MEN AND WOMEN IN HYPOXIA: THE INFLUENCE OF TISSUE OXYGENATION ON REPEATED-SPRINT ABILITY

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Dedications

I would like to dedicate this Master's thesis to my wife and two children for their endless love and support. It is without question that through their counsel and understanding the following works were made possible.

"*One may go a long way after one is tired*." *~French Proverb*

Abstract

This thesis examined the impact of oxygen (O_2) availability on prefrontal cortex and muscle tissue oxygenation during repeated-sprint exercise (RSE) in men and women. Men and women matched for initial-sprint mechanical work performed during ten, 10-s sprints (30s of rest) in normoxia (21% F_IO_2) and acute hypoxia (13% F_IO_2). Mechanical work and arterial O_2-saturation (S_PO_2) were obtained for every sprint. Oxy- and deoxygenated haemoglobin concentrations (O_2Hb, HHb) were obtained via near-infrared spectroscopy. Hypoxia elicited lower S_PO_2 and work (14.8% & 7.4%, $P < 0.05$), larger (45.1%, $P < 0.05$) and earlier reductions in cortical oxygenation, and no differences between sexes. Cortical de-oxygenation and work decrement were strongly correlated (R^2=0.85, $P < 0.05$). Muscle de-oxygenation was greater in men than women (67.3%, $P < 0.05$). These results show that O_2 availability influences cortical oxygenation and performance equally in men and women, and suggest a more efficient muscle O_2 uptake in women.

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-CHAPTER 1-

INTRODUCTION

Team sports are increasingly popular with millions of participants worldwide. For example, the 2009 Stanley Cup finals averaged 8 million viewers, and the Canada versus US Olympic hockey final broke the Canadian television broadcast record for a single period when 6.6 million people worldwide tuned in to watch the event ("Plus/Minus," 2009). The popularity of the 2010 world cup of soccer, considered the mecca of international team sports tournaments, is expected to break the previous viewership records at 26 billion throughout the month long tournament ("THE BIZNESS," 2009). As such, the past two decades have seen a tremendous increase in the number of research studies devoted to identifying and enhancing the athletic abilities related to team-sports performance (for review see (Glaister, 2005; Spencer, Bishop, Dawson, & Goodman 2005)). Athletes engaged in hockey, rugby, basketball, and soccer are required to repeatedly produce maximal or near maximal efforts (*i.e.*, sprints), interspersed with brief recovery intervals (consisting of complete rest or low- to moderate-intensity activity), over an extended period of time (1 to 4 hours) (Bishop, Lawrence, & Spencer, 2003; Enemark-Miller, Seegmiller, & Rana, 2009; Gabbett, 2010; Hamlin, Hinckson, Wood, & Hopkins 2008; Montgomery 2006; Spencer et al., 2005; Wadley & Le Rossignol 1998). Therefore, an important fitness component of these multiple-sprint sports has been termed repeated-sprint ability (RSA) (Bishop et al., 2001, Fitzsimons et al., 1993).

When sprints are repeated, it is necessary to distinguish two different types of exercise, *i.e.*, intermittent-sprint and repeated-sprint exercise (RSE). Intermittent-sprint exercise is characterised by short-duration sprints ($\leq$ 10 seconds), interspersed with recovery periods long enough (60 to 300 s) to allow near complete recovery of sprint performance (Balsom et al., 1992; Duffield et al., 2009). On the other hand, RSE is characterised by short-duration sprints ($<$ 10 seconds)

interspersed with brief recovery periods (usually ≤ 60 seconds). Such a distinction is important as the factors limiting performance are likely to be different for these two types of exercise.As such, intermittent-sprint exercise results in little or no performance decrement (Balsom et al., 1992; Bishop & Claudius, 2005), whereas RSE exhibits marked performance decrements (Figure 1: showing performance decrement during sprints) (Balsom et al., 1992; Billaut et al., 2006; Bishop et al., 2004; Gaitanos et al1993; Mendez-Villanueva et al., 2008).

RSE therefore provides researchers with a unique task mimicking the physiological demands of team sports, and thereby offering the possibility to investigate the determinants of RSA. Although there is limited research regarding RSA (as compared with endurance exercise), studies show that success in team sports is a complicated issue because of the complex interaction between technical, tactical, psychological and physiological factors (Bangsbo, 1994). However, the ability to sustain high-intensity, repeated loads throughout a match is believed to be crucial for the final outcome of the match. Owing to its importance, many studies have investigated the physiological responses or the effects of ergogenic aids on this mode of exercise (for review see (Billaut & Bishop, 2009; Glaister, 2005; Spencer et al., 2005)).

It has also been demonstrated that neuromuscular fatigue is influenced by the biological sex of the individual (for review see Drinkwater, 1984; Hicks et al., 2001; Shephard, 2000). The involvement of women in physical training programs and amateur and professional sport, where high-intensity, exhaustive exercise is commonly performed has dramatically increased in the last ten years, and many sport “idols” include women. In concert with this trend, Sport Canada and advisory groups involved in sport promotion have also established goals focusing on increasing sport participation among women.

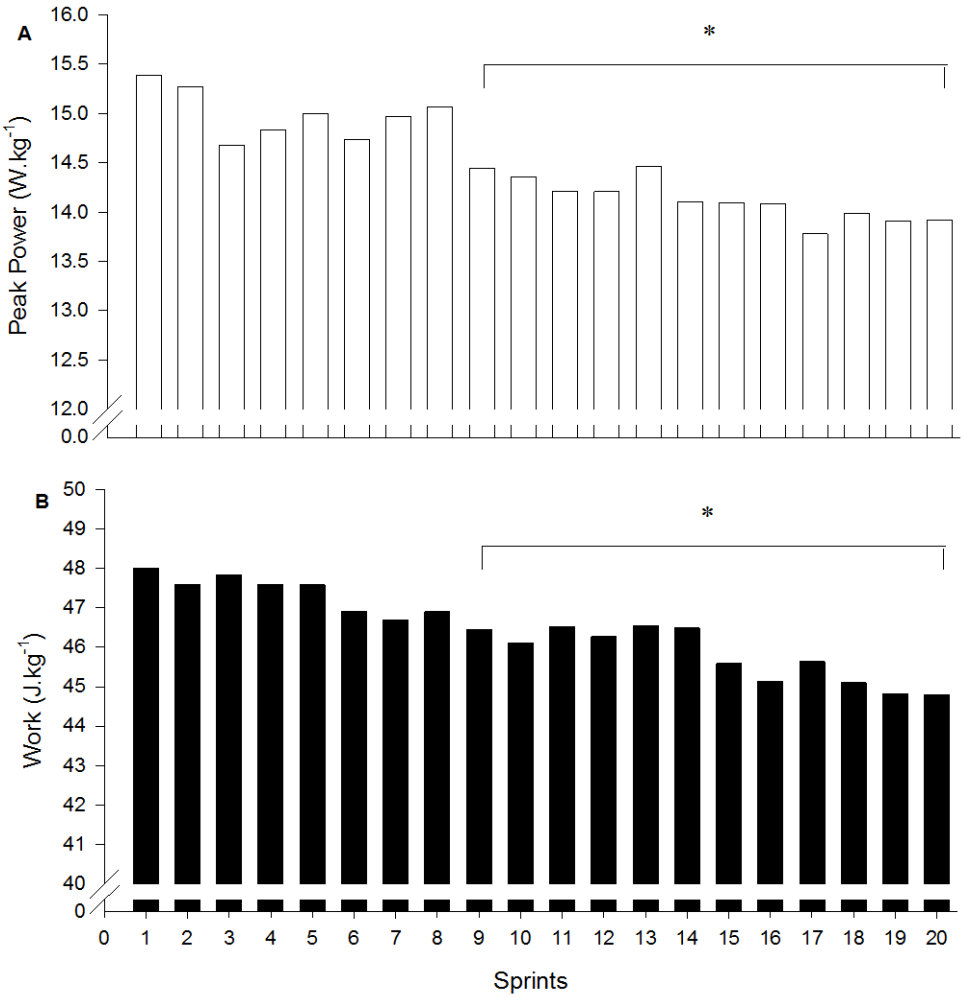

Fig.1. Example of mechanical (A: peak power & B: work) performance decrements relative to lean body mass during a repeated-sprint exercise (adapted from Billaut & Smith, (2009); Gaitanos et al., (1993);Mendez-Villenueva (2007). * denotes typical pattern of significance related to initial-sprint performance.

However, although exercise tolerance and its resultant muscle fatigue are topics that have fascinated physiologists since the early 1900s, most of the studies examining the causes of muscle fatigue have used male subjects. Compared to the relatively novel investigation of male physiology during maximal exercise it would be fair to say that the female aspect is still in its infancy (Billaut & Bishop 2009). Applying evidence obtained from males to training programs for females has little benefit as studies show they take advantage of different metabolic and phenotypic characteristics (Billaut & Bishop 2009; Hicks et al., 2001). Just as pharmacology of drugs differs between individuals, so may the physiological adaptations to acute and chronic exercise. A sex-specific approach to training program design is crucial to fully develop the physical potential of female athletes.

There is growing evidence that women experience lower performance decrement over exercise repetitions than their male counterparts (Billaut et al., 2003; Yanagiya et al., 2003). "Historically", this sex-related muscle fatigue has been attributed to muscle phenotypes (e.g. variation in fibre types and distribution) and metabolism (e.g. variation in substrate use and enzyme activity) (Esbjörnsson-Liljedahl et al., 2002: Hill & Smith, 1993), and little consideration has been given to the brain's ability to activate skeletal muscles. A potential sex difference in central fatigue cannot be ignored anymore.

1.2 Fatigue

For the purpose of this thesis, fatigue is defined as a RSE-induced reduction in the maximal power output (*i.e*. during cycling exercise) or speed (*i.e*. during running exercise) despite the task can that can be sustained. Fatigue during RSE typically develops rapidly after the first sprint. It is now accepted that fatigue can be caused by a variety of factors, ranging from the generation of an inadequate motor command in the motor cortex (*i.e*. neural factors) to the accumulation of metabolites within muscle fibres (*i.e*. muscular factors), and that there is no one global mechanism

responsible for all manifestations of fatigue (Figure 2). For example, studies have identified perturbations of the respiratory (Glaister, Stone, Stewart, Hughes, & Moir 2006; Price & Halabi, 2005) circulatory (Buchheit, Laursen, & Ahmaidi, 2007), metabolic (Bishop, Edge, Davis, & Goodman, 2004; Spencer et al., 2005) and nervous systems (Billaut & Basset, 2007; Billaut et al., 2006; Billaut & Smith, 2010; Buchheit et al, 2007; Mendez-Villanueva, Hamer, & Bishop, 2008; Perrey, Racinais, Saimouaa, & Girard, 2010; Racinais, Girard, Micallef, & Perrey, 2007). However, their respective contributions are continually being debated. The complex nature of fatigue is also highlighted by the diversity of approaches, models or indices (see section 2.2) that have been used to account for the decline in muscular performance.

In recent years there has been an exponential growth of interest in factors underlying fatigue during RSE. This is probably due to technological advances, the study of new potential limiting factors, and the inclusion of diverse RSE protocols. However, there is still no clear explanation for the mechanisms that limit RSA (Billaut & Bishop, 2009; Glaister, 2005). A model that clearly differentiates between central (central nervous system) and peripheral (muscle) factors associated with fatigue during RSE has yet to be established to enhance our knowledge of the integrative physiological responses to RSE.

1.3 Relevance

Understanding the factors contributing to fatigue and hence limiting performance is obviously the first step in order to design interventions (*i.e.*, training programs, ergogenic aids) that could delay the onset of fatigue and improve RSA. The ability of players to recover and reproduce performance is considered by many coaches to be a crucial fitness component of many sports, and it is thought that RSA may determine the final outcome of a game, by influencing the ability to win possession of the ball or to concede goals. In fact, ~0.8% impairment in sprint velocity would have a substantial detrimental effect in the chance of one player losing possession advantage

against an opponent, when both players sprint for the ball (Paton et al., 2001). Furthermore, Billaut et al. (2003) calculated that only a 7% drop of mechanical work during a short sprint would represent a loss of 5 to 7 meters in a race for the ball. While performance in multiple-sprint sports is largely dependent upon technical and tactical proficiencies, the importance of neuro-physiological factors to sustain consistent successful performance in a variety of sporting disciplines cannot be denied. For instance, it has been reported that RSA mean time predicts both the distance of very high-intensity running (> 19.8 $km \cdot h^{-1}$) and the total sprint distance during a professional soccer match (Rampinini et al., 2007).

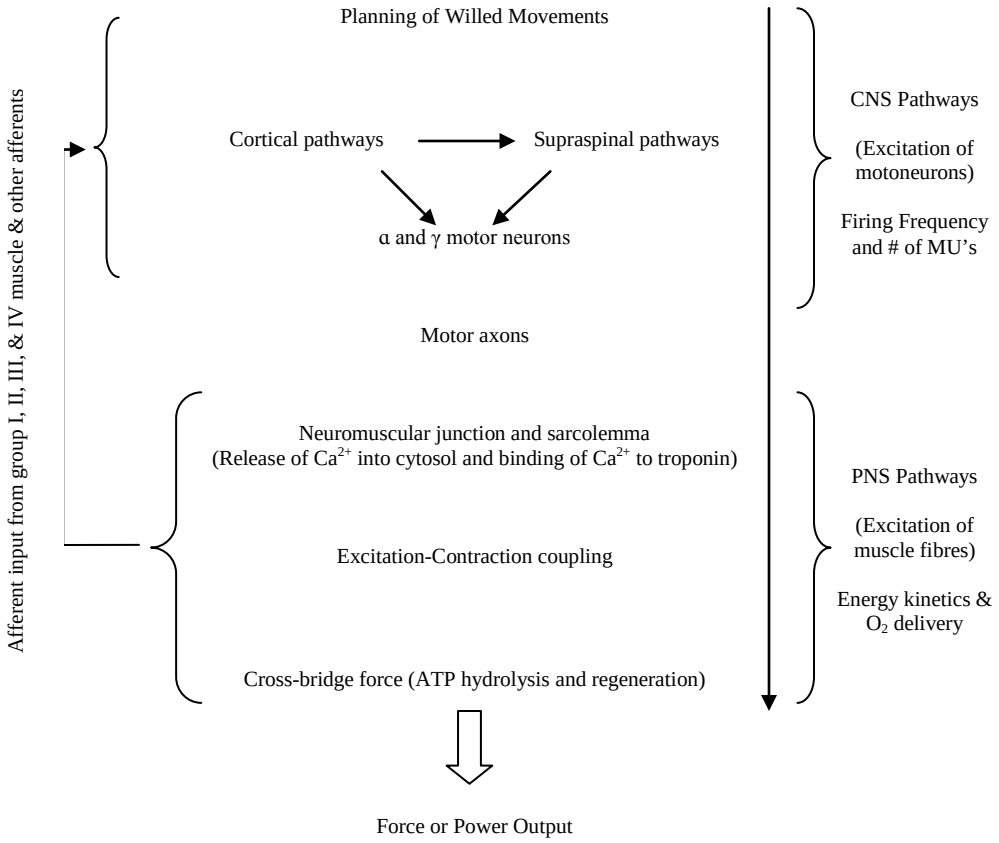

Fig.2. Model demonstrating the pathways of muscular activation and modulation beginning with the upstream cortical planning centers (pre-frontal and frontal cortices) located in the supra-corticol sections of the central nervous system (CNS). Excitation of motor pathways results in downstream firing of motor units (MU) and leading to peripheral nervous system (PNS) action potential (AP) propagation across neuromuscular junction. Muscle fibre excitation triggered by CNS stimulated AP's trigger mechanical ATP driven muscle contractions leading to force and power output. Energy kinetics and O_2 delivery result in afferent feedback to CNS and PNS possibly influencing excitation of motor pathways and MU recruitment altering force and power output.

-CHAPTER 2-

REVIEW OF LITERATURE

2.1. Activity profiles of team sports

The profile of many field-based sports (soccer, field hockey, rugby, gaelic and Australian rule football) indicate that athletes engage in strenuous exercise throughout a match as frequently as 20–60 times, covering on average 10–20 m per sprint, with each sprint respectively lasting a period of ≤ 10 s. The average distance covered by sprinting in team sports games is approximately 700–1000 m (Spencer et al., 2005). Variance in times and distances are related to individual sports and player position and an expected variance in the distribution of individual sport values considering differences in field sizes as well as skilled components and rules (Spencer et al., 2005). The frequency of these sprints is another important component of the athletic profile. Spencer et al. (2004) used a defined repeatability index, requiring 3 sprints with no more than 21 seconds separating each sprint to demonstrate the average number of repeated-sprint bouts during a field-hockey game was 4±1 with a mean recovery of 14.9±5.5 seconds. Oxygen consumption during repeated sprints is believed to be a maximal (90% VO_2max) and often supra maximal oxygen consuming activity (>100% VO_2max) (Dupont & Berthoin 2004; Glaister, Stone, Stewart, Hughes, & Moir 2007; Shibuya, Tanaka, Kuboyama, & Ogaki 2004). Spencer et al. (2004) identified that recovery during a field hockey match was performed as an active recovery, most likely because of dynamic environment of team sport match play. Active recovery during repeated sprints has been shown to have an increased duration of time spent at or above VO_2max compared to passive recovery activities (Dupont & Berthoin 2004). Therefore the recovery type and duration is another key component that should be considered when conducting a reliable repeated sprint test. Owing to this complex interplay of all-out efforts and submaximal runs, studies have consistently demonstrated that both aerobic and anaerobic metabolisms contribute significantly to the energy production during RSE (Balsom, Gaitanos, Soderlund, &

Ekblom, 1999; Balsom, Seger, Sjodin, & Ekblom 1992; Bishop & Claudius, 2005; Bishop & Spencer, 2004; Dupont & Berthoin, 2004; Esbjornsson-Liljedahl et al., 1999; Gaitanos, Williams, Boobis, & Brooks, 1993; Glaister et al., 2006; Price & Halabi, 2005; Wadley & Le Rossignol, 1998).

