.67	0.011
MARTpower	8.28	0.024
MARTspeed	7.54	0.037
MARTtime	11.21	0.012
MARTla	2.45	1.217
DO2 Total	1.59	0.218
DO2 II	1.67	0.195
DO2 III	7.53	0.038

Table A.23 Correlations Pre intervention for the training group.

		VO2MLKG	TVENT	T1ON	T1OFF	T2ON	T2OFF	A1ONNN	A1OFFF	A2ONN	A2OFFF	YOYOO	Mapo	Masp	Mati	MARTLa
VO2MLKG	Pearson Corr	1.00														
	Sig. (2-tailed)	.														
	N	8.00														
TVENT	Pearson Corr	0.68	1.00													
	Sig. (2-tailed)	0.05	.													
	N	8.00	8.00													
T1ON	Pearson Corr	-0.67	0.64	1.00												
	Sig. (2-tailed)	0.05	0.05	.												
	N	8.00	8.00	8.00												
T1OFF	Pearson Corr	-0.51	-0.49	0.63	1.00											
	Sig. (2-tailed)	0.14	0.18	0.08	.											
	N	8.00	8.00	8.00	8.00											
T2ON	Pearson Corr	-0.16	0.12	0.36	-0.31	1.00										
	Sig. (2-tailed)	0.82	0.88	0.41	0.45	.										
	N	8.00	8.00	8.00	8.00	8.00										
T2OFF	Pearson Corr	-0.08	-0.45	0.35	0.05	0.11	1.00									
	Sig. (2-tailed)	0.91	0.22	0.37	0.96	0.88	.									
	N	8.00	8.00	8.00	8.00	8.00	8.00									
A1ONNN	Pearson Corr	0.71	0.66	-0.24	0.29	0.46	0.17	1.00								
	Sig. (2-tailed)	0.04	0.05	0.62	0.58	0.25	0.80	.								
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00								
A1OFFF	Pearson Corr	0.42	0.18	0.44	0.16	-0.10	-0.46	0.59	1.00							
	Sig. (2-tailed)	0.19	0.79	0.18	0.86	0.89	0.17	0.12	.							
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00							
A2ONN	Pearson Corr	0.14	0.36	0.19	0.44	-0.21	-0.33	-0.11	0.43	1.00						
	Sig. (2-tailed)	0.84	0.47	0.78	0.19	0.70	0.54	0.87	0.21	.						
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00						
A2OFFF	Pearson Corr	-0.17	-0.23	0.32	0.51	0.33	0.47	-0.27	-0.45	-0.07	1.00					
	Sig. (2-tailed)	0.81	0.68	0.52	0.12	0.54	0.18	0.66	0.20	0.90	.					
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00					
YoYo	Pearson Corr	0.69	-0.68	-0.65	-0.32	0.28	0.39	0.48	0.05	-0.14	0.44	1.00				
	Sig. (2-tailed)	0.03	0.45	0.03	0.68	0.63	0.47	0.17	0.94	0.83	0.05	.				
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	0.96	8.00				
MARTPo	Pearson Corr	0.10	0.26	-0.06	0.10	-0.42	-0.01	-0.33	0.05	-0.33	-0.05	0.81	1.00			
	Sig. (2-tailed)	0.89	0.69	0.92	0.90	0.23	0.98	0.48	0.96	0.49	0.95	0.04	.			
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00			
MARTSp	Pearson Corr	0.14	0.16	0.19	-0.11	0.08	-0.13	-0.17	0.15	0.06	0.05	0.74	0.85	1.00		
	Sig. (2-tailed)	0.83	0.79	0.72	0.88	0.91	0.85	0.76	0.83	0.95	0.96	0.03	0.00	.		
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00		
MARTTi	Pearson Corr	0.27	0.35	0.16	0.10	-0.17	0.13	-0.05	0.27	-0.06	0.04	0.81	0.88	0.89	1.00	
	Sig. (2-tailed)	0.27	0.43	0.79	0.88	0.77	0.86	0.96	0.62	0.92	0.97	0.02	0.00	0.00	.	
	N	0.48	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	
MARTLa	Pearson Corr	0.16	0.47	0.14	0.12	0.19	0.09	0.16	0.45	0.15	0.14	0.27	-0.13	0.11	0.21	1.00
	Sig. (2-tailed)	0.78	0.18	0.85	0.88	0.69	0.90	0.78	0.20	0.82	0.86	0.35	0.85	0.88	0.18	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8

Table A.24 Correlations post the intervention for the training group.

		VO2KG	TVENT	T1ON	T1OFF	T2ON	T2OFF	A1ON	A1OFF	A2ON	A2OFF	YOYO	Mapo	Masp	Mati	Mala
VO2KG	Pearson Corr	1.00														
	Sig. (2-tailed)	.														
	N	8.00														
TVENT	Pearson Corr	0.79	1.00													
	Sig. (2-tailed)	0.00	.													
	N	8.00	8.00													
T1ON	Pearson Corr	-0.72	-0.68	1.00												
	Sig. (2-tailed)	0.04	0.05	.												
	N	8.00	8.00	8.00												
T1OFF	Pearson Corr	-0.34	-0.17	0.67	1.00											
	Sig. (2-tailed)	0.28	0.83	0.05	.											
	N	8.00	8.00	8.00	8.00											
T2ON	Pearson Corr	-0.32	0.41	0.29	0.18	1.00										
	Sig. (2-tailed)	0.36	0.25	0.55	0.83	.										
	N	8.00	8.00	8.00	8.00	8.00										
T2OFF	Pearson Corr	-0.25	0.22	-0.11	-0.36	-0.05	1.00									
	Sig. (2-tailed)	0.61	0.62	0.89	0.41	0.93	.									
	N	8.00	8.00	8.00	8.00	8.00	8.00									
A1ON	Pearson Corr	-0.63	-0.57	0.42	-0.28	0.36	0.15	1.00								
	Sig. (2-tailed)	0.06	0.08	0.24	0.64	0.44	0.87	.								
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00								
A1OFF	Pearson Corr	0.19	0.45	0.31	0.46	0.22	-0.12	0.65	1.00							
	Sig. (2-tailed)	0.81	0.23	0.32	0.21	0.63	0.89	0.05	.							
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00							
A2ON	Pearson Corr	-0.49	-0.37	0.36	-0.09	0.48	0.33	0.12	0.16	1.00						
	Sig. (2-tailed)	0.19	0.29	0.39	0.92	0.18	0.37	0.93	0.86	.						
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00						
A2OFF	Pearson Corr	0.39	0.19	0.18	-0.18	0.37	-0.47	0.08	0.42	0.16	1.00					
	Sig. (2-tailed)	0.41	0.80	0.79	0.80	0.33	0.23	0.93	0.24	0.87	.					
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00					
YOYO	Pearson Corr	0.70	0.68	-0.66	-0.28	-0.16	-0.31	-0.44	-0.39	0.24	0.32	1.00				
	Sig. (2-tailed)	0.03	0.05	0.05	0.67	0.85	0.38	0.20	0.31	0.69	0.29	.				
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00				
MARTPo	Pearson Corr	-0.18	0.11	-0.15	0.12	0.11	0.08	-0.07	-0.13	0.32	-0.05	0.89	1.00			
	Sig. (2-tailed)	0.83	0.89	0.92	0.87	0.90	0.93	0.94	0.88	0.28	0.96	0.01	.			
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00			
MARTSp	Pearson Corr	0.18	0.05	0.09	0.14	-0.09	0.07	0.04	0.06	0.28	0.06	0.84	0.89	1.00		
	Sig. (2-tailed)	0.81	0.96	0.90	0.85	0.91	0.93	0.97	0.93	0.66	0.93	0.01	0.00	.		
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00		
MARTTi	Pearson Corr	0.12	-0.07	0.19	0.18	0.21	0.09	0.20	0.21	-0.10	-0.08	0.85	0.88	0.94	1.00	
	Sig. (2-tailed)	0.88	0.92	0.76	0.77	0.33	0.90	0.63	0.62	0.88	0.93	0.01	0.00	0.00	.	
	N	18.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	
MARTLa	Pearson Corr	0.11	0.15	0.12	0.15	0.06	0.04	-0.21	-0.16	0.05	0.36	0.11	-0.45	-0.41	-0.13	1.00
	Sig. (2-tailed)	0.88	0.82	0.87	0.82	0.90	0.95	0.68	0.76	0.94	0.41	0.88	0.19	0.23	0.86	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8

Table A.25 Correlations pre the intervention for the control group.