2.2 Manifestation of fatigue in repeated-sprint exercise

As previously mentioned, during RSE, fatigue manifests as a decline in maximal or mean sprint velocity (*i.e*., running), or a decrease in peak power or total work (*i.e*., cycling), over sprint repetitions (figure 1). (Bishop, Spencer, Duffield, & Lawrence 2001; McGawley & Bishop 2006; Price & Moss 2007; Spencer, Fitzsimons, Dawson, Bishop, & Goodman2006). The onset of fatigue is thus indicated by a drop in these mechanical indices from the initial-sprint values. Bishop et al. (2001) performed an analysis of performance decrements during RSE compared to those seen in a simulated game. The results highlighted a significant correlation ($r=0.71$, $P < 0.05$) between the fatigue occurring during five 6-s cycling sprints and the fatigue occurring during a game while players covered a distance of 15 m. Therefore, to quantify the amount of fatigue experienced during RSE, researchers have tended to use one of two terms, the fatigue index or the percentage decrement score. The fatigue index has generally been calculated as the drop-off in performance from the best to worst sprint performance during a RSE. In comparison, the percentage decrement score attempts to quantify fatigue by comparing actual performance to an imagined "ideal performance" (*i.e*. where the best effort would be replicated in each sprint) (Bishop et al. 2001;Spencer et al. 2006). A possible advantage of the percentage decrement score is that it takes into consideration all sprints, whereas the fatigue index will be influenced more by a particularly good or bad first or last sprint. By comparing eight different approaches, Glaister et al. (2008) also support the percentage decrement calculation as the most valid and reliable method to quantify fatigue in tests of RSA.

Fatigue has also been shown to develop along with signs of mechanical deficiency, such as decreases in the pedalling rate or moment (Billaut et al. 2005) and slower time to reach peak power (Billaut et al. 2003). Strength losses - *i.e.* reductions in the maximal isometric voluntary contraction torque - ranging from ~10 to 16% have typically been reported following repeated sprint cycling (Billaut et al., 2006; Racinais et al., 2007) or running (Girard et al., 2007) tests.

2.3 Possible sites of fatigue during repeated-sprint exercise

Intense muscle contractions impose a major strain on a wide variety of physiological systems. To generate the high force levels accompanying intense activity, maximal or near-maximal activation of all of the synergistic muscles is a fundamental requirement. Voluntary force generation results from a sequence of events (Figure 2) and each of these events is a potential limiting factor for force development (Gandevia, 2001;Vollestad, 1997).

During intense and repetitive muscular activity, maximal exercise can only be sustained for a brief period of time before fatigue quickly sets in. For example, during a maximal cycling sprint, power output peaks during the first 2–3 s and thereafter declines (Bogdanis et al., 1995; Gaitanos et al., 1993). If maximal effort continues to 30 s, power output typically falls by about 50% (Bogdanis et al. 1995, 1996). Under such exercise conditions, the inability to maintain or produce power output can be due to a failure of one or more steps in the chain of events leading to muscle contraction. The following sections do not intend to review all the putative factors that have been related with the development of fatigue, thus only a few selected candidates that have been suggested to contribute to muscle performance during RSE will be considered. These include factors describing peripheral and central fatigue, with a particular emphasis on the contribution from the central nervous system (CNS).

2.3.1 Peripheral Fatigue

At the peripheral level (*i.e.*, beyond the neuromuscular junction), an inability to repeatedly generate action potentials at the high frequency required for the intense contraction activity during sprinting may result in excitation failure or a failure to fully translate the neural signal to the interior of the fibre, finally resulting in lower muscle force. It is now well established that muscular fatigue during RSE can result from factors as varied as substrate utilization, energy turnover-rate, intramuscular bi-product accumulation and excitation-contraction coupling (Balsom, et al. 1999; Bishop & Claudius, 2005; Bishop et al. 2004; Glaister et al., 2006; Matsuura, Arimitsu, Kimura, Yunoki, & Yano 2007; Perrey et al. 2010; Racinais et al. 2007; Rockwell, Rankin, & Dixon 2003).

Muscular metabolic demands during a single maximum sprint are respectively large, requiring a high initial energy demand in order to obtain peak mechanical output scores; Phosphocreatine (PCr), adenosine triphosphate (ATP), and glycogen degradation in fast twitch muscle fibres during a 30-s Wingate protocol have been shown to be 83%, 50%, and 35% respectively. PCr is a primary factor involved in the re-synthesis of ATP anaerobically, a requirement in order to generate rapid amounts of ATP required to obtain peak mechanical scores (Glaister, 2005). The aerobic breakdown of glycogen and utilization of blood glucose are the remaining energy systems found to be involved in the re-synthesis of ATP (aerobic and anaerobic synthesis) during maximal exercise. Medbø and Tabata (1993) demonstrated during a 30-s cycling sprint that the relative metabolic contributions of the aerobic, anaerobic lactic and anaerobic alactic energy systems (ATP, PCr) to maximum mechanical output was approximately 55%, 45%, respectively. During shorter sprint durations ($\leq$ 10s), the relative energy contributions shift to a more equal utilization of PCr (55%) and ATP (45%) (Spencer et al., 2005), and during repeated sprints PCr contribution increases to 82% during the final sprint of a ten sprint protocol.

Furthermore, in vitro measurement of PCr turnover rates (approximately 9 mmol ATP/kgdm/sec), shows a complete depletion of intramuscular ATP stores, approximately 80 mmol ATP/kgdm/sec within 10 seconds of maximal exercise, indicating the need for other forms of substrate utilization in producing and maintaining maximal force production during RSE (Glaister 2005). Therefore, if energy pathways during single sprints are impaired to the point of observable mechanical decrement, it is therefore likely that the interplay between maximal exercise and recovery be vital to the force generating capacity when sprints are repeated. Coincidently, the recovery of power output during RSE has been described as following a similar time-course to that of PCr replenishment (Glaister 2005). In relation to this finding and the required contribution of PCr to peak power output Gaitanos et al. (1993) were able to indicate that a 30-s recovery period, coinciding with most RSA tests (Billaut & Bishop 2009; Glaister 2005; Spencer et al. 2005), is a sufficient time frame for PCr recovery to still contribute adequately (>50%) to the anaerobic production of ATP during subsequent sprints and most likely not the main factor in repeated sprint fatigue. Depletion of intramuscular ATP stores during maximal exercise (30-s sprint) has been shown to be reduced to approximately 45-50% pre-exercise levels; and as well, ATP reductions during brief intermittent exercise are believed to still be well maintained during RSE as a product of the interaction between ATP hydrolysis and PCr energy buffering kinetics (Glaister 2008; Spencer et al. 2005). Interestingly, a study performed by Balsom (1999)explored glycogen availability and its effect on RSA through the administration of a high and a low carbohydrate supplement prior to exercise. The data showed that increased glycogen availability improved performance in the later portion of the sprints during a RSE, thus, indicating a significant contribution from the other energy systems. Similarly, a study investigating the changes in metabolism during RSE, (Gaitanos et al. 1993), investigated the relative energy contributions, was unable to disregard ATP provision from aerobic sources in conjunction with the major PCr (sp1= 49.6%, sp2=80.1%) and anaerobic glycolytic contributions (sp1=44.1%, sp2=16.1%). The

effect of glycogen on RSA as seen by a 37% reduction from resting levels following RSE implicates glycogen as an important energy substrate in repeated sprint performance.

The contribution from the anaerobic metabolic pathways to the total energy production during RSE subsequently leads to the accumulation of metabolic by-products that are, in turn, also responsible for some loss of performance (Balsom, Wood, Olsson, & Ekblom, 1999; Bishop & Claudius, 2005; Bishop, Edge, & Goodman, 2004; Bishop, Edge, Mendez-Villanueva, Thomas & Schneiker 2009; Gaitanos et al., 1993). Metabolite accumulation (i.e., hydrogen ions [H^+] and inorganic phosphates[P_i]) stemming from the ATP producing metabolic pathways, and lower pH levels have been shown to inhibit the enzyme activities, muscle buffer capacity, muscle contractile properties and neurotransmitter (Ca^{2+}, K^+, Na^+) kinetics collectively summarized by Glaister (2005) and Spencer et al. (2005). Overall peripheral accumulation of H^+ is believed to impair performance because of correlation findings demonstrating a relationship between RSA and both muscle buffer capacity and changes in blood pH (Bishop, et al., 2004; Bishop et al., 2009; Matsuura, et al., 2007). Studies investigating the physiological response to enhanced (training and supplemental $NaCHO_3$) muscle buffer capacity and intracellular pH regulation via the sodium-hydrogen exchanger have further implicated the role of H^+ accumulation in the impairment of performance through the observation of a paralleled improvement in RSA (Bishop & Claudius, 2005; Bishop et al., 2004; Bishop et al., 2009; Edge, Hill-Haas, Goodman, & Bishop, 2006; Mohr et al., 2007). Accumulation of other metabolites such as interstitial potassium (K^+) and intracellular P_i although theorized to have adverse effects on metabolism and performance, have yet to be performed with the goal of linking such factors with RSA.

At the skeletal muscle level, marked ionic disturbances arising secondary to decreases in Na^+-K^+-ATPase activity have been observed following intense dynamic contractions (Vollestad & Sejersted 1988). In such cases, the Na^+-K^+ pump cannot readily re-accumulate the potassium (K^+)

efflux into the muscles cells, inducing at least a doubling of muscle extra-cellular [K^+] (Juel, , Pilegaard, Nielsen, , & Bangsbo, 2000). These modifications will impair cell membrane excitability and depress force development (Fuglevand, Zackowski , Huey & Enoka, 1993). Since most of our knowledge, to date, has been gained from *in vitro* studies it is still unclear whether these ionic disturbances contribute to fatigue during RSE. Nevertheless, unpublished observations indicate that plasma [K^+] increases significantly (~30%) following 5 x 6-second sprints (30 seconds of recovery). However, the potentially-negative impact that this increase in plasma [K^+] might have on RSE performance is not clear.

The contribution of oxidative phosphorylation to the total energy expenditure during a single, short sprint is rather limited (< 10%; Jacobs et al., 1983; Gaitanos et al.,1993;Parolin et al., 1999). As sprints are repeated, however, the level of aerobic ATP provision progressively increases such that the O_2 uptake (VO_2) achieved during the final repetitions of a RSE is often close to 70-80% VO_2 max (Balsom et al., 1999; Hamilton et al., 1991). It is therefore not surprising to note that aerobic metabolism may contribute as much as 40% of the total energy supply during RSE (Balsom et al., 1999; Gaitanos et al., 1993;Hamilton et al., 1991;Tomlin & Wenger, 2002). This suggests that increasing VO_2 max via appropriate training (Edge et al., 2005) and/or ergogenic aids (*e.g.*, erythropoietin; Balsom et al., 1994) may allow for a greater aerobic contribution during the latter sprints as well as during the recovery phase between sprints, potentially minimising fatigue and improving performance. This may explain why subjects with a greater VO_2 max display a better RSA (Bishop et al., 2003), and is supported by moderate correlations between VO_2 max and RSA (r = -0.20 to -0.75) (Bishop et al., 2004; Bishop & Edge 2006;Rampinini et al., 2009).

Finally, the use of the near-infrared spectroscopy (NIRS) technique allows monitoring tissue oxygenation directly in an attempt to explain the relationship between muscular oxidative

properties and RSA. In this perspective, several authors have shown that the increase in muscle de-oxyhemoglobin engendered by sprint repetitions remains fairly constant (Buchheit, Cormie, Abbiss, Ahmaidi, Nosaka, & Laursen., 2009; Racinais et al., 2007). This indicates that despite a progressive deoxygenation the ability of the subjects to use the available O_2 may be well preserved during RSE. Future studies combining pulmonary gas exchange kinetics and muscle/cerebral oxygenation measurements during RSE are needed to determine whether both cardiovascular (i.e., O_2 delivery) and muscle (i.e., O_2 extraction) impairments to oxidative metabolism contribute to performance decrements.

2.3.2 Central Fatigue

Peak power production is achieved through the optimal and dynamic interaction between muscle (excitation-contraction processes), neural recruitment strategies (type of motor units, size of motor unit pool, frequency and pattern of discharge), and the metabolic sources of ATP production (see above). Repetitive muscle contractions of high intensity have been shown to affect the synchrony between these interacting factors. And in some cases, fatigue during RSE might be of central origin.

Central fatigue can be defined as a reduction in force or power occurring through inadequate activation on motor neurons (Bigland-Ritchie, Jones, Hosking, & Edwards, 1978;Kent-Braun et al., 2002; Loscher, Cresswell, & Thorstensson, 1996) External stimulation techniques of the peripheral nerve/muscle and/or the motor cortex are employed to assess central fatigue (Kremenic, Glace, Ben-Avi, Nicholas, & McHugh, 2009; Russ, Towse, Wigmore, Lanza, & Kent-Braun, 2008; Szubski, Burtscher, & Loscher, 2007; Todd, Taylor, & Gandevia, 2004), but on other occasions the normalcy of the CNS behaviour may be assessed more functionally via surface electromyography (EMG) (Billaut et al., 2006;Billaut, Basset, & Falgairette, 2005; Matsuura, 2007 ; Mendez-Villanueva et al., 2008). The following sections aim to summarise the

varied techniques used by researchers to explore neural drive and muscle recruitment as they pertain to the study of fatigue during exercise, with a particular interest into RSE

2.3.2.1 Neural drive/Voluntary activation

Central fatigue can be detected by measuring voluntary activation via supramaximal stimulation of the motor nerve and/or the muscle (Belanger &McComas 1981; Kent-Braun & LeBlanc, 1996). Electrical stimulation has been widely used in the field of sport training to enhance strength training and to assess central fatigue (Taylor et al., 2000). The superimposition of an electrical stimulus onto a maximal voluntary muscle contraction can theoretically activate more motor units than a voluntary effort performed alone, which can engender an increase of the contraction force. An increment in force implies that some motor units have not been recruited by the voluntary effort or are not firing fast enough to achieve maximal force output: voluntary activation is less than 100%. If the increment in force evoked by the stimulus enlarges with exercise (from pre- to post-intervention), then voluntary activation is decreasing. A progressive, exercise-related failure of voluntary activation indicates central fatigue (Gandevia et al., 1995;Kremenic et al.,2009;Loscher et al., 1996;Loscher et al., 2002). The increased "artificial" force produced is due to a failure at a site within the CNS.

Stimulation of the motor cortex (i.e., transcranial stimulation) can also be used to estimate voluntary activation, and can further localise the site of failure of voluntary drive to at or above the level of the motor cortical output (Figure 1) (Taylor et al., 2000; Todd et al., 2003). Supraspinal fatigue is a component of central fatigue and is attributable to a suboptimal output from the motor cortex (Gandevia 2001; Taylor et al., 2000).

Recently, electrical stimulation has been used during RSE to assess central fatigue (Racinais et al., 2007; Perrey et al., 2010). A slight 3% reduction in voluntary activation

(interpolated-twitch technique) was observed after ten 6-s sprints on a cycle ergometer (Racinais et al., 2007). However, this was accompanied by a 15% drop in the RMS/M-wave ratio (*i.e.*, central to peripheral properties) providing further support to the reduced neural drive during RSE. Similar results of a slight reduction in voluntary activation (interpolated twitch; 2.7%) were reported by Perrey et al., (2010) following twelve 40-m repeated running sprints. This corroborates other studies demonstrating a reduction in EMG activity (i.e., net motor unit recruitment) during RSE (see section on "Muscle recruitment strategies") (Billaut et al., 2006; Billaut & Smith, 2009; Mendez-Villanueva et al., 2008). Overall, these results indicate a combination of central and peripheral components to fatigue during RSE. However, although relevant and valuable, the contributions and extent of central and peripheral fatigue may not be adequately assessed using post-fatigue stimulation techniques. Other innovative methods such as magnetic resonance imaging, electroencephalography, and near-infrared spectroscopy, in compliment to surface electromyography, may provide a more valid representation of "in-time" central impairments and subsequent modulation of motor neuron excitability.

2.3.2.2 Muscle recruitment strategies

Literature regarding muscle recruitment strategies has primarily relied on electromyographic (EMG) activity to quantify adjustments in the amplitude and frequency of muscle activity. In theory, failure to fully activate the contracting musculature –as assessed by lowered surface EMG– will decrease force production and therefore reduce RSA. While not a universal finding (Billaut et al., 2005;Billaut & Basset 2007; Girard et al., 2007, Hautier et al., 2000; Matsuura et al., 2007), a concurrent decline in sprint performance and the amplitude of EMG signals (*e.g.*, root mean square (RMS) and integrated EMG values) has been reported in several studies (Billaut & Smith, 2009, 2010; Mendez-Villanueva et al., 2007, 2008; Racinais et al., 2007) (Table 1). This has been interpreted as a net inhibition of the α-motor neuron pool (Billaut & Smith, 2009). This suggests that under conditions of fatigue development, the failure to

fully activate the contracting muscles may become an important factor limiting performance during RSE. In fact, changes in quadriceps EMG amplitude have been shown to explain 83%–97% of the variance in total work performed during cycling sprints of 6 s repeated with 30 s of rest (Billaut & Smith 2010; Mendez-Villanueva et al., 2008). Billaut and Smith (2009) also add to the current knowledge that this close relationship is valid in both men and women (R^2 = 0.97 and 0.86 in men and women, respectively; $P < 0.05$). Although the correlation of muscle activation and power output is obvious and strong, the EMG activity (serving as a surrogate for central fatigue) is only reduced by a few percents (from 10% to 20%) in the studies. This demonstrates that peripheral fatigue also accounts for part of the decline in RSA.

Despite reports suggesting that EMG may not be a robust method of distinguishing between central and peripheral mechanisms, it has been proven to be efficient at measuring agonist and antagonist muscle activation timing (ultimately regulated by the CNS) during sprint cycling (Billaut et al., 2005). It was first reported in studies investigating agonist and antagonist muscle coordination properties during fatigue that neural attenuations may contribute to performance decrements during RSE (Billaut et al., 2005). In fact, the authors noted a reduction in the time delay between agonist and antagonist muscle contractions, indicating that co-contraction of antagonist (bicep femoris) and agonist (vastus lateralis) muscles during RSE reduced the synergistic ability of the agonist muscles to drive maximal force production. This was considered as evidence of neural regulation. On the contrary, the findings of an increased EMG/force ratio during RSE (Hautier et al., 2000) could be considered as an indication of peripheral fatigue. Because no decrease in mean activation levels (EMG amplitude) of the active muscles (vastus lateralis, gastroc lateralis, and biceps femoris) were recorded despite a drop in peak power during the final sprints, the authors concluded that fatigue was not related to a neuromuscular attenuation.

Table 1. Repeated sprint studies investigating lower limb neuromuscular components of fatigue

Study	Mode of exercise	Protocol	Principal findings
Hautier et al., 2000	Cycle	15 x 5s RSE (25 s rest); EMG	↓ EMG sprints 14 and 15,↑ EMG/force (peripheral fatigue).
Billaut et al., 2005	Cycle	10x6s RSE (30s rest); EMG AG;ANT	↓AG and ANTcontraction coordination (possibly associated with progressive ↓PPO).
Billaut et al., 2006	Cycle	10x6s RSE (30s rest); Pre-post MVC	↑ EMG and lower post MVC suggest a modification in recruitment pattern.
Giacomoni et al., 2006	Cycle	10x6s RSE (30s rest); Pre- post MVC	↓ EMG and NME during post MVC (brought on by RSE).
Matsuura et al., 2006	Cycle	2[10x10s RSE](35s and 350s rest); Lac^{-1} and VO2; EMG	↑Lac^{-1} and ↓VO_2 associated with lower EMG in 35s condition. PPO (NS) btw conditions
Racinaiset al., 2007	Cycle	10x6s RSE(30s rest); EMG, pre – post MVC	↓ PP output and EMG during RSE; Pre>Post MVC (VAR and RMS/M-wave ratio); PNS and CNS fatigue components
Billaut & Basset, 2007	Cycle	10x6s RSE (inc, const, dec recovery); MVC	Recovery patterns post sprints leads to varying magnitudes of RS performance and post MVC.
Mendez-Villanueva et al., 2008	Cycle	10x6s RSE;EMG	↓PPO and MPO linearly related to ↓EMG (R^2=0.97).
Billaut & Smith, 2010	Cycle	20x5s RSE (30s rest); iEMG; S_pO_2	Linear relationship with W and iEMG (R^2=0.83). S_pO_2 associated with W and iEMG (R^2=0.68, R^2= 0.64)
Perreyet al., 2010	Running	12x40m RSE; stimulated MVC	↓ all twitch parameters and PT in post MVC (attributed to reduced muscle activation)

AG=agonist muscle; **ANT**=antagonist muscle; **CNS**=central nervous system; **cons**=constant recovery; **dec**=decreasing recovery; **EMG**=electromyography; **iEMG**=integrated EMG; **inc**=increasing recovery; **Lac^{-1}**=blood lactate concentration; **MPO**=mean power output; **MVC**=maximal voluntary contraction; **NS**=no significance observed; **PNS**=peripheral nervous system; **PPO**=peak power output; **twitch parameters**= H_{max}/M_{max}, P80/MVC ratio, RMS/M-wave, voluntary activation; **VO_2**= volume of oxygen consumption; **W**=work; ↓↑ indicates decreasing and increasing values.

or changes in the co-contraction of agonist and antagonist muscles. Hautier et al., (2000), however, speculated that the small reduction in antagonistic force output could be related to a reflex mechanism of central origin that monitors the fatigue of agonist muscles. These findings highlight therefore the presence of both central and peripheral integrative mechanisms associated with the mechanical decrements observed during RSE.

A shift of median frequency values (obtained during maximal isometric voluntary contractions) towards lower frequencies has been reported post-RSE (Billaut et al., 2006; Mendez-Villanueva et al., 2007). This has been interpreted as a modification in the pattern of muscle fibre recruitment; *i.e*. decreased recruitment of fibres with faster conduction velocities. That being said, one should exercise caution when interpreting these data since they were collected during tests of maximal voluntary contractions and may not reflect sprinting performance (Billaut et al., 2006).

Reduced activation of the α-motor neuron during RSE could be the result of a variety of central and peripheral mechanisms. One possibility is that inhibitory signals from small-diameter group III and IV metabolic afferents lower the α-motor neuron pool discharge, as the result of accumulated myo-cellular metabolites (H^+, P_i) (Amann & Kayser, 2009; Gandevia, 2001). Another mechanism that has been proposed is a down regulation of neural drive (efferent) to active musculature originating in upstream cortical and spinal centres possibly to accommodate firing rate to the increase in twitch duration in order to maintain an optimal pattern of stimulation, the phenomenon being called “muscle wisdom” (Enoka & Stuart 1992; Rupp, Girard, & Perrey, 2010; Todd, Taylor, & Gandevia, 2003; Todd et al., 2004).

Overall, it is now well accepted that the fatigue that develops during RSE (figure 3) is not merely a result of metabolic and contractile changes within the working muscles (peripheral

fatigue); some loss of force occurs because voluntary activation of the motor neuron pool in the brain declines (central fatigue) (Billaut et al., 2005; Billaut & Smith 2010; Racinais et al., 2007). However, what triggers these acute changes in the CNS behaviour remains to be determined, and this is a major aim of the current thesis. In doing so, we have concentrated on only the O_2 delivery and utilization by the tissues because of its potential relationship to motor unit recruitment, and our findings do not exclude the likely influence of other potential modulators (Jammes & Balzamo, 1992, Sinoway et al., 1989).

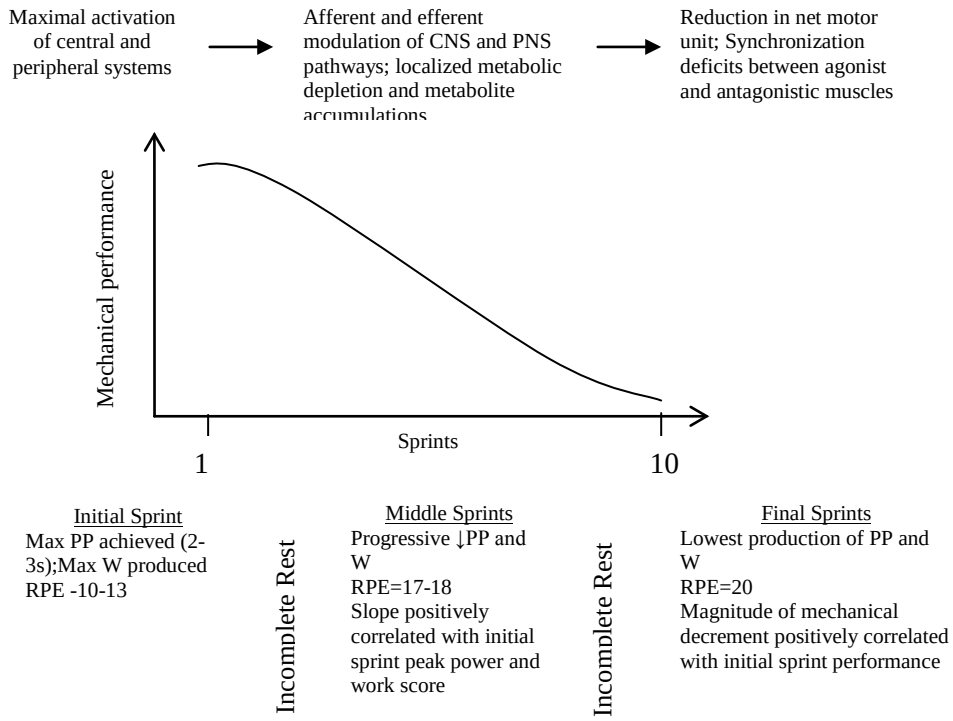

Fig.3. Example of the physiological and mechanical components to repeated sprint performance: **PP**= peak power, **W**= work, **RPE**= ratings of perceived exertion.

2.4 - The influence of oxygen availability on repeated-sprint performance

In conditions of hypoxia the partial pressure of O_2 falls. Faced with a reduction in O_2 availability, the rate of activity of O_2-consuming pathways falls and the balance of human cellular metabolism can switch from mitochondrial oxidative phosphorylation towards cellular anaerobic glycolysis. Consequently, exposure to hypoxia compromises the ability of humans to perform physical aerobic exercise (Amann & Calbet, 2008; Amann & Kayser, 2009). Alterations in oxygen availability are induced by combinations of environmental/ artificial manipulation and exercise factors. Systemic circulatory changes during exercise triggers chemo- and baro-receptors responding to changes in oxygen saturations, artero-venous tensions and metabolite accumulation, reflexively stimulating sympathetic pathways via afferent sensory feedback to supra-cortico, cortico-spinal and spinal locations in the CNS. Sympathetic reflexes are responsible for modifying various homeostatic mechanisms such as vascular resistance required to maintain efficient blood flow and O_2 delivery to central and peripheral systems (Amann & Calbet, 2008; Amann et al., 2006; Amann, Romer, Subudhi, Pegelow, & Dempsey, 2007; Calbet, 2000). Although an important mechanism in maintaining oxygen perfusion ratios at the appropriate cellular levels, it has been indicated that the effectiveness of the homeostatic mechanism is inadequate during maximal exercise intensities resulting in severe arterial hypoxemia (arterial saturation reduction < 75%). Failure to maintain blood flow and oxygen supply sufficiently to meet increased metabolic demand during maximal exercise has been identified as a key contributor to performance decrements.

The ability to generate maximal power output during a single sprint (up to 60-s duration) is, however, well preserved in acute hypoxia (Calbet et al., 2003; McLellan et al., 1990; Weyand et al., 1999) owing to an enhancement of the anaerobic energy supply. In contrast, the ability to reproduce performance in subsequent sprints during RSE has been shown to be impaired in hypoxia (Balsom et al., 1994). This is not surprising if one considers the contribution of the

aerobic metabolism to the total energy production when sprints are repeated with short recovery periods (see above section). Studies investigating the impact of O_2 availability on RSA are sparse. The study performed by Balsom et al., (1994) was the first of its kind and used a reduced O_2 content (13%) to investigate the anaerobic and aerobic contributions during RSE. The authors demonstrated that by reducing the O_2 availability, subjects were unable to reproduce similar levels of peak power compared with the controlled trial. Since 1994, several other studies have explored the influence of the use of O_2 on RSA (Balsom, Ekblom, & Sjodin, 1994; Billaut & Smith, 2010; Buchheit et al., 2009; Buchheit, Laursen, & Ahmaidi, 2009; Dupont & Berthoin, 2004), although these studies did not use hypoxia as a control condition. Nonetheless, these reports demonstrated that O_2 availability was related to metabolic and performance changes. A measure of crucial importance to the current thesis was the finding that O_2 availability was also significantly correlated with changes in muscle recruitment (accessed via changes in EMG activity) during RSE (Billaut & Smith 2009, 2010). In a pilot study, Billaut and Smith (2010) identified a correlation of determination of 0.64 ($P < 0.05$) between changes in integrated EMG and the fall in arterial O_2 saturation during RSE performed in normoxia. Two plausible explanations were proposed for this relationship: 1) increased reflexive inhibition of the alpha motoneuron pool through the facilitation of groups III/IV afferent neurons found in the carotid bodies, stemming from exercise induced hypoxaemia (Virkki et al., 2007); or 2) a homeostatic mechanism located in the CNS that could regulate neural drive and force output during maximal exhaustive exercise in response to increasing metabolic costs associated with hypoxaemia, in order to prevent irreversible organ failure (Noakes, Peltonen, & Rusko, 2001; St Clair Gibson et al., 2006). More research is therefore urgently needed to understand the impact of arterial hypoxaemia on the CNS function and the associated consequence for physical exercise performance. In this perspective, the non-invasive technique of near-infrared spectroscopy (NIRS) offers real-time measurement of oxygenation and haemodynamics in tissues (Neary 2004; Van Beekvelt et al., 2001), and thus,

constitutes a relevant tool to enhance our current knowledge of central (CNS) and peripheral (muscle) determinants of RSA.

2.4.1 The usefulness of near infrared spectroscopy in analyzing fatigue

Near-infrared spectroscopy (NIRS) is a non-invasive technique that utilizes infrared lasers to penetrate through skin, muscle and bone several centimetres deep into muscle and cerebral capillary beds. NIRS allows for the quantification of muscle and cerebral tissue oxygenation by measuring and quantifying changes in oxy and de-oxygenated haemoglobin molecules located within the identified vasculature (for review see (Neary, 2004; Perrey, 2008). The NIRS instrumentation uses specific wavelength frequencies (650-950 mm) transmitting infrared photons through electrodes placed on the skin to monitor the concentrations of oxy- and deoxy-haemoglobin through the absorptive and reflective actions of the chromophores (colour compound) using the Beer and Lambert law: $A=\log(I_o/I)= \varepsilon cd$ where A is the absorption of light expressed as optical density (log of the ratio between intensities of incident and transmitted light I), c the chromophore concentration, ε it extinction coefficient, and d the thickness (optical path length) of the solution. This formula describes the relationship between the absorption and concentration of the solution measured. The application of this formula allows for the manipulation of infrared photons to be transmitted through the superficial tissues deep into the corresponding vascular tissues resulting in a unique absorbance and reflection of the photons based on the whether or not the haemoglobin is saturated or unsaturated with oxygen molecules. The alteration or change in infrared light based on absorbance or reflection is then received through a second detector optode attached a specified distance from the transmission electrode. The need for this optical path length is due to the behaviour of photons, and their uncanny ability to act like photons which results in a scattering effect in all directions. Therefore in order to accurately quantify the respective NIRS signal, a longer optical path length is required to receive the appropriate changes in chromophore concentrations. In the brain the optode distance ranges

from 40–60 mm resulting in a banana shaped optical path length. The photons detected are therefore only the photons that were scattered in the path of the shallow arc. In order to resolve the differences in scattered and transmitted light a further modification of the Beer-Lambert law is required to calculate optimal spacing. The modified Beer-Lambert law allows researchers to use a more appropriate differential path length factor (DPF), which operates as a mediator between absorber concentrations and attenuations in the transmitted light. The explanation above is a simplistic breakdown of the mathematical foundations of the NIRS system, further explanation of the complexities of the Beer-Lambert equation and its utilization in the NIRS system is discussed in exhaustive detail elsewhere (Perrey, 2008).

As mentioned earlier, the NIRS technique offers real-time measurement of oxygenation and haemodynamics in tissues (Ide, Horn, & Secher, 1999; van Beekvelt, Borghuis, van Engelen, Wevers, & Colier, 2001; van Beekvelt, van Engelen, Wevers, & Colier, 2002), and thus, may prove very useful to explore further the central and peripheral determinants of performance. Muscular and cerebral tissue oxygenation is quantified by measuring the concentrations of oxy- (O_2Hb) and deoxy-haemoglobin (HHb) as well as total haemoglobin (THb) at rest or during exercise. Furthermore, haemoglobin difference (Δ[Hbdiff] = [O_2Hb] – [HHb]) can be calculated and used as an additional indicator of oxygenation due to its high correlation with blood flow (Tsuji et al., 2000). The difference between the two reflect the kinetics involved in oxygen demand and availability to the measured tissue. Validation studies using indocyanide green intravascular tracer support the quantification of total HHb as a reliable measure of regional tissue blood flow (Boushel et al., 2000). Furthermore, NIRS has been successfully validated against functional magnetic resonance imaging and positron emission tomography measures (Kleinschmidt et al., 1996, Villringer et al., 1997). The oxygen related metabolic cost of cerebral cortex neural activation during novel tasks were also confirmed in a study comparing NIRS oxygenation to the gold standard for measuring cerebral metabolic and electrical activity

(magnetic resonance imaging [MRI] and electroencephalography [EEG]) (Gusnard & Raichle, 2001). In addition, NIRS is more practical to use during sprinting activity than MRI that is more suitable for small muscle investigations. Overall, these data highlight the effective and relevant utilization of NIRS as a primary measure of cerebral hemodynamic and neural activation during exercise.

Oxygen supply to the brain during conscious rest is believed to approximate 11 – 15 % of the cardiac output, equivalent to approximately 20% of oxygen consumption (Wolf et al., 2008). During maximal exercise, however, due to a increase cardiac output, a larger portion is diverted from the brain to the active muscle to maintain the required level of O_2 delivery of activated neurons and muscle tissue (Ainslie, Barach, Cummings, Murrell, Hamlin, & Hellemans, 2007). Data on cerebral oxygenation exist for constant workload exercise (Amann et al., 2007), incremental test to maximal effort (Subudhi et al., 2007), time trial (Billaut, Davis, Smith, Marino & Noakes, 2009) and supramaximal exercise (Nielsen et al., 1999; Shibuya et al., 2004). Its role in the cessation of exercise or, at least, the reduction of exercise intensity is highly dependent upon the availability of O_2. While in normoxia cerebral oxygenation is usually maintained and is not readily considered as a limiting factor of performance (Billaut et al., 2009; Rasmussen et al., 2010; Subudhi, Dimmen, & Roach, 2007; Subudhi, Lorenz, Fulco, & Roach, 2008), it has been shown to be affected by mild to severe arterial hypoxemia (arterial saturation, $S_PO_2 < 89\%$) (Subudhi et al 2008, 2009, Amann & Kayser 2009). Further support for the critical role of cerebral hypoxia in fatigue comes from studies showing that a reversal of cerebral deoxygenation during exercise actually maintains performance (Nielsen, Boushel, Madsen & Secher, 1999; Subudhi et al., 2008). More than 10 years ago, Nielsen and colleagues (1999) were the first to demonstrate that by increasing the inspired fraction of O_2 (FiO_2=.30), elite rowers were able to improve their performance in a time trial on a rowing ergometer, as compared with normoxia.