		VMAX	TVENT	T10N	T10FF	T2ON	T2OFF	A1ON	A1OFF	A2ON	A2OFF	YOYO	Mapo	Masp	Mati	MARTLa
VMAX	Pearson Corr	1														
	Sig. (2-tailed)	.														
	N	8														
TVENT	Pearson Corr	0.73	1.00													
	Sig. (2-tailed)	0.03	.													
	N	8.00	8.00													
T10N	Pearson Corr	-0.64	0.60	1.00												
	Sig. (2-tailed)	0.04	0.04	.												
	N	8.00	8.00	8.00												
T10FF	Pearson Corr	-0.32	0.24	0.18	1.00											
	Sig. (2-tailed)	0.12	0.16	0.32	.											
	N	8.00	8.00	8.00	8.00											
T2ON	Pearson Corr	0.03	-0.01	0.25	0.19	1.00										
	Sig. (2-tailed)	0.31	0.94	0.56	0.84	.										
	N	8.00	8.00	8.00	8.00	8.00										
T2OFF	Pearson Corr	-0.17	0.01	0.07	-0.04	0.11	1.00									
	Sig. (2-tailed)	0.32	0.96	0.70	0.82	0.53	.									
	N	8.00	8.00	8.00	8.00	8.00	8.00									
A1ON	Pearson Corr	0.22	0.14	0.55	0.19	-0.30	0.03	1.00								
	Sig. (2-tailed)	0.21	0.33	0.00	0.19	0.12	0.75	.								
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00								
A1OFF	Pearson Corr	0.25	0.13	0.35	0.10	0.38	-0.14	0.46	1.00							
	Sig. (2-tailed)	0.54	0.93	0.32	0.96	0.36	0.31	0.01	.							
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00							
A2ON	Pearson Corr	0.08	0.23	0.29	0.29	-0.12	0.01	0.26	0.45	1.00						
	Sig. (2-tailed)	0.93	0.55	0.51	0.51	0.89	0.97	0.53	0.27	.						
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00						
A2OFF	Pearson Corr	0.22	-0.12	-0.09	-0.08	-0.30	-0.23	0.23	0.15	0.16	1.00					
	Sig. (2-tailed)	0.59	0.88	0.93	0.93	0.37	0.58	0.57	0.86	0.86	.					
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00					
YOYO	Pearson Corr	0.71	0.65	-0.75	-0.39	0.15	-0.17	-0.13	-0.09	0.19	0.25	1.00				
	Sig. (2-tailed)	0.04	0.05	0.03	0.37	0.88	0.85	0.92	0.92	0.84	0.57	.				
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00				
MARTPo	Pearson Corr	0.35	0.27	0.13	-0.08	0.07	0.05	-0.05	0.02	0.25	-0.22	0.78	1.00			
	Sig. (2-tailed)	0.31	0.49	0.91	0.94	0.94	0.96	0.96	0.98	0.53	0.56	0.02	.			
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00			
MARTSp	Pearson Corr	0.20	0.14	0.03	0.18	-0.09	-0.29	-0.17	-0.08	-0.19	-0.13	0.72	0.86	1.00		
	Sig. (2-tailed)	0.59	0.89	0.97	0.24	0.86	0.49	0.84	0.91	0.77	0.86	0.03	0.01	.		
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00		
MARTTi	Pearson Corr	-0.24	0.26	-0.35	-0.46	0.19	0.23	-0.09	0.41	-0.07	-0.22	0.76	0.84	0.92	1.00	
	Sig. (2-tailed)	0.57	0.55	0.31	0.23	0.77	0.54	0.91	0.95	0.25	0.21	0.03	0.01	0.00	.	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	
MARTLa	Pearson Corr	0.32	0.41	0.29	0.25	0.19	0.08	0.22	0.45	0.34	-0.17	0.15	0.12	0.23	0.17	1.00
	Sig. (2-tailed)	0.37	0.25	0.55	0.59	0.74	0.91	0.61	0.0.22	0.33	0.79	0.87	0.93	0.61	0.81	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00

Table A.26 Correlations post the intervention for the control group.

Correlations																
		VO2KG	TVENT	T1ON	T1OFF	T2ON	T2OFF	A1ON	A1OFF	A2ON	A2OFF	YOYO	Mapo	Masp	Mati	MARTLa
VO2KG	Pearson Corr	1.00														
	Sig. (2-tailed)	.														
	N	8.00														
TVENT	Pearson Corr	0.75	1.00													
	Sig. (2-tailed)	0.03	.													
	N	8.00	8.00													
T1ON	Pearson Corr	-0.63	-0.61	1.00												
	Sig. (2-tailed)	0.04	0.04	.												
	N	8.00	8.00	8.00												
T1OFF	Pearson Corr	-0.48	-0.41	0.59	1.00											
	Sig. (2-tailed)	0.09	0.15	0.05	.											
	N	8.00	8.00	8.00	8.00											
T2ON	Pearson Corr	0.19	0.12	0.21	0.26	1.00										
	Sig. (2-tailed)	0.63	0.67	0.57	0.52	.										
	N	8.00	8.00	8.00	8.00	8.00										
T2OFF	Pearson Corr	-0.12	0.17	0.22	-0.23	0.14	1.00									
	Sig. (2-tailed)	0.78	0.59	0.44	0.49	0.68	.									
	N	8.00	8.00	8.00	8.00	8.00	8.00									
A1ON	Pearson Corr	0.57	0.53	0.22	-0.23	-0.19	0.28	1.00								
	Sig. (2-tailed)	0.07	0.08	0.45	0.68	0.73	0.43	.								
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00								
A1OFF	Pearson Corr	0.54	0.55	-0.34	-0.16	0.26	0.11	0.61	1.00							
	Sig. (2-tailed)	0.07	0.06	0.32	0.69	0.44	0.81	0.05	.							
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00							
A2ON	Pearson Corr	-0.33	0.26	0.08	0.07	0.13	-0.11	-0.32	0.39	1.00						
	Sig. (2-tailed)	0.26	0.71	0.93	0.86	0.76	0.91	0.29	0.22	.						
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00						
A2OFF	Pearson Corr	0.38	-0.13	0.14	0.21	-0.24	0.08	-0.11	0.31	0.07	1.00					
	Sig. (2-tailed)	0.21	0.82	0.80	0.55	0.69	0.94	0.87	0.29	0.91	.					
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00					
YOYO	Pearson Corr	0.73	-0.61	-0.71	-0.34	0.15	0.21	0.17	0.28	0.19	0.31	1.00				
	Sig. (2-tailed)	0.03	0.05	0.03	0.25	0.74	0.48	0.71	0.42	0.69	0.23	.				
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00				
MARTPo	Pearson Corr	0.23	0.26	0.16	0.14	0.15	-0.02	-0.07	0.13	0.11	-0.07	0.75	1.00			
	Sig. (2-tailed)	0.53	0.47	0.83	0.81	0.82	0.94	0.95	0.86	0.89	0.96	0.03	.			
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00			
MARTSp	Pearson Corr	0.25	0.12	0.16	0.15	0.08	-0.13	0.06	0.09	0.19	0.13	0.72	0.71	1.00		
	Sig. (2-tailed)	0.49	0.92	0.77	0.88	0.90	0.88	0.94	0.91	0.76	0.85	0.03	0.03	.		
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00		
MARTTi	Pearson Corr	0.07	0.13	0.36	0.18	0.25	0.10	-0.27	0.28	-0.12	-0.04	0.77	0.76	0.87	1.00	
	Sig. (2-tailed)	0.70	0.91	0.27	0.78	0.48	0.95	0.52	0.49	0.92	0.91	0.02	0.03	0.01	.	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	
MARTLa	Pearson Corr	0.12	0.18	0.41	0.23	0.30	0.15	-0.22	0.33	-0.07	0.01	0.21	0.18	0.11	0.21	1.00
	Sig. (2-tailed)	0.94	0.76	0.22	0.53	0.42	0.86	0.63	0.44	0.96	0.98	0.47	0.76	0.96	0.54	.
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00