The improved performance during the hyperoxic trial was paralleled by a higher cerebral oxygenation in the absence of muscle changes.

2.4.2 Near-infrared spectroscopy and repeated-sprint exercise

NIRS data during sprint exercise are difficult to find in the literature. Some studies have reported a reduction in Mox during RSE (Buchheit et al., 2009; Racinais, Bishop, et al., 2007), yet, because of the fairly constant level of deoxygenation observed in the active musculature, the authors have suggested that muscular oxygen uptake is sufficient and, thus, is not likely to limit RSA in normoxia. That being said, studies performed in hypoxia to ascertain this hypothesis are still missing.

As far as the cerebral tissue is concerned, no published data are available at this stage despite its potentially-strong role in muscle activation and RSA. Shibuya et al (2004) examined cerebral oxygenation during seven 30-s supramaximal exercises at 150% VO_2 max and separated with 15 s of rest. The initial rise (+5.7 µM [Hb_{Diff}]) in Cox at the onset of exercise was significantly reduced during the final sprint (-8.4 µM [Hb_{Diff}]). However, this decrement was not associated with usual systemic changes associated with cerebral oxygenation impairment (i.e., arterial hypoxemia, higher end-tidal CO_2 concentration). Although these results indicate that cerebral hypoxia may affect performance during intermittent supramaximal exercises, the protocol used by Shibuya et al. (2004) did not replicate a team-sport pattern. Therefore, the above perturbations have yet to be explored during RSE to determine whether cerebral oxygenation influences RSA. In this perspective, it is interesting to note that Billaut and Smith (2010) demonstrated that RSE induces arterial hypoxemia similar to the levels reported in other studies and that impair Cox (Amann & Kayser, 2009; Nielsen et al., 1999; Nybo & Rasmussen, 2007). This exciting perspective provided the rationale for the measurement of cerebral oxygenation, in both normoxia and hypoxia, during RSE.

2.5 General sex differences in response to exercise

It is widely accepted that sex differences exist in absolute muscle strength and peak power output. These differences have been shown through a wide range of exercises, muscle groups. The research regarding the female muscular fatigue response has shown that the female muscle is capable of longer endurance times (*i.e.*, improved fatigue resistance) and faster recovery of force output compared to the male muscle (Billaut & Bishop, 2009; Hunter, 2009; Russ et al., 2008). These differences have been observed during single joint isometric and isokinetic contractions as well as complex dynamic movements such as endurance and sprint activities. Aside from the endurance and strength differences it has been shown during repeated sprint exercise that women are better able to maintain peak power and mechanical work during repeated sprint fatigue. There have been several possible suggestions explaining the sex differences pertaining to exercise related fatigue. Body composition (lean and fat mass), muscle metabolism (e.g., hormonal regulation, enzymatic activities, substrate utilization), muscular phenotype (fibre type characteristics) and, finally, motor unit recruitment (firing frequency and number) are detailed below. In addition to these factors, it is of interest to investigate these physiological differences during exercise with changing environments. Therefore potential sex differences in the environmental responses will also be discussed.

2.5.1 Morphological and Body Composition

The morphological and body composition differences in men and women are among the most identifiable and apparent differences observed when comparing the two. The mean height of men in comparable age range is significantly larger (+11cm) compared to their female counterparts. Mean weight (+13 kg), lean muscle mass (+18kg) and fat mass (-5kg) are also different when comparing men and women (Glenmark, Hedberg & Jansson, 1992; Hill & Smith, 1993; Kumagai et al., 2000; Maughan, Watson & Weir, 1983; Mayhew & Salm, 1990). Research

methodologies therefore use these differences in body composition to explain sex differences in performance as well as attempting to match men and women for relative sex related performance decrements. Cross-sectional area (CSA) of single or multiple muscles, specific CSA tension (possibly fibre type distribution differences), and possible anthropometric differences in joint and lever kinematics are believed to be the main contributors to maximum strength (Hakkinen & Keskinen, 1989). These determinants of voluntary max strength are again in favour of the male gender, with men having higher mean segment length and muscle CSA, leading to a higher absolute muscle force and power output than women. Studies investigating BM differences and total mechanical work during repeated sprints has shown a significant correlation ($r=0.91$; $p<0.05$) between higher scores in athletic populations (Bishop, 2003). It is reasonable to expect that when utilizing relative performance measures (body mass, lean body mass, leg volume or CSA) smaller sex differences are witnessed. Despite the control of absolute morphological properties during sex difference related protocols, the performance differences between men and women although reduced are not fully accounted for when attempts are made to match between group factors. Morphological factors during repeated sprint protocols are also reduced, yet males remain more powerful than their female counterparts during two consecutive -8-second cycling sprints when expressed relative to lean body mass (+17%) and lean lower limb volume (LV, +16%). As follows, it is apparent that performance differences between men and women are also related to other physiological factors.

2.5.2 Endocrine Status and Hormonal Fluctuations

Male and female sex hormone secretion is another major difference between the two genders. The ability to synthesize protein to increase muscle size (hypertrophy) is regulated by hormones known as androgens (i.e., testosterone) and is significantly higher in men (Ahtiainen, Pakarinen, Alen, Kraemer, & Hakkinen, 2003). Estrogen concentrations (i.e., estrone, estradiol and estriol) responsible for the increased growth hormone production have been shown to be

higher in young females at rest compared to similarly aged men (Nygaard, 1981). Growth hormone increases stimulation of lipolysis and reduced glycogenolytic activity via decreased plasma adrenaline secretion, or epinephrine, however during exercise GH release appears to be equivocally evoked in both men and women. Activation of the sympathetic system during exhaustive exercise presents another possible attributable performance related sex difference (Giustina & Veldhuis, 1998). Supra-maximal exercise results in the sympathetic lowering of plasma catecholamine levels (epinephrine and nor-epinephrine) in females compared to men when matching subjects for relative intensities (Gratas-Delamarche, Le Cam, Delamarche, Monnier, & Koubi, 1994; L. J. Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990).

The effect of menstruation and its magnitude on athletic performance and fatigue resistance is still debated as a possible sex related factor (Lebrun & Rumball, 2001). Evidence has been provided in support and opposition for physiological and mechanical performance enhancements such as improved O_2 consumption, blood lactate removal, voluntary muscle strength and mechanical work during the luteal phase of menstruation (Sandoval & Matt, 2002). Thus no consensus has yet to be reached on the influence of monthly hormonal fluctuations in men and women and therefore needs to be taken into consideration comparing male and female athletes. Despite a lack of consensus on enhanced vs stable findings regarding O_2 availability during menstruation the findings regardless of outcome raise an interesting possibility in the improved fatigue resistance in men and women. An enhanced oxygen availability, or at least a decreased sensitivity to arterial desaturation as suggested by Billaut and Smith (2009), may influence the aerobic oxidative advantage of women during maximal exercise. A summary of the research investigating the overall contribution of aerobic and anaerobic energy systems during fatiguing 30-s maximal Wingates acknowledges sex differences in the ratio of aerobic: anaerobic energy system contributions is found in Billaut and Bishop (2009; table 1).

2.5.3 Enzymatic activities

Invasive measurements of the enzymatic activities of muscle cells, performed to measure whole-body metabolism is thought to be another possible method in quantifying the physiological differences in the male and female response to exercise. Investigations into the sex differences in ATP provision through in vitro studies measuring the activities of myosine adenosine triphosphatase (ATPase) and creatine phosphokinase (CPk) have suggested that men have a greater utilization of high energy phosphates beneficial in exercise performance. Breakdown of glycogen and glucose molecules during maximal exercise is the product of glycolytic and glycogenolytic enzymatic activity (glycogen phosphorylase, phosphofructokinase, and lactate dehydrogenase), which have also been found to be reduced in females (Billaut & Bishop, 2009). Interestingly, morphological and body composition components (height, mass, muscle CSA and relative type II fibre area) were reported to be associated with maximal glycolytic enzymes in males than in females (Jaworowski, Porter, Holmback, Downham, & Lexell, 2002). This difference in anaerobic potential was attributed to greater participation in high intensity activities during childhood, or perhaps a representation of daily activity patterns and training status. Anaerobic potential and high intensity performance in men and women then must be carefully measured, since increases in muscle mass and muscle recruitment related to anaerobic potential may be only measuring the differences in training status and not bio-genetic sex differences (Carter, Rennie, Hamilton, & Tarnopolsky, 2001). Continued investigation into enzymatic sex differences uncovers further controversy in the assessment of the ATP energy pathway utilization discrepancies in men and women. Oxidative enzyme activities of the citric acid cycle have been reported as both significant and non significant increases in men, with no sex differences being reported between male and female lipid oxidation. These findings again fall under scrutiny for failing to discriminate between training status and bio-genetic sex differences as the cause for improved enzymatic activity. This is suggested because of studies indicating endurance training

enzymatic improvement to be similar in both men and women; this is in disagreement with the expected outcome if a bio-genetic difference was present in enzyme activity.

2.5.4 Substrate utilization

Sex differences in enzymatic activity during exercise will ultimately lead to the investigation of metabolism, primarily the relation of substrate utilization differences in males and females during exercise. The literature surrounding sex differences and metabolism attributes a possible difference in performance to an increased aerobic contribution to the energy balance during prolonged maximal exercise and isometric contractions (Esbjornsson, Sylven, Holm, & Jansson, 1993; Hill & Smith, 1993). According to Hill and Smith (1993) aerobic contribution to total work performed during a 30-s sprint is 5% higher in women than in men. If a greater activation of glycolytic pathways is present in men compared to women, it is a fair assessment to attribute differences in peak power and fatigue resistance to substrate utilization. Higher provision of ATP from anaerobic glycolytic sources would explain a larger initial peak power, but also explain the larger fatigue decrement and lower amounts of mechanical work produced compared to the higher oxidative phosphorylation of ATP used to produce and maintain maximal power and total work in women (S. K. Hunter & R. M. Enoka, 2001; J. A. Kent-Braun, A. V. Ng, J. W. Doyle, & T. F. Towse, 2002). Higher phosphorylation and a reduction in the speed of glycogen breakdown via smaller glycolytic enzymatic activity as well as improved fat oxidation have been the focus of explaining an improved fatigue resistance in females, since these reactions would result in glycogen sparing as well as lower accumulation of blood lactate concentrations it remains of interest to mention these sex related metabolic factors. Lower utilization of glycogen would ultimately increase the available supply of glycogen to fuel oxidative respiration during fatigue, in addition to increased glycogen smaller amount of blood lactate and hydrogen ions accumulation would not be as strong of an enzymatic and neural inhibitor ultimately increasing the females fatigue resistance ability during a 30-s sprint (Esbjornsson-Liljedahl et al., 1999;

Jacobs, Tesch, Bar-Or, Karlsson, & Dotan, 1983). PCr and ATP contribution in type I and type II fibres during maximal sprint exercise was reported to be similar in men and women, however contrary to this finding a lower ATP reduction in female type II fibres during three repeated 30-s Wingate tests were also reported (Esbjornsson-Liljedahl, Bodin, & Jansson, 2002).

Differential reliance on muscle fibres in men and women, measured by histochemical staining properties for the enzyme myofibrillar myosin ATPase have been reported again under a controversial blanket. CSA in all fibre types (type I, IIA and IIX fibres) has been accepted to be indicative of training status, as seen by a general reduction in CSA in untrained females compared to untrained females (Esbjornsson-Liljedahl et al., 1999; Esbjornsson-Liljedahl et al., 2002) . Conversely, fibre percentage is not generally accepted or reported as different men and women as varying results have indicated both significant and non significant differences in the distribution of type I (greater) and type IIX (lower) fibres in women compared to men. The review performed by Billaut and Bishop (2009) again summarizes these discrepancies within the literature as related to subject population (training status, sample size), however variation in fibre size and recruitment properties between sexes is an important constituent to the development of peak power and the ability to maintain it. Although elusive, the identification of physiological sex differences has proven to be possible when performed under strict control for the varying influence of the integrated physiologies of male and female athletes. Interestingly, the numerous activities and sports that are open to both males and females, especially the repeated sprint sports, studies regarding sex differences during repeated sprint activities are still infrequent.

2.5.5 Neural activation

Typical trends involving neuromuscular recruitment of men and women during maximal exercise have proven to be elusive and difficult to validly measure. There have been an increasing number of publications relating sex differences in performance related to

neuromuscular adjustments associated with specific tasks (Table 2). Surface EMG measured as a maximum voluntary decrease of muscle electrical activity was found to be greater impaired in males following 20 maximal squat lifts (100% of one repetition maximum). The decreased EMG has been thought of as an attenuation of neuromuscular recruitment initiated through strenuous heavy-resistance exercise when comparing men and women.

Interpolated twitch of afferent motor neurons at the motor end plate, a technique measuring voluntary activation has also been examined in men and women (Gandevia et al., 2001). Russ and Kent-Braun (2003) observed a larger decrease in voluntary activation (central activation ratio; difference between MVC and stimulated MVC with no resultant change in the compound muscle action potential or muscular recruitment) in men than women. This suggests a lower fatigue resistance in males during maximal, intermittent, isometric contractions of the dorsiflexor muscles. Interestingly the authors noticed a reduction in the sex effect when contractions were performed under ischemic conditions (*i.e.*, when no O_2 is available). This indicates that the sex difference in neuromuscular fatigue may be a result of an oxidative advantage or decreased sensitivity to oxygen reduction. Investigation of supraspinal factors relating to neuromuscular fatigue (assessed using transcranial magnetic stimulation) has shown no sex dimorphism (Hunter, Butler, Todd, Gandevia, & Taylor, 2006). Martin and Rattey (2007) also found sex-specific adaptive responses of the peripheral (↓ excitation-contraction coupling accessed via resting twitch amplitude) and the central (⇓ voluntary activation accessed via motor nerve stimulation) systems during 100-s sustained MVC.

Overall, the clear distinction of neuromuscular activation patterns in men versus women in exercise physiology is not an easy task, as they are underpinned by the task characteristics. Therefore, it is likely that the neural drive to locomotor muscles will differ during RSE, as well as the ‘central’ and/or ‘peripheral’ nature of fatigue mechanisms. Finally, when looking at the

literature of sex differences in fatigue and exercise performance, whatever the mechanism that prevails, it is interesting to note that the sex difference in muscle fatigue is exhibited only when muscle perfusion is apparent (*i.e.*, when O_2 is available) (Clark et al., 2005; Fulco et al., 2001; Hunteret al., 2004; Russ & Kent-Braun 2003).

2.5.6 Sprint ability in men and women

Women were shown to exhibit a lower peak power decrement than men (4% vs. 8%, respectively, $P < 0.05$) during three 30-s Wingate tests (Esbjornsson-Liljedahl et al., 2002; Esbjornsson-Liljedahl et al., 1999). This sex difference in sprint ability has been purported to be caused by a greater aerobic contribution to energy production during sprint in women, which would have allowed for a faster resynthesis of ATP stores in women compared the more powerful men.

A summary of the limited knowledge of RSA in men and women was performed by Billaut and Bishop (2009). Table 2 summarizes their findings as well as the latest studies investigating the possible factors involved in RSA in men and women. The first study investigating sex differences during RSE was conducted over a decade ago, and looked at the hormonal fluctuations during RSE (Brooks, et al., 1990). The ten 6-s sprint (30-s recovery) protocol showed a peak difference in plasma adrenaline as well as peak absolute power and total work (+25%) in men. The differences in absolute power and total work were ascribed to be the result of hormonal growth factors as well as anaerobic metabolism and CSA of highly fatigable type II fibres. It was deemed that the observed sex differences may have been the result of training status; however the reported physical characteristics were insufficient to support this explanation. Using a similar protocol (10 x 5-s cycling sprints with 10-s rest) Yanagiya et al. (2003b) found supporting evidence that the fatigue index was greater in teenage boys than girls. Billaut and Smith (2009) were also able to show a lower decrement in mechanical work (29.6%

vs. 18.9%; $P < 0.05$) in men and women, as did Bishop et al. (2004). Additionally, a sex difference in the relationship of lower limb EMG activity to arterial saturation indicated a possible lower sensitivity to exercise induced arterial desaturation in women than men (Billaut & Smith 2009). Other studies, investigating oxygen availability as well as central and peripheral nervous fatigue in men and women during single joint stationary and dynamic contractions have identified similar sex differences in time to fatigue and mechanical performance decrements (Fulco, et al., 2001; Fulco, et al., 1999; Hunter, et al., 2004). Oxygen availability is an important component while investigating sex differences; by reason of previously identified sex differences (Esbjornsson-Liljedahl, et al., 1999; Russ, Lanza, Rothman, & Kent-Braun, 2005; Tarnopolsky, 2000) believed to be dependent on different sensitivities to changes in venous and arterial oxygen tensions in men and women(Billaut & Smith, 2009; Hunter et al., 2004; Russ & Kent-Braun, 2003). In consideration of sex differences and oxygen availability during repeated sprint exercise only a few studies have indirectly attempted to explain oxygen availability on metabolism and nervous activity. Further observations are required to investigate if changes in oxygen sensitivities in men and women arise from changes at both muscular and cerebral tissue levels during repeated sprint exercise, and if further aggravation is elicited when oxygen availability is manipulated.

2.6 Influence of the initial-sprint performance

While the above sections have discussed the possible contributors to fatigue during RSE, research has also shown a correlation between the initial-sprint mechanical score and the performance decrement in subsequent sprints (Bishop et al., 2003; Hamilton et al., 1991; Mendez-Villanueva et al., 2008; Yanagiya et al., 2003). In other words, the higher the mechanical output at the onset (1^{st} sprint) of a RSE, the larger the decline in performance towards the end of the test. This can probably be attributed to the observation that subjects with greater initial-sprint performance will have greater changes in muscle metabolites in response to a higher anaerobic

metabolism contribution, which in turn have been related to larger performance decrements (Gaitanos et al., 1993). However, initial-sprint mechanical output *per se* cannot solely account for performance decrements during RSE. For example, Bishop and Edge (2006) found a greater fatigability (*i.e.*, larger work decrement) across five 6-s cycling sprints repeated every 30 s in low*vs.* moderately aerobically-trained women matched for single-sprint performance. Similarly, Yanagiya et al. (2003b) explained the larger mechanical decrement in teenage boys compared to aged-matched girls by their greater initial-sprint performance. Finally, sex differences in the absolute changes in ATP concentration during sprint exercise (with men developing higher mechanical work than women)_were found to be greatly reduced when expressed relative to the mean power output developed by each sex (Esbjornsson-Liljedahl, et al., 2002; Esbjornsson-Liljedahl, et al., 1999b). Thus, the influence of the initial-sprint performance has to be taken into account when attempting to compare performances and physiological responses of a given group of athletes in different environments or when comparing men and women (Billaut and Bishop 2009).

Table2. Sex differences in mechanical and physiological responses to repeated-sprint exercise

Study	Mode of exercise	Protocol	Principal significances
Esbjornsson-Liljedahl et al. (1993)	Cycle	3x 30 s Wingate (20 mins rest)	PPO & MPO (abs. & rel. to BM): M>F FI(abs & rel. to PPO): M>F; NS Post LDH activity: M>F
Bodin et al. (1994)	Cycle	3 x 30 s Wingate(20 min rest)	Post Δ[PCr] & [ATP]:NS AL ATP turnover rate: M=F
Esbjornsson-Liljedahl et al. (2002)	Cycle	3 x 30 s Wingate (20min rest)	PPO & MPO (abs.): M > F sp1 to sp3 decrease in PPO: M > F sp1 to sp3 decrease in MPO: M = F Post Δ type II [ATP] & [IMP]: M > F Post Δ type I [glycogen] I: M > F
Winter et al. (1991)	Cycle	4x8s sprint (5 min rest)	PPO (abs, & rel LLV): M > F;NS PPO ANOVA (rel, to LLV): M > F
Martin et al. (2004)	Cycle	2x5-8 s sprint (3 min rest)	V_{opt} ANOVA(rel. to LL): M > F (14-17.5 y of age)
Dore et al. (2005)	Cycle	3x5-8 s sprint (4 min rest)	PPO ANOVA (rel. to LLV): M > F (16-20 y of age)
Falgairette et al. (2004)	Cycle	2x8 s sprint (15-120 s rest)	PPO & W: M>F t-PPO: M<F
Esbjornsson et al. (2006)	Cycle	3x 30s sprint (20 min rest)	PPO & MPO: M>F $[NH_3]$:M>F a-v(abd)/a) of $[NH_3]$: M<F $[NH_3]$ vs. a-v(abd)/a) of $[NH_3]$ corr. slp.:M<F
Billaut and Smith (2009)	Cycle	20x5s sprint (30s rest)	PPO & sum-iEMG: M<F NME:M=F S_PO_2 corr. Sum-iEMG: M=F (R^2=0.87 & 0.91) corr. slp.: M>F

abs, = absolute value; **a-v(abd)/a) of $[NH_3]$**= extraction rate of **$[NH_3]$**; **BM** = body mass; **F** = females; **corr.=** correlation; **IMP** = inosine monophosphate: **Lac^-**= lactate; **LBM** = lean body mass; **LDH** = lactate dehydrogenase; **LL** = leg length; **LLV** = lean leg volume; **M** = males; **min** = minutes; **MPO** = mean power output; **NS** = not significant; PCr^phosphocreatine; **PPO** = peak power output; **rel,** = relative value; **NH_3=** plasma ammonia **run-track** = over-ground running; **s** = seconds; **slp**.=slope; **sp1,3** = sprint 1 & 3; sum-iEMG: sum of iEMG for lower limb muscles; **W** = work; **V_{opt}** = optimal velocity (i,e, velocity to reach peak power output); [.,,] indicates concentration; **Δ** indicates delta changes from pre (rest) to post exercise.

2.7 General aims and hypotheses

The research studies detailed above has identified a possible link between central fatigue and oxygen availability during endurance exercise and sprints longer than 30 s; however it has yet to be explored during RSE. The first aim of this thesis is therefore to use NIRS to monitor changes in both central and peripheral oxygenation during a RSA test, in order to build upon the theories surrounding central vs. peripheral components of repeated sprint fatigue. The single-blind experimental approach allows for a valid investigation of tissue oxygenation during RSE under different concentrations of inspired oxygen levels. To our knowledge, it is the first study to monitor simultaneous changes in cerebral and muscle oxygenation under both normoxic and hypoxic conditions during RSE. In accordance with the growing literature on systemic circulation and tissue oxygenation, it is hypothesized that cerebral, but not muscle, deoxygenation would be associated with impairments in RSA. The lack of distinguishing data surrounding the male and female responses to fatiguing protocols calls for a stronger approach to matching men and women for fatigue related variables. The contributions of lean body mass and performance achieved in the initial sprint and training status has been identified as contributors to identifiable sex differences. It is of further interest to monitor live simultaneous changes in central and peripheral components of repeated sprint fatigue. The second study conducted in this thesis is designed to investigate the possibility of sex-specific central and peripheral responses of male and female athletes matched for mechanical work. Based on the assumption that sex differences in performance may be related to a greater utilisation of any available O_2 by women, we reasoned that performance during RSE would be less impaired during acute exposure to hypoxia in women than in men. It is also hypothesised that this sex dimorphism in performance would be accompanied by sex differences in tissue oxygenation.

-Chapter 3-

METHODS AND RESULTS

3.1.Influence of cerebral and muscle oxygenation on repeated-sprint ability

[This study was adapted from Smith, K., & Billaut, F. (2010). Influence of cerebral and muscle oxygenation on repeated-sprint ability. *European Journal of Applied Physiology* 109:989-999]

Abstract The study examined the influence of cerebral (prefrontal cortex) and muscle (vastus lateralis) oxygenation on the ability to perform repeated, cycling sprints. Thirteen team-sport athletes performed ten, 10-s sprints (with 30 s of rest) under normoxic (F_IO_2 0.21) and acute hypoxic (F_IO_2 0.13) conditions in a randomised, single-blind fashion and crossover design. Mechanical work was calculated and arterial O_2 saturation (S_pO_2) was estimated via pulse oximetry for every sprint. Cerebral and muscle oxy-(O_2Hb), deoxy-(HHb), and total haemoglobin (THb) were monitored continuously by near-infrared spectroscopy. Compared with normoxia, hypoxia induced larger decrements in S_pO_2 and work (11.6% and 7.6%, respectively; $P<0.05$). In the muscle, we observed a fairly constant level of deoxygenation across sprints, with no effect of the condition. In normoxia, regional cerebral oxygenation increased during the first two sprints and slightly fluctuated thereafter. In contrast, this initial cerebral hyper-oxygenation was attenuated in hypoxia. Changes in [O_2Hb] and [HHb] occurred earlier and were larger in hypoxia compared with normoxia ($P<0.05$), while regional blood volume (Δ[THb]) remained unaffected by the condition. Changes in cerebral [HHb] and mechanical work were strongly correlated in normoxia and hypoxia ($R^2=0.81$ and $R^2=0.85$, respectively; $P<0.05$), although the slope of this relationship differed (normoxia: -351.3 ± 183.3 vs. hypoxia: -442.4 ± 227.2; $P<0.05$). The results of this NIRS study indicate that O_2 availability influences oxygenation of the prefrontal cortex during repeated, short sprints. By using a hypoxia paradigm, the study demonstrates that cerebral, unlike muscle, oxygenation imposes a limitation to repeated-sprint ability.

Introduction

Support for the role of a failure of the central nervous system (CNS) to excite the motor neurons adequately (i.e., central fatigue) in fatigue during tests of repeated-sprint ability (RSA) has been provided by the finding that voluntary activation of skeletal muscles (assessed via the twitch-interpolation technique) is reduced after ten 6-s sprints separated by 30 s of rest (Racinais, Bishop, et al., 2007). This suboptimal muscle activation has also been functionally observed via lowered surface electromyographic (EMG) activity on several occasions during repeated sprints (Billaut & Smith, in press; Mendez-Villanueva, Hamer, & Bishop, 2007; Mendez-Villanueva, et al., 2008). However, what triggers these acute changes in the CNS behaviour remains to be determined.

Central fatigue may be elicited by low brain oxygenation, i.e., by insufficient O_2 delivery and/or low pressure gradient to drive the diffusion of O_2 from the capillaries to the mitochondria. Direct and indirect evidence supports the contention that inadequate cerebral oxygenation depresses cortical neuron excitability, although the mechanisms remain debated (for review see (Amann & Calbet, 2008; Amann & Kayser, 2009; Nybo & Rasmussen, 2007). The non-invasive technique of near-infrared spectroscopy (NIRS) offers real-time measurement of oxygenation and haemodynamics in tissues (Colier, Quaresima, Oeseburg, & Ferrari, 1999; Van Beekvelt, Colier, Wevers, & Van Engelen, 2001), and thus, constitutes a relevant tool to enhance our current knowledge of central (CNS) and peripheral (muscle) determinants of RSA.

Some studies have reported that muscle deoxygenation occurs during cycling and running RSA tests (Buchheit, et al., 2009; Racinais, Bishop, et al., 2007). However, exercises of this nature appear to induce a fairly constant level of deoxygenation in prime mover muscles across sprints, and therefore, authors have suggested that muscle O_2 uptake is well preserved and is not

likely to represent a limiting factor of RSA. Data on cerebral oxygenation changes during RSA tests are currently inexistent. Based on studies conducted during constant workload exercise (Amann, et al., 2007), incremental test to maximal effort (Subudhi, et al., 2007), and supramaximal exercise (Nielsen, et al., 1999; Shibuya, Tanaka, Kuboyama, Murai, & Ogaki, 2004; Shibuya, Tanaka, Kuboyama, & Ogaki, 2004), the deoxygenation of the cerebral cortex has, in general, been incriminated in the cessation of exercise, or at least the reduction of exercise intensity. This finding, however, is confounded by the availability of O_2 (Amann, et al., 2007; Subudhi, et al., 2007). Although an association exists between cerebral oxygenation and performance in varied exercises, no studies have yet determined if a critical level of cerebral deoxygenation impairs RSA. Since we postulated that muscle activation, and subsequently motor performance, during RSA tests might be influenced by the level of cerebral oxygenation, an additional observation needs to be emphasised. Systemic hypoxaemia, which is considered as an aggravating factor of cerebral deoxygenation (Amann & Calbet, 2008; Amann & Kayser, 2009; Nielsen, et al., 1999; Nybo & Rasmussen, 2007), has been found to occur during a RSA test (Billaut & Smith, 2010), and may have contributed towards reducing EMG activity in the quadriceps muscles (Billaut & Smith, 2010). This correlative evidence and the observation that arterial hypoxaemia exacerbates cerebral deoxygenation constituted an additional argument to examine the potential influence of cerebral oxygenation on RSA.

The aim of this study was therefore to use NIRS to monitor changes in both central and peripheral oxygenation during a RSA test, in order to gain further understanding of the factors associated with fatigue during such task and how best to improve RSA. This study was the first to monitor simultaneous changes in cerebral and muscle oxygenation under both normoxic and hypoxic conditions. The possibility to blind subjects to manipulations of F_IO_2 represents a relevant experimental approach to investigate the effects of tissue oxygenation on RSA. It was

hypothesised that cerebral, but not muscle, deoxygenation would be associated with impairments in RSA.

Materials and methods

Subjects

Thirteen, male soccer and rugby players were recruited from university and local sports clubs (mean ± SD: 23.6 ± 3.7 y, 181.5 ± 5.5 cm, 81.5 ± 11.3 kg, 13.6 ± 1.2 % body fat). These subjects were chosen because they were accustomed to high-intensity exercise and familiar with laboratory testing. All subjects were healthy and with no known neurological or cardiovascular diseases. The study was conducted with the ethical approval of the Human Subject Research Committee of the University of Lethbridge. Before the trials, all subjects were informed of the nature of the investigation, after which they gave written informed consent.

Experimental design

Athletes visited the laboratory three times. During the first visit, anthropometric measurements (stature, body mass, and body fat percentage) were recorded, and athletes were re-accustomed with sprint cycling (five, 5-s sprints) until fully confident of producing an all-out effort from a stationary start.

Two to three days following the familiarisation session, subjects were randomised in a single-blind, cross-over design and asked to perform a RSA test under normoxic (F_IO_2 0.21) and acute hypoxic (F_IO_2 0.13) conditions. Trials were conducted at the same time of day and were separated by one week.

Exercise testing

All testing was performed on a friction-loaded cycle ergometer (Monark 874E, Stockholm, Sweden) with a braking, resistive force applied on the flywheel set at 0.9 $N.kg^{-1}$ of body mass. The instantaneous power output, corrected for flywheel acceleration, was recorded at 50 Hz, and the mechanical work performed (kJ) was calculated by integrating the power curve over the 10 s of the sprint for every sprint. Subjects were instrumented with necessary probes and sensors, and were then asked to close their eyes, eliminate extraneous thoughts, and rest completely in the exercising position on the ergometer to obtain a 2-min baseline period. Immediately after the baseline period, subjects were equipped with the breathing apparatus, and a 10-min exposure period to the gas was observed while seating on the ergometer (10 min was enough to reach a steady state in every subject). After a 5-min warm-up at 60–70 watts, subjects rested for another 1 min, and the RSA test (10 x 10-s sprints with 30 s of rest) was initiated. The cycle ergometer was equipped with toe-clips to prevent the subjects' feet from slipping. Sprints were initiated with the subject's dominant leg, with the crank arm located 45 deg forward to the vertical axis. Subjects were asked to remain seated during every sprint and during the recovery periods. Following the test, all instrumentation was removed and subjects performed a self-paced cool down under ambient condition.

Humidified, experimental gases (normoxia = compressed air; hypoxia = 13% O_2, 87% N_2) were administered using a system of plastic tubing and 150-liter Douglas bag reservoir, with the O_2-N_2 dilution constantly controlled by a PO_2 probe (Hypersphere Pro, AltitudeTech Inc., Kingston, ON, Canada).

Responses to exercise

Arterial oxygen saturation (S_pO_2).

S_pO_2 was estimated via pulse oximetry (Nellcor N-200, Nellcor Inc., Hayward, CA) with adhesive optodes placed on the forehead. This technique has been shown to be in good agreement

(intraclass correlation coefficient = 0.88) with haemoglobin O_2 saturation based on arterial blood analysis (Romer, Haverkamp, Lovering, Pegelow, & Dempsey, 2006), and has been used elsewhere (Amann, et al., 2007; Billaut & Smith, in press; Romer, et al., 2006). SpO_2 was recorded during baseline, exposure, and immediately after every sprint.

NIRS measurements and analysis.

During all tests, subjects were instrumented with two pairs of NIRS probes to monitor absorption of light across cerebral and muscle tissue (Oxymon MKIII, Artinis, The Netherlands). The theory, limitations and reliability of measurement obtained with this device during exercise are detailed elsewhere (Subudhi, et al., 2007; Van Beekvelt, Colier, et al., 2001). One NIRS emitter and detector pair was placed over the left prefrontal lobe, between Fp1 and F3 (international EEG 10-20 system), and placement was further adjusted (less than 5 mm) to obtain strong signal strength on every subject. Spacing between optodes was fixed at 5 cm using a black, plastic spacer held in place via double-sided, stick disks and a black, tensioning headband to reduce the intrusion of extraneous light and the loss of transmitted NIR light from the field of investigation. It has previously been shown that an emitter–detector distance of 5 cm increases spatial resolution, allowing a light penetration depth of ~2.3–2.5 cm (Ferrari, Mottola, & Quaresima, 2004). It has also been reported that extracranial contribution to the NIRS signal is negligible when the inter-optode distance is > 4.5 cm (Smielewski, Kirkpatrick, Minhas, Pickard, & Czosnyka, 1995). Pictures of the optode position were taken to ensure accurate replacement on subsequent visits.

A second NIRS emitter and detector pair was fixed on the distal part of the left vastus lateralis muscle belly (approximately 15 cm above the proximal border of the patella) using a black, plastic spacer with optode distance of 4.5 cm. Skinfold thickness was measured between the emitter and detector using a skinfold calliper (Harpenden Ltd.) to account for skin and adipose

tissue thickness covering the muscle. Probes were secured to the skin using double-sided, stick disks and shielded from light using black, elastic bandages. An indelible pen was used to mark the position of the optodes for subsequent visits.

A modified form of the Beer-Lambert Law was used to calculate micromolar changes in tissue [O_2Hb] and [HHb] across time using received optical densities from two continuous wavelengths of NIR light (763 and 855 nm). An age-dependent differential optical pathlength factor for cerebral cortex (Duncan et al., 1996; Shibuya, Tanaka, Kuboyama, Murai, et al., 2004; Shibuya, Tanaka, Kuboyama, & Ogaki, 2004) and of 4.95 for muscle (Duncan et al., 1995; Subudhi, et al., 2007) were used in this study. Changes in total Hb ([THb]) were calculated by the sum of [O_2Hb] and [HHb] and used as an index of change in regional blood volume within the illuminated area (Van Beekvelt, Colier, et al., 2001). When regional blood volume (i.e., Δ[THb]) is constant, [O_2Hb] and [HHb] exist in equilibrium. Thus, decreases in [O_2Hb] and increases in [HHb] reflect relative deoxygenation in the underlying tissue (Ferrari, et al., 2004; Grassi et al., 2003; Subudhi, et al., 2007).