Table A.27 Correlations between change in variables for the Pro group

		VO2KG	TVENT	T1ON	T1OFF	T2ON	T2OFF	A1ON	A1OFF	A2ON	A2OFF	YOYO	Mapo	Masp	Mati	Mala
YOYO	Pearson Corr	0.14	-0.11	0.19	-0.21	-0.14	0.22	0.06	-0.09	0.11	0.22	1.00				
	Sig. (2-tailed)	0.85	0.87	0.72	0.65	0.86	0.64	0.93	0.90	0.88	0.64	.				
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00				
MARTPo	Pearson Corr	0.12	0.10	0.16	-0.14	-0.22	0.09	-0.16	-0.13	0.14	0.22	0.89	1.00			
	Sig. (2-tailed)	0.86	0.89	0.79	0.84	0.65	0.91	0.79	0.86	0.84	0.62	0.01	.			
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00			
MARTSp	Pearson Corr	0.21	0.08	0.12	0.17	-0.06	0.10	0.07	0.09	0.31	0.09	0.90	0.89	1.00		
	Sig. (2-tailed)	0.63	0.90	0.86	0.76	0.93	0.89	0.92	0.90	0.59	0.91	0.01	0.00	.		
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00		
MARTTi	Pearson Corr	-0.15	-0.16	-0.14	0.11	0.12	-0.26	0.21	0.14	-0.19	-0.08	0.87	0.88	0.94	1.00	
	Sig. (2-tailed)	0.82	0.79	0.86	0.88	0.86	0.59	0.63	0.84	0.71	0.92	0.01	0.00	0.00	.	
	N	18.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	
Martla	Pearson Corr	0.26	0.35	0.48	0.51	-0.13	-0.11	0.19	-0.21	-0.07	0.22	-0.17	-0.21	-0.52	-0.14	1.00
	Sig. (2-tailed)	0.65	0.51	0.29	0.12	0.85	0.88	0.72	0.67	0.92	0.67	0.76	0.65	0.19	0.84	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8

Table A.28 Correlations between change in variables for the Cn group

		VMAX	TVENT	T10N	T10FF	T2ON	T2OFF	A1ON	A1OFF	A2ON	A2OFF	YOYO	Mapo	Masp	Mati	Mala
YOYO	Pearson Corr	0.17	0.15	0.13	0.19	0.22	0.06	0.32	-0.17	-0.11	0.29	1.00				
	Sig. (2-tailed)	0.78	0.82	0.85	0.71	0.63	0.93	0.53	0.77	0.87	0.52	.				
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00				
MARTPo	Pearson Corr	0.31	0.29	-0.16	0.22	0.09	0.16	0.13	0.22	0.29	-0.17	0.32	1.00			
	Sig. (2-tailed)	0.51	0.56	0.78	0.63	0.88	0.79	0.85	0.62	0.56	0.76	0.44	.			
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00			
MARTSp	Pearson Corr	0.16	0.10	-0.01	0.14	-0.13	-0.33	-0.21	-0.12	-0.23	-0.17	0.58	0.92	1.00		
	Sig. (2-tailed)	0.79	0.89	0.97	0.85	0.87	0.49	0.66	0.87	0.67	0.76	0.19	0.01	.		
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00		
MARTTi	Pearson Corr	0.08	-0.13	-0.36	-0.23	0.11	0.12	-0.42	0.21	-0.19	-0.18	0.32	0.89	0.92	1.00	
	Sig. (2-tailed)	0.57	0.55	0.31	0.23	0.77	0.54	0.91	0.95	0.25	0.21	0.45	0.01	0.00	.	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	
MARTLa	Pearson Corr	0.21	0.27	0.15	0.15	0.22	-0.11	-0.16	0.34	-0.05	0.22	0.27	0.10	0.08	0.09	1.00
	Sig. (2-tailed)	0.68	0.54	0.83	0.83	0.66	0.89	0.82	0.46	0.96	0.67	0.52	0.90	0.91	0.90	
	N	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00