NIRS data were acquired at 10 Hz and transferred online from the Oxymon MKIII to a PC (Figure 4). Data were averaged over the last 5 s within every sprint to obtain one value per sprint, and normalised to express the magnitude of changes from the baseline period (arbitrarily defined as 0 μM) (Shibuya, Tanaka, Kuboyama, Murai, et al., 2004; Shibuya, Tanaka, Kuboyama, & Ogaki, 2004; Subudhi, et al., 2007; Subudhi, Miramon, Granger, & Roach, 2009).

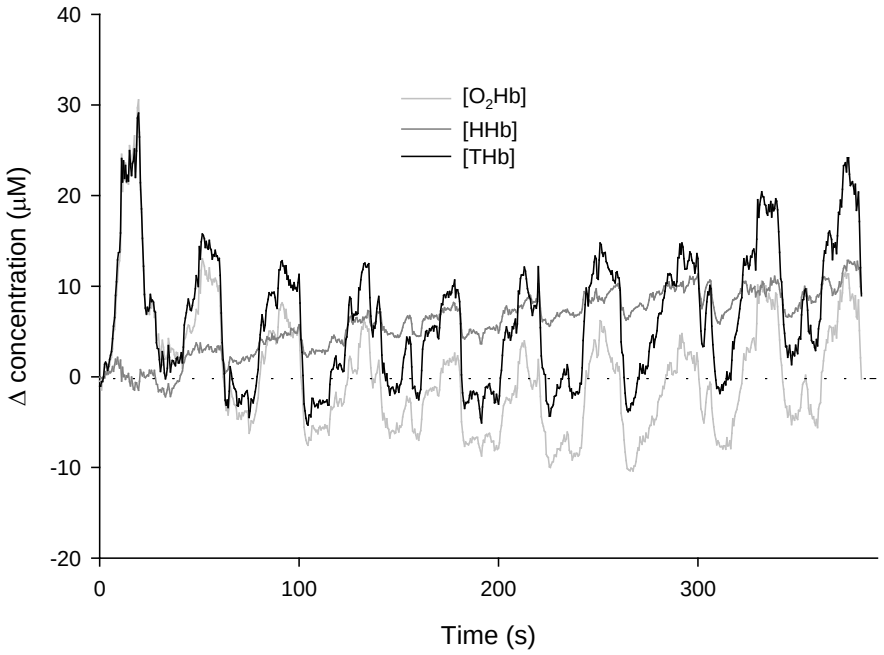

Fig. 4 Representative concentration changes in cerebral oxy-haemoglobin ([O_2Hb]), deoxy-haemoglobin ([HHb]), and total haemoglobin ([THb]) from a single subject during the sprints in normoxia.

Electromyography.

The EMG signals were recorded from the vastus medialis muscle of the right lower limb via surface electrodes (DE-2.1 single differential electrodes, DelSys Inc., Boston, MA). Recording electrodes were fixed longitudinally over the area of greatest muscle bulk, and aligned parallel to the underlying muscle fibre direction. The reference electrode was fixed over an electrically-neutral site (epicondyle of femur). Electrode site preparation was thoroughly performed before the beginning of every test (skin impedance < 2 kΩ) and electrode location was marked with a waterproof felt-tip pen to ensure reliable electrode replacement in subsequent testing sessions. To ensure low levels of movement artefact, electrode cables were fastened to the subjects' limb with medical adhesive tape and wrapped in elastic bandage. The raw EMG signal was pre-amplified and filtered (bandwidth frequency = 20–450 Hz, gain = 1000). A 50-Hz line filter was applied to the EMG data to prevent interference from electrical sources. The filtered EMG signal was sampled at 2 kHz (Bagnoli EMG system, DelSys Inc., Boston, MA). Muscle activity was considered as the integrated EMG of the signal between the onset and offset of activation of every burst in the last 5 s of every sprint to obtain one value per sprint. Data collected during the initial sprint were described as 100% with all subsequent data normalised by using the corresponding initial value as the denominator.

Statistical analysis

Statistical analyses were performed using Statistica 5.5 for Windows (Statistica, Statsoft Inc., Tulsa, OK). Two-way, repeated measures, analyses of variance (ANOVAs) (sprint x condition) were used to compare the following dependent variables between normoxia and hypoxia across sprints: mechanical work, iEMG, S_pO_2, $\Delta[O_2Hb]$, Δ[HHb], and Δ[THb]. One-way ANOVAs (condition) were used to compare the slope of the relationships between mechanical work and Δ[HHb] and iEMG and Δ[HHb] between normoxia and hypoxia. Tuckey's HSD *post-hoc* analyses were used to locate differences among pairs of means when ANOVAs revealed a

significant *F* ratio for main or interactive effects. Pearson's product–moment coefficients were used to determine relationships. The level of significance was set at $P < 0.05$. Data are reported as mean ± SD.

Results

Mechanical measurements

The mechanical work values recorded during the RSA tests are displayed in Fig. 5. No effect of the condition was observed for the initial-sprint score (normoxia: 8.6 ± 1.1 kJ vs. hypoxia: 8.4 ± 1.1 kJ; $P > 0.05$). When compared with the first sprint of the series, there was a significant decline in work (both conditions compounded) in sprints 2–10 (overall decrement: 31.2%, $P < 0.05$). We also noted a significant main effect of condition on the work performed (normoxia: 67.2 ± 5.5 vs. hypoxia: 62.1 ± 5.4 kJ; $P < 0.05$). No significant interaction sprint x condition was observed for this parameter. All but two subjects were able to correctly identify the order of treatment, and only two subjects reported adverse side effects of hypoxia (dizziness and/or nausea) during or following exercise.

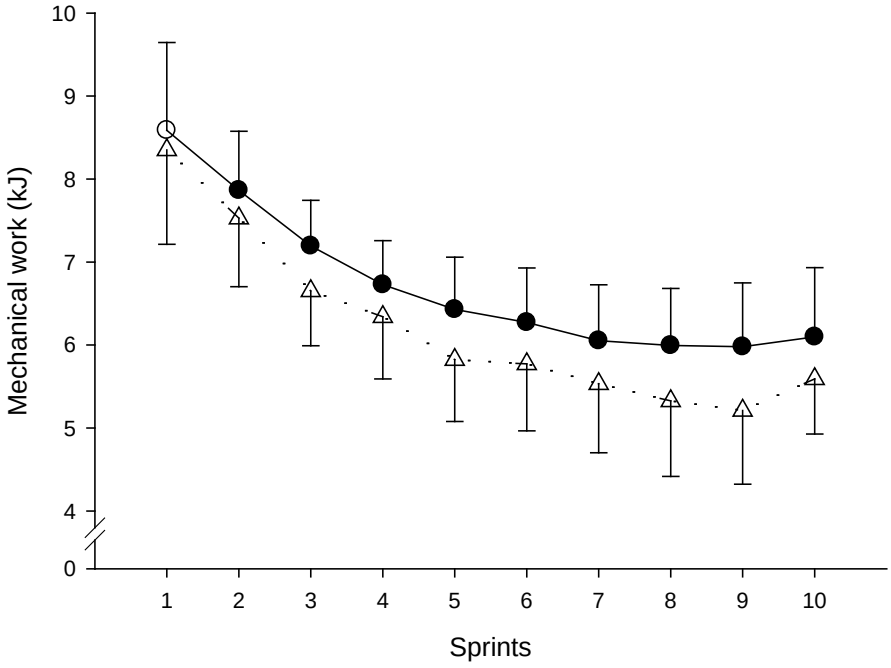

Fig. 5 Mechanical work performed during the sprints (n = 13) in normoxia (●) and hypoxia (Δ). There was a decrease in work during the sprints (main effect of sprint: $P < 0.05$); however, decrements were larger in hypoxia than in normoxia (main effect of condition: $P < 0.05$).

Arterial O_2 saturation

Mean data at rest, exposure, and over the sprints are displayed in Fig. 6. At rest, there was little variation among subjects and conditions (average: 98.6 ± 0.9%) with all participants within the normal range. Compared with resting baseline, S_pO_2 (all conditions compounded) fell significantly in sprints 1–10 (overall decrement: 14.5%, $P < 0.05$). This parameter was profoundly affected by the condition, with lower values recorded in hypoxia than in normoxia (average: 12.1%, $P < 0.05$). Furthermore, the pattern of desaturation across sprints varied between conditions, with significant decrements occurring in sprints 2–10 (overall decrement: 8.3%, $P < 0.05$) in normoxia, and in sprints 1–10 (overall decrement: 20.7%, $P < 0.05$) in hypoxia.

NIRS measurements

Fig. 7 displays the average concentration changes in cerebral and muscle NIRS signals across sprints during normoxia and hypoxia.

Muscle analysis.

In normoxia, regional muscle oxygenation decreased rapidly during the first sprint ($\downarrow \Delta[O_2Hb]$, $\uparrow \Delta[HHb]$), and this was accompanied by a reduction in regional blood volume ($\downarrow \Delta[THb]$). From sprints 2–10, $\Delta[HHb]$ remained unchanged, yet $\Delta[O_2Hb]$ and $\Delta[THb]$ rose significantly, indicating a reduction in the rate of muscle deoxygenation in subsequent sprints. In hypoxia, regional muscle oxygenation followed a very similar pattern as seen in normoxia (interaction sprint x condition: $P > 0.05$). The magnitude of muscle deoxygenation ($\downarrow \Delta[O_2Hb]$, $\uparrow \Delta[HHb]$) was the same in both conditions (main effect of condition: $P > 0.05$).

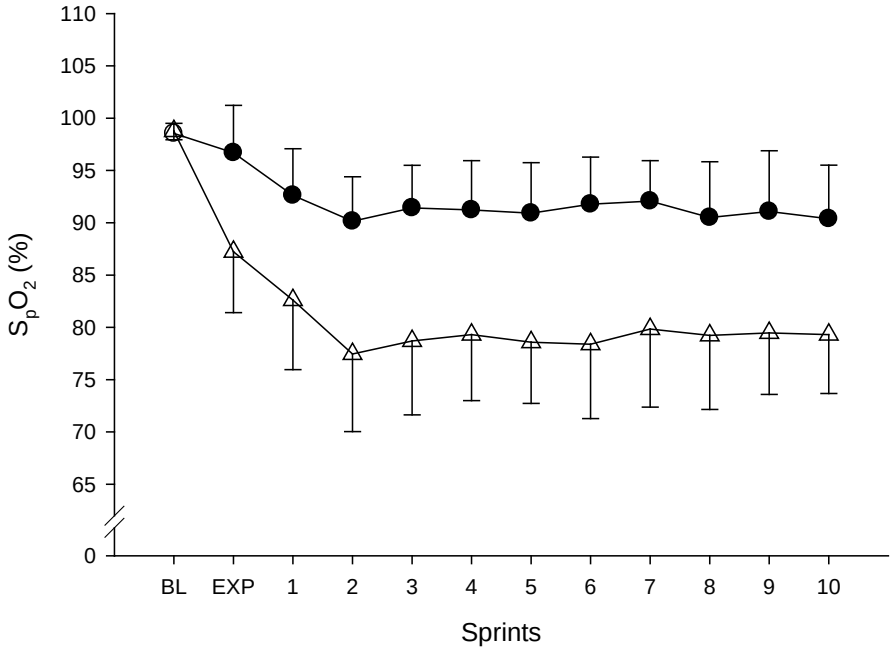

Fig. 6 Mean arterial O_2 saturation at baseline (BL), exposure (EXP), and throughout the sprints (n = 13) in normoxia (●) and hypoxia (Δ). There was a decrease in arterial saturation during the sprints (main effect of sprint: $P < 0.05$); however, changes were larger (main effect of condition: $P < 0.05$) and occurred earlier (interaction sprint x condition: $P < 0.05$) in hypoxia than in normoxia.

Cerebral analysis.

In normoxia, regional cerebral oxygenation increased rapidly in sprints 1–2 (↑ Δ[O_2Hb], ↔ Δ[HHb], and ↑ Δ[THb]; Table 3), indicating cerebral vasodilation during initial sprints. Thereafter, NIRS signals fluctuated without showing clear evidence of deoxygenation between sprints 3 and 10 (from ↔ to ↑ Δ[O_2Hb] and ↑ Δ[HHb]), while regional blood volume displayed a slight, although not significant, increase from sprint 1 to 10 (P = 0.21). In contrast, regional cerebral oxygenation was markedly attenuated during sprint 1 in the hypoxic trial (↔ Δ[O_2Hb], ↑ Δ[HHb], and ↑ Δ[THb]), and decreased progressively across subsequent sprints (↔ Δ[O_2Hb], ↑ Δ[HHb]), whereas regional blood volume did not show a significant alteration (P = 0.21). Changes in Δ[O_2Hb] and Δ[HHb] were larger (main effect of condition) in hypoxia compared with normoxia throughout the RSA test (average: 118.5% and 45.1%, respectively; P < 0.05; Table 3). Between normoxia and hypoxia, there was no change in Δ[THb] across the sprints (main effect of condition: P = 0.58).

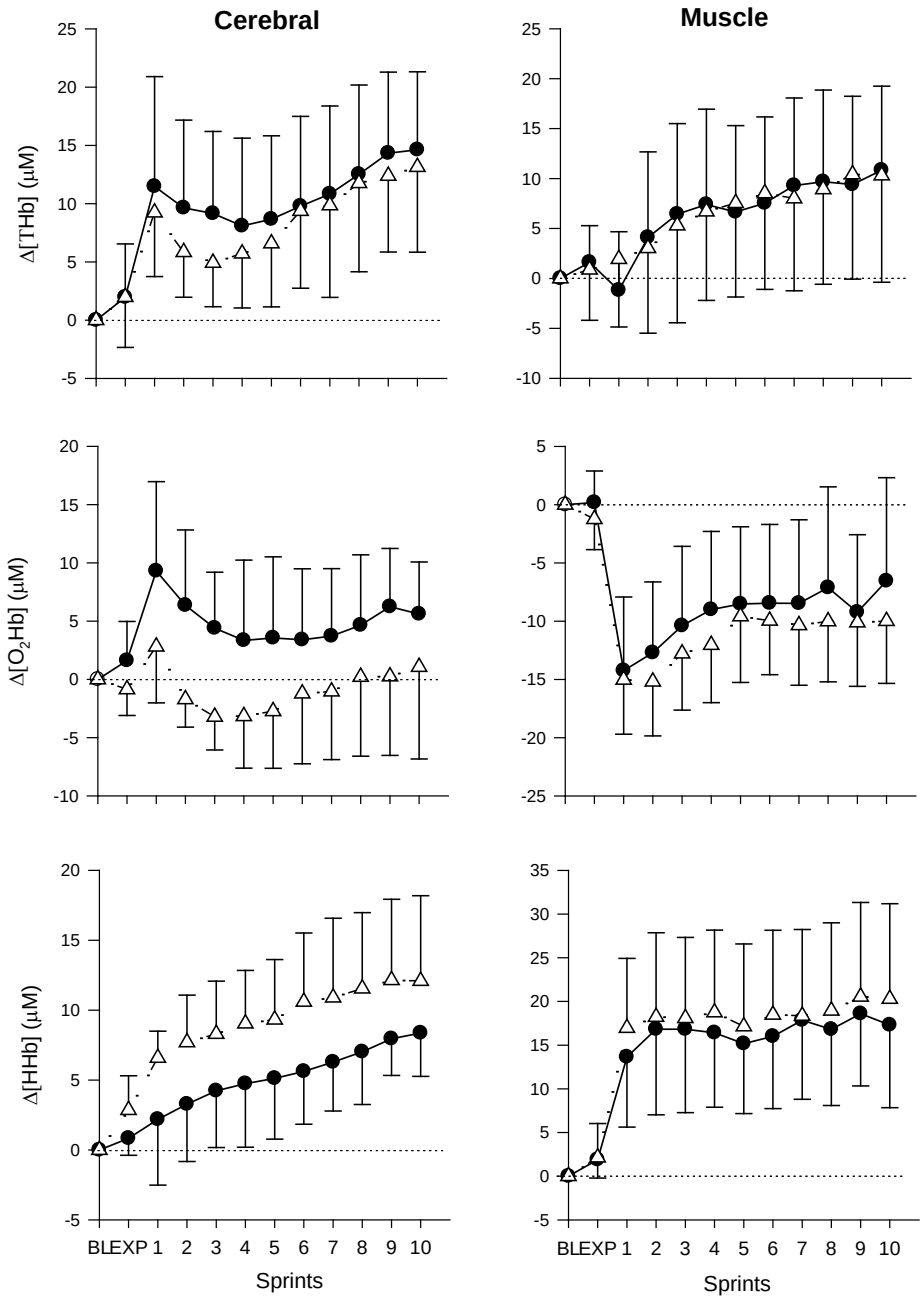

Fig. 7 Near-infrared spectroscopy concentration changes from resting baseline at baseline (BL), exposure (EXP), and throughout the sprints (n = 13) in normoxia (●) and hypoxia (Δ). O_2Hb: oxy-haemoglobin; HHb: deoxy-haemoglobin; THb: total haemoglobin. Brackets indicate concentration.

Electromyographic measurements

Compared with the initial sprint, there was a significant decline in iEMG (both conditions compounded) for sprints 6–10 (overall decrement: 19.4%, $P < 0.05$). Although only a tendency for the main effect of condition was observed ($P = 0.054$), results showed a significant interaction sprint x condition. Specifically, values of iEMG were lowered in sprints 8–10 in normoxia (9.1%, $P < 0.05$), whereas the hypoxic trial exhibited lowered values in sprints 5–10 (27.4%, $P < 0.05$).

Relationships between variables

The reduction in mechanical work over the sprints was found to correlate negatively with the increase in [HHb] in the prefrontal lobe in both normoxia and hypoxia ($R^2 = 0.81$ and $R^2 = 0.85$, respectively, $P < 0.05$; Fig. 8A). However, the slope of this relationship was greater ($P < 0.05$) in hypoxia (-442.4 ± 227.2) than in normoxia (-351.3 ± 183.3). Changes in cerebral [HHb] also correlated negatively with changes in iEMG in both the normoxic and hypoxic trials ($R^2 = 0.78$ and $R^2 = 0.92$, respectively, $P < 0.05$; Fig. 8B), and the slope of this relationship also differed (normoxia: -0.003 ± 0.002 vs. hypoxia: -0.007 ± 0.002, $P < 0.05$).

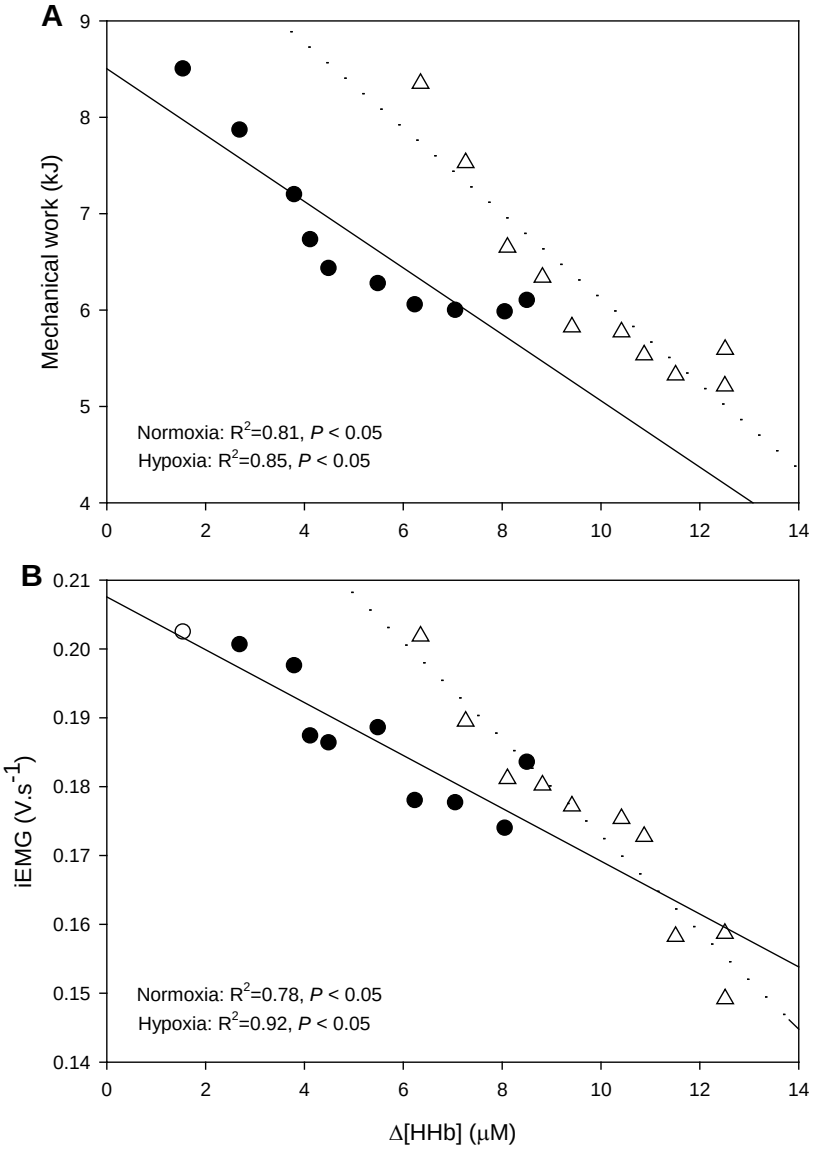

Fig. 8 Relationship of cerebral deoxy-haemoglobin concentration changes (Δ[HHb]) to mechanical work (panel A) and to iEMG of the vastus medialis (panel B) over the sprints (n = 13 for each data point) in normoxia (●) and hypoxia (Δ) . Main effect of condition on slope of linear regressions: $P < 0.05$.

Discussion

The major finding in this study was that acute hypoxia induced by a blinded change in F_IO_2 profoundly affected the pattern of prefrontal cortex oxygenation during a RSA test. Results demonstrated for the first time that repeated, short sprints interspersed with incomplete recovery intervals elicited an earlier and larger degree of cerebral deoxygenation in hypoxia compared with normoxia. These findings indicate that changes in cerebral, unlike muscle, oxygenations appear to limit RSA in team-sport athletes under hypoxic conditions, since hypoxia-induced performance decrements were not accompanied by an aggravated muscle deoxygenation.

Technical considerations

We acknowledge that prefrontal cortex oxygenation is a regional measurement that may not be reflective of global cerebral oxygenation. However, the deoxygenation that occurs during whole-body, strenuous exercise is not confined to the prefrontal cortex but seems widespread to motor areas as well (Subudhi, et al., 2009). Also, while this area of the brain is not directly involved in the neural control of movement as motor areas do, the deoxygenation of the prefrontal cortex has been purported to contribute to fatigue on different occasions (Amann, et al., 2007; Shibuya, Tanaka, Kuboyama, Murai, et al., 2004; Shibuya, Tanaka, Kuboyama, & Ogaki, 2004; Subudhi, et al., 2007). Finally, since the differential pathlength factors were only estimated, it must be reminded that absolute NIRS measurements and actual tissue oxygenation values remain unknown.

As far as the muscle tissue is concerned, no post-exercise, vascular occlusion was performed in the current study because Chance et al. (1992) reported little additional deoxygenation (< 2%) during supra-systolic cuff occlusion of the vastus lateralis muscle immediately following strenuous exercise. We also chose not to perform arterial occlusion to stay consistent with previous studies that have investigated muscle oxygenation trends during RSA

tests (Buchheit, et al., 2009; Racinais, Bishop, et al., 2007). Nevertheless, if a leg cuff ischemia technique had allowed obtaining a low-oxygenation reference point it would have been similar in both conditions, and thus, would not have altered our results and conclusions.

Hypoxia and repeated-sprint ability

The ability to generate maximal power during isolated, all-out exercise is preserved in acute hypoxia (J.A. Calbet, De Paz, Garatachea, Cabeza de Vaca, & Chavarren, 2003) owing to an enhancement of the anaerobic energy supply. The current results are in good agreement with this finding since the initial-sprint performance was not affected by hypoxia. In the hypoxic trial, every subject was able to reproduce the initial mechanical score achieved during the trial performed in normoxia although S_pO_2 was significantly lowered. In contrast, and in accord with previous research on RSA (Balsom, Gaitanos, et al., 1994), the ability to reproduce total mechanical work in subsequent sprints was impaired in the current hypoxic conditions (Fig. 5) after we induced large changes in S_pO_2 by the experimental treatment (Fig. 6). Therefore, our results confirm that a reduction in O_2 availability impairs RSA (Balsom, Gaitanos, et al., 1994), and further strengthen the recent finding that arterial O_2 desaturation may be considered as a limiting factor of RSA in team-sport athletes (Billaut & Smith, 2010). Finally, this is in keeping with the hypothesis that the subsequent, potential mismatch between O_2 delivery and requirement explains part of the progressive reduction in the absolute contribution from aerobic sources and mechanical output observed during such exercise (Balsom, Gaitanos, et al., 1994; G.C. Gaitanos, C. Williams, L.H. Boobis, & S. Brooks, 1993; Tomlin & Wenger, 2001).

In this perspective, it is worth mentioning that a correlation between the initial mechanical score and performance decrement over subsequent sprints has consistently been reported and demonstrated as a confounding factor when investigating the mechanisms underlying RSA impairment (Bishop, Lawrence, & Spencer, 2003b; Mendez-Villanueva, et al., 2007a). Since the initial-sprint performance was similar in normoxia and hypoxia in the current study, it is likely

that the lower RSA observed in hypoxia has been elicited by the decrease in S_pO_2, i.e., by O_2-dependent mechanisms of fatigue.

Muscle oxygenation and repeated-sprint ability

Muscle oxygenation patterns have been described during cycle- and run-based RSA tests (Buchheit, et al., 2009; Racinais, Bishop, et al., 2007). In agreement with these reports, our data showed that under normoxic conditions muscle oxygenation decreased rapidly at the beginning of the exercise and displayed a plateau across sprints. Since NIRS measurements have been correlated with changes in intracellular O_2 tension (Tran et al., 1999) and venous O_2 saturation (Esaki et al., 2005), a plateau in oxygenation may be interpreted as evidence of maximal O_2 extraction (Esaki, et al., 2005; Tran, et al., 1999). A unique feature of the current study was to use a hypoxia paradigm to exacerbate the reduction in O_2 availability (as shown in Fig. 6), and thereby test directly the influence of muscle oxygenation on RSA. Our results showed that patterns of change and the magnitude of tissue deoxygenation were near identical in normoxia and hypoxia (Fig. 7). This indicates no additional deoxygenation in the quadriceps muscle during the hypoxic trial compared with the normoxic trial, which demonstrates that muscle O_2 uptake was maintained in acute hypoxia despite a reduced O_2 availability. Interestingly, by comparing whole-body (cycling) with small-muscle (one-leg, knee extension) exercises, Calbet and colleagues (2009) have demonstrated that the main mechanism limiting performance in hypoxia appears to be the systemic delivery of O_2, whereas muscle O_2 diffusing capacity (and therefore muscle O_2 uptake) may only have a secondary role. These univocal findings have thus demonstrated that the level of muscle oxygenation is not likely to limit RSA, as previously hypothesised (Buchheit, et al., 2009; Racinais, Bishop, et al., 2007), and highlight that the hypoxia-induced RSA decrement was caused by factors other than purely the muscle O_2 metabolism. Our assessment is primarily based upon muscle Δ[HHb] because it is shown to be essentially independent of changes in blood volume during exercise (De Blasi, Cope, Elwell, Safoue, & Ferrari, 1993; Grassi, et al., 2003).

Δ[HHb] is therefore a reliable estimator of changes in intramuscular oxygenation and O_2 extraction in the area investigated (De Blasi, Alviggi, Cope, Elwell, & Ferrari, 1994). Our results showed, however, that regional blood volume (given by Δ[THb]) fluctuated across sprints, as previously reported (Racinais, Bishop, et al., 2007), which may raise a concern about the current physiological conclusions. That being said, because subjects exhibited very similar patterns and magnitudes of change for every NIRS parameter under normoxic and hypoxic conditions, our conclusion would be unaffected: these observed levels of tissue deoxygenation would not be directly responsible for RSA impairments. Although an association exists between O_2 availability and RSA (Billaut & Smith, 2010), other avenues for potential physiological mechanisms must be considered, which include O_2-dependent CNS fatigue (Amann & Calbet, 2008; Amann & Kayser, 2009; Nybo & Rasmussen, 2007; Subudhi, et al., 2007).

Cerebral oxygenation and repeated-sprint ability

We observed that regional cerebral oxygenation and blood volume were fairly well maintained during ten, 10-s sprints performed in normoxia and interspersed with 30 s of rest (Fig. 7 and Table 3). In contrast, Shibuya and colleagues (2004) reported a progressive cerebral deoxygenation during intermittent exercises. Specifically, these authors observed a reduction in Δ[O_2Hb] and Δ[THb], while Δ[HHb] increased, over the course of seven, 30-s cycling exercises performed at an intensity corresponding to 150%VO_2max and interspersed with 15 s of rest. It was thus concluded that fatigue resulting from such intermittent, supramaximal efforts was related to a decrease in the cerebral oxygenation level (Shibuya, Tanaka, Kuboyama, & Ogaki, 2004). At first glance, the results of the current study and that of Shibuya et al. (2004) may appear contradictory, but could be explained by major differences in protocols such as duration and intensity of the efforts performed.

Here, we questioned whether a reduction in cerebral oxygenation influences RSA, which is a major fitness requirement for team-sport athletes (Bishop, Lawrence, & Spencer 2003; Mendez-Villanueva, et al., 2007). To test this hypothesis, we compared the patterns of change in cerebral oxygenation obtained during normoxia and hypoxia. The deoxygenation of the prefrontal cortex occurred earlier and to a larger extent in hypoxia than in normoxia. All subjects exhibited a fall in cerebral oxygenation when performing the hypoxic trial. In addition, the relationship of Δ[HHb] to mechanical work exhibited a strong coefficient of determination in both conditions (Fig. 8A), which shows a direct link between the oxygenation of the brain and the ability to perform work at supramaximal intensity (Nielsen, et al., 1999; Shibuya, Tanaka, Kuboyama, Murai, et al., 2004) and repeatedly (Shibuya, Tanaka, Kuboyama, & Ogaki, 2004). The slope change of this relationship in normoxia vs. hypoxia further indicates that the larger RSA decrement observed in the hypoxic trial was presumably caused, in part, by the larger metabolic disturbances of the cerebral function recorded in this trial. These data therefore add to the current knowledge that, during repeated sprints in normoxia, the changes in cerebral oxygenation *per se* may not reach a level low enough to cause the reduction in work (Amann & Calbet, 2008; Amann & Kayser, 2009; Subudhi, et al., 2007). However, larger changes seen in acute hypoxia (F_IO_2 0.13) support the thought that prefrontal cortex oxygenation imposes a limitation to RSA. This subsequently raises the question: how can the lower oxygenation of the brain explain the greater RSA decrement in hypoxia?

Motor neuron activity is dramatically influenced by O_2 availability (Amann, et al., 2007; B. R. Bigland-Ritchie, Dawson, Johansson, & Lippold, 1986; Dillon & Waldrop, 1992; Szubski, Burtscher, & Loscher, 2006), which plays a pivotal role in the protective mechanisms against muscle fatigue (Bigland-Ritchie, et al., 1986; Enoka & Stuart, 1992). More specifically, this is supported by the observation of a positive correlation between S_pO_2 and the surface EMG activity (serving as surrogate for muscle recruitment) in prime mover muscles during a RSA test (Billaut

& Smith, 2010). The influence of cerebral oxygenation on motor neuron excitability during exercise has also been described, although *in vivo* evidence is limited so far (Amann & Calbet, 2008; Amann & Kayser, 2009; Nybo & Rasmussen, 2007). Our data in Fig. 8B support such a phenomenon during a RSA test since the hypoxia-induced, marked increase in cerebral Δ[HHb] led to the largest decline in iEMG of the vastus medialis. Changes in cerebral Δ[HHb] explained 92% of the variance in iEMG activity in the hypoxic trial. Therefore, this seems to suggest that the level of cerebral oxygenation impaired RSA via an effect on muscle activation. At this point however, it is important to acknowledge that the use of surface EMG as a sole determinant of the neural response is questionable and requires caution, even though its rate of change throughout the exercise may be used as an index of the rate of motor unit recruitment (Amann, et al., 2007; Enoka & Stuart, 1992; Mendez-Villanueva, et al., 2008). Taken as it is (i.e., without neuromuscular stimulation technique), these data are compatible with the findings that hypoxia has a direct effect on cortical and subcortical function of the CNS, and thereby, on motor neuron excitability and synaptic neurotransmission. Insufficient oxygenation of the cerebral cortex has been shown to affect neurotransmitter turnover and depress neuronal electrical activity of the brain, which has been incriminated in the occurrence of central fatigue in challenging levels of arterial hypoxaemia (i.e., S_pO_2 levels < 80%) (Amann & Kayser, 2009; Amann, et al., 2007; Chaudhuri & Behan, 2000). However, since the entire RSA test was performed with reduced F_IO_2, this likely also accelerated the development of peripheral fatigue (Balsom, Gaitanos, et al., 1994; Gaitanos, et al., 1993). Consequently, there is also the possibility that the greater RSA impairment in hypoxia is explained by sensory afferent feedback (groups III/IV muscle afferents), originating in the fatiguing locomotor muscles, to the CNS, which have a powerful input to the regulation of the CNS response in moderate hypoxia (Amann & Calbet, 2008; Amann & Kayser, 2009; Bigland-Ritchie, et al., 1986). Therefore, it is impossible to determine whether the RSA impairment in hypoxia was attributable to a directly-mediated decrease in CNS motor output (i.e., due to reduced cerebral oxygenation) or to inhibitory, peripheral feedback to the CNS from

increased disturbances in the fatiguing muscles. Quantifying the relative contributions of these central fatigue-causing mechanisms will advance the current body of knowledge in the area of RSA. Finally, while the current results highlight a role for changes in cerebral oxygenation in the reduction in EMG activity during repeated sprints in hypoxia, it is difficult to ascertain that it was also the case in other studies showing a decline in the net motor unit activity in active muscles (Billaut & Smith, 2010; Mendez-Villanueva et al., 2007, 2008; Racinais, Bishop, et al., 2007), since these studies were performed in normoxia.

Conclusion

The results of the current study have shown that it is unlikely that changes in cerebral oxygenation limit RSA in normoxia, yet such changes contribute to performance decrement in hypoxia. Since muscle oxygenation was not affected by hypoxia, we conclude that cerebral, unlike muscle, oxygenation imposes a limitation to the maintenance of performance during repeated-sprint efforts. The consequences of reduced oxygenation of the brain are not only reflected in a debilitated exercise capacity, but also in compromised fine, psychomotor skills and cognitive abilities (Amann & Kayser, 2009; Leon-Carrion et al., 2008). The implications that these findings have for team-sport performance warrant further investigation. The ability to produce and maintain high power outputs during prolonged periods is a strong determinant of performance in many sports. The current results highlight the potentially-considerable benefit of intermittent hypoxic conditioning in this respect, since this form of conditioning is shown to increase arterial O_2 content at a given altitude (Ainslie, Barach, Cummings et al., 2007; Muza, 2007), and thus, may attenuate tissue deoxygenation during a game played in an hypoxic environment.

Table 4. NIRS cerebral concentration changes during the RSA test in normoxia and hypoxia.

Variable	Exposure		Sprints									
			1	2	3	4	5	6	7	8	9	10
Δ[THb]	Normoxia	1.9±4.3	11.4±0.6	9.6±9.0	9.1±8.4	8.0±10.1	8.6±9.7	9.7±9.2	10.8±8.9	12.5±8.6	14.3±7.8	14.6±7.6
	Hypoxia	2.0±4.2	9.2±5.2	5.8±4.0	4.9±3.6	5.7±4.5	6.6±5.2	9.3±7.2	9.8±7.7	11.8±8.5	12.4±8.2	13.1±10.0
Δ[O_2Hb]	Normoxia	1.6±3.4	9.39±7.7*	6.4±6.5*	4.4±4.8	3.3±6.9	3.5±6.9	3.4±6.1	3.7±5.8	4.6±6.0	6.2±5.1*	5.6±4.5*
	Hypoxia	-0.9±2.2	2.8±4.8	-1.7±2.3	-3.2±2.8	-3.2±4.4	-2.7±4.9	-1.2±6.0	-1.1±5.8	0.2±6.8	0.2±6.8	1.1±7.9
Δ[HHb]	Normoxia	0.8±1.2	2.2±4.7	3.3±4.1	4.2±4.1	4.7±4.5*	5.1±4.3*	5.6±3.8*	6.3±3.5*	7.0±3.8*	7.9±2.6*	8.4±3.1*
	Hypoxia	2.9±2.5*	6.6±1.9*	7.7±3.4*	8.3±3.8*	9.0±3.8*	9.3±4.3*	10.5±4.9*	10.9±5.7*	11.5±5.4*	12.1±5.8*	12.0±6.1*

Values are micromolar changes from resting baseline (n = 13). O_2Hb: oxy-haemoglobin; HHb: deoxy-haemoglobin; THb: total haemoglobin. Brackets indicate concentration. * Different from resting baseline value (interaction sprint x condition: $P < 0.05$)

3.2. Tissue oxygenation during repeated sprints in men and women matched for mechanical work

[This manuscript is in preparation for the journal of *Medicine and Sciences in Sport and Exercise*]

Abstract Women usually exhibit lower performance decrement than men during exercise possibly because of differences in O_2 utilisation. Here, we determined the effect of sex on cerebral (prefrontal lobe) and muscle (vastus lateralis) oxygenation during repeated sprints. Men (n = 10) and women (n = 10) matched for initial-sprint mechanical work performed ten, 10-s sprints (30 s of rest) under normoxic (NM: 21% F_IO_2) and acute hypoxic (HY: 13% F_IO_2) conditions in a randomized, single-blind fashion and crossover design. Mechanical work was calculated and arterial O_2 saturation (S_pO_2) was estimated via pulse oximetry for every sprint. Cerebral and muscle oxy-(O_2Hb), deoxy-(HHb), and total haemoglobin (THb) were monitored continuously by near-infrared spectroscopy, and haemoglobin difference ([Hbdiff] = [O_2Hb] – [HHb]) was calculated. In both NM and HY there was no sex difference in mechanical work performed during the initial sprint (men: 114.1–116.3 J.kgLBM^{-1}; women: 116.4–118.3 J.kgLBM^{-1}; P = 0.81). Compared with NM, HY induced greater performance decrement for both sexes (7.4%, $P < 0.05$), and this was associated with lower S_pO_2 and cerebral [Hbdiff] (14.8% and 102.1%, respectively; $P < 0.05$). These HY-induced changes were nearly identical in these men and women matched for initial-sprint work. However, muscle [Hbdiff] differed: the sprint-induced muscle deoxygenation was less for the women compared with the men in each condition (63.7%, $P < 0.05$). Results indicate that men and women matched for initial-sprint work experience similar levels of fatigue and systemic and cerebral adjustments during repeated sprints performed in NM and HY, despite differences in muscle O_2 metabolism.

Introduction

Women can present lower mechanical impairments during varied types of exercise (i.e., less fatigue) compared with men, and accordingly, the mechanisms that constrain performance of a fatiguing exercise differ between sexes (for review see (Billaut & Bishop, 2009; Hunter, 2009)). Among the purported mechanisms that may explain this sex difference, the delivery and/or use of O_2 is one of the most intriguing, and increasing evidence points to this direction. In fact, women are often reported to perform better than men in normoxic and hypoxic environments (i.e., when O_2 is available) (Fulco et al., 2001; Fulco, et al., 1999; Hunter, et al., 2006; Hunter, et al., 2004b; Hunter & Enoka, 2001; Saito, Iemitsu, Otsuki, Maeda, & Ajisaka, 2008), but not under conditions of ischemia (Clark, Collier, Manini, & Ploutz-Snyder, 2005; D.W. Russ & J.A. Kent-Braun, 2003). This is consistent with studies showing no performance difference when the sexes are matched for strength (Hatzikotoulas, Siatras, Spyropoulou, Paraschos, & Patikas, 2004; Hunter, Critchlow, Shin, & Enoka, 2004a), which implies similar levels of muscle perfusion. This therefore implies a key role for tissue (both muscle and cerebral) perfusion and/or metabolism in explaining the sex-related differences in performance. As yet, however, studies addressing the functional consequences of reduced O_2 supply in men and women have been rare (Fulco, et al., 2001), and discussion of potential physiological mechanisms remains hypothetical at this time.

The non-invasive technique of near-infrared spectroscopy (NIRS) offers real-time measurement of oxygenation and haemodynamics in tissues (Ide, et al., 1999; Van Beekvelt, Colier, et al., 2001; Van Beekvelt, et al., 2002), and thus, constitutes a relevant tool to enhance our current knowledge of central (brain) and peripheral (muscle) determinants of performance in women. Again, while studies insinuate the importance of O_2 metabolism in underpinning the sex-specific fatigue (Fulco, et al., 2001; Fulco, et al., 1999; Hunter, et al., 2006; Hunter, et al., 2004b;

Hunter & Enoka, 2001; Saito, et al., 2008), the conclusions remain speculative, since tissue oxygenation was not measured.

Strenuous physical exercise involving a large muscle mass is a situation shown to stress the O_2 transport system, and thereby, represents a relevant situation to investigate the oxygenation status of active tissues during exercise. For instance, the NIRS technique has been successfully used during repeated sprints interspersed by short rest periods, which is an activity pattern known to induce large changes in muscle (Buchheit, et al., 2009; Racinais, Bishop, et al., 2007; Smith & Billaut, 2010) and cerebral oxygenation (Smith & Billaut, 2010). Furthermore, the ability of women to repeatedly generate maximum power outputs has often been reported to be higher than that of men (Billaut & Smith, 2009; Esbjörnsson-Liljedahl, Bodin, & Jansson, 2002; Esbjornsson-Liljedahl, Sundberg, Norman, & Jansson, 1999a; Falgairette, Billaut, Giacomoni, Ramdani, & Boyadjian, 2004b; Yanagiya, Kanehisa, Kouzaki, Kawakami, & Fukunaga, 2003a), which makes the use of these tasks relevant to study the rate-limiting physiological adjustments that cause the sex difference in performance. By investigating the effects of arterial hypoxaemia on sprint performance, Billaut and Smith (2009) further postulated that women could be less sensitive to a reduction in O_2 availability, which may delay the occurrence of fatigue during strenuous exercise. However, although this metabolic difference may highlight a potentially-important mechanism, the design and techniques used in the study did not allow the investigators to ascertain this finding.

The current study was therefore designed to determine whether women exhibit a higher tissue oxygenation than men do during exercise. To focus on the specific role of tissue oxygenation for fatigue, exercise was carried out both during normoxia and hypoxia. Based on the assumption that sex differences in performance may be related to a greater utilisation of any available O_2 by women, we reasoned that performance during repeated sprints would be less impaired during acute exposure to hypoxia in women than in men. It was also hypothesised that

this sex dimorphism in performance would be accompanied by sex differences in tissue oxygenation.

Materials and methods

Subjects

Current research suggests that it is necessary to consider differences in absolute performance (and training background) when investigating the sex difference in physiological responses, performance and fatigue (Billaut & Bishop, 2009; Fulco, et al., 1999; Hunter, et al., 2004b; Tarnopolsky, 2000). This appears particularly important during repeated sprints where a correlation between the initial mechanical score and performance decrement has consistently been reported (Bishop, Lawrence, & Spencer 2003; Mendez-Villanueva et al., 2007). Previous sex comparisons have simply used absolute performance scores, and large differences in initial muscular power have contributed to the inability to definitely establish sex differences in fatigue rates.

Fifteen male and sixteen female rugby and soccer athletes (age: 18–25 yr) from the University of Lethbridge volunteered for the study. All subjects were healthy and with no known neurological or cardiovascular diseases. These subjects were chosen because they were accustomed to high-intensity exercise. After being fully informed of the requirements, benefits, and risks associated with the participation, each subject gave a written informed consent. Ethical approval for study's procedures was obtained from the Human Subject Research Committee of the University of Lethbridge. This first sample of subjects (n = 31) performed a single, 10-s, all-out sprint on a friction-loaded cycle ergometer (described below) for matching purposes. Matching was accomplished by ranking the results for each subject as closely as possible for mechanical work of the lower limb (measured in joules per kg.lean body mass^{-1}) and discarding the extreme data (Bishop & Edge, 2006). An equal number of men (n = 10) and women (n = 10)

was matched (Table 7). As a result, the initial sprint was conducted at approximately the same mechanical work and, very likely, at similar rates of metabolic demand for both sexes (Bishop & Edge, 2006; Fulco, et al., 1999; Tarnopolsky, 2000). Percentage of body fat was calculated from skinfold thickness measured with a calliper (Harpenden Ltd.) and the 7-site Jackson-Pollock equation (Jackson & Pollock, 1978).

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Table 7: Anthropometric, physical and training characteristics of athletes

	Men	Women	P values
n	10	10	
Age, yr	23.2 ± 2.6	22.4 ± 2.3	NS
Height, cm	182.3 ± 7.5	167.6 ± 5.5	< 0.05
Body mass, kg	82.5 ± 11.3	68.4 ± 12.8	< 0.05
Body fat, %	13.6 ± 2.0	22.2 ± 2.1	< 0.05
Lean body mass, kg	70.7 ± 6.3	51.0 ± 6.1	< 0.05
Training profile, yr	10.3 ± 5.3	10.1 ± 3.5	NS
Physical activity level, $h.week^{-1}$	11.7 ± 6.2	12.1 ± 2.8	NS

P values denote the level of significance between men and women. NS, not significant.

Experimental design

Athletes matched for mechanical work visited the laboratory three times. During the first visit, anthropometric measurements (stature, body mass, and body fat percentage) were recorded, and athletes were re-accustomed with sprint cycling (five, 5-s sprints) until fully confident of producing an all-out effort from a stationary start.

Two to three days following the familiarisation session, subjects were randomised in a single-blind, cross-over design and asked to perform a repeated-sprint exercise under normoxic (NM: 21% F_IO_2) and acute hypoxic (HY: 13% F_IO_2) conditions. Trials were conducted at the same time of day and were separated by one week.

Repeated-sprint exercise testing

All testing was performed on a friction-loaded cycle ergometer (Monark 874E, Stockholm, Sweden) with a braking, resistive force applied on the flywheel set at 0.9 $N.kg^{-1}$ of body mass (Billaut & Smith, 2010; Racinais, et al., 2007). The instantaneous power output, corrected for flywheel acceleration, was recorded at 50 Hz, and the mechanical work performed (J) was calculated by integrating the power curve over the 10 s of the sprint for every sprint. The method used to calculate the absolute (total work, J) and relative (% decrement) scores during repeated sprints has been described elsewhere (Bishop, Edge, & Goodman, 2004b). Subjects were instrumented with necessary probes and sensors, and were then asked to close their eyes, eliminate extraneous thoughts, and rest completely in the exercising position on the ergometer to obtain a 2-min baseline measurement. Immediately after the baseline period, subjects were equipped with the breathing apparatus, and a 10-min exposure period to the gas was observed while seated on the ergometer (10 min was enough to reach a steady state in every subject). After a 5-min warm-up at 50–70 watts, subjects rested for another 1 min, and the test (10 x 10-s sprints each with 30 s of rest) was initiated. The cycle ergometer was equipped with toe-clips to prevent the subjects' feet from slipping. Sprints were initiated with the subject’s dominant leg, with the

crank arm located 45 deg forward to the vertical axis. Subjects were asked to remain seated during every sprint and during the recovery periods. Following the test, all instrumentation was removed and subjects performed a self-paced cool down under ambient condition.

Humidified, experimental gases (NM = compressed air; HY = 13% O_2, 87% N_2) were administered using a system of plastic tubing and 150-liter Douglas bag reservoir, with the O_2-N_2 dilution constantly controlled by a PO_2 probe (Hypersphere Pro, AltitudeTech Inc., Kingston, ON, Canada).

Perceptual and physiological responses to exercise

Rating of perceived exertion (RPE). As an index of overall feeling of subjective perceived exertion, the RPE was assessed with a 15-point category ratio scale (Borg, 1970). The scale was anchored so that 6 represented the resting state and 20 corresponded to maximal exertion. Subjects were thoroughly instructed in the use of the RPE scale before performing every trial. The scale was located in full view of the subjects throughout testing, and subjects pointed to the perceptual ratings that best reflected their conscious effort sensations. RPE readings were taken at rest and immediately after every sprint.

Arterial oxygen saturation (S_pO_2). S_pO_2 was estimated via pulse oximetry (Nellcor N-200, Nellcor Inc., Hayward, CA) with adhesive optodes placed on the forehead. This technique has been shown to be in good agreement (intraclass correlation coefficient = 0.99) with haemoglobin O_2 saturation based on arterial blood analysis (Romer et al., 2007), and has been used during repeated sprints (Billaut & Bishop, 2009; Smith & Billaut, 2010). SpO_2 was recorded during baseline, exposure, and immediately after every sprint.

NIRS measurements and analysis. During all tests, subjects were instrumented with two pairs of NIRS probes to monitor absorption of light across cerebral and muscle tissue (Oxymon MKIII, Artinis, The Netherlands), as previously described (Smith & Billaut, 2010). The theory, limitations and reliability of measurement obtained with this device during exercise are detailed elsewhere (Subudhi, et al., 2007; Van Beekvelt, Colier, et al., 2001). One NIRS emitter and detector pair was placed over the left prefrontal lobe, between Fp1 and F3 (international EEG 10-20 system), and placement was further adjusted (less than 5 mm) to obtain strong signal strength on every subject. Spacing between optodes was fixed at 5 cm using a black, plastic spacer held in place via double-sided, stick disks and a black, tensioning headband to reduce the intrusion of extraneous light and the loss of transmitted NIR light from the field of investigation. It has previously been shown that an emitter–detector distance of 5 cm increases spatial resolution, allowing a light penetration depth of ~2.3–2.5 cm (Ferrari, et al., 2004). It has also been reported that extracranial contribution to the NIRS signal is negligible when the inter-optode distance is > 4.5 cm (Smielewski, et al., 1995). Pictures of the optode position were taken to ensure accurate replacement on subsequent visits.

A second NIRS emitter and detector pair was fixed on the distal part of the left vastus lateralis muscle belly (approximately 15 cm above the proximal border of the patella) using a black, plastic spacer with optode distance of 4.5 cm. Skinfold thickness was measured between the emitter and detector using a skinfold calliper (Harpenden Ltd.) to account for skin and adipose tissue thickness covering the muscle. Probes were secured to the skin using double-sided, stick disks and shielded from light using black, elastic bandages. An indelible pen was used and pictures were taken to mark the position of the optodes for subsequent visits.

A modified form of the Beer-Lambert law was used to calculate micromolar changes in tissue [O_2Hb] and [HHb] across time using received optical densities from two continuous wavelengths of NIR light (763 and 855 nm). An age-dependent differential optical pathlength

factor for cerebral cortex (Duncan, et al., 1996; Shibuya, Tanaka, Kuboyama, Murai, et al., 2004; Shibuya, Tanaka, Kuboyama, & Ogaki, 2004) and of 4.95 for muscle (Duncan, et al., 1995; Subudhi, et al., 2007) were used in this study. Changes in total Hb ([THb]) were calculated by the sum of [O_2Hb] and [HHb] and used as an index of change in regional blood volume within the illuminated area (Van Beekvelt, et al., 2001). When regional blood volume (i.e., Δ[THb]) is constant, [O_2Hb] and [HHb] exist in equilibrium. Thus, decreases in [O_2Hb] and increases in [HHb] reflect relative deoxygenation in the underlying tissue (Ferrari, et al., 2004; Subudhi, et al., 2007). Furthermore, haemoglobin difference (Δ[Hbdiff] = [O_2Hb] – [HHb]) was calculated and used as an additional indicator of oxygenation due to its high correlation with blood flow (Tsuji et al., 2000) and because it is more reliable than other NIRS variables when [THb] is not constant (van Beekvelt, et al., 2002).

NIRS data were acquired at 10 Hz and transferred online from the Oxymon MKIII to a PC. Data were averaged over the last 5 s within every sprint to obtain one value per sprint (Smith & Billaut, 2010), and normalised to express the magnitude of changes from the baseline period (arbitrarily defined as 0 μM) (Shibuya, Tanaka, Kuboyama, Murai, et al., 2004; Shibuya, Tanaka, Kuboyama, & Ogaki, 2004; Smith & Billaut, 2010; Subudhi, et al., 2007).

Statistical analysis

Analyses were performed using Statistica 5.5 for Windows (Statistica, Statsoft Inc., Tulsa, OK). Separate one-way ANOVAs (sex) were used to compare anthropometric characteristics, training profile, physical activity level, initial-sprint mechanical work, and the percent decrement in mechanical work between men and women. Separate three-factor ANOVAs (sprint x condition x sex) with repeated measures on sprint were used to compare the following dependent variables between men and women across repetitions: mechanical work, S_pO_2, RPE, Δ[O_2Hb], Δ[HHb], Δ[THb] and Δ[Hbdiff]. Tukey's HSD *post-hoc* analyses were used to locate differences among

pairs of means when ANOVAs revealed significant *F*-ratio for main or interactive effects. The level of significance was set at $P < 0.05$. Data are reported as mean ± SD.

Results

Mechanical measurements

The mechanical work completed during every sprint and the total work accumulated over the series in NM and HY are displayed in Figure 9 and Table 5. No effect of sex or condition was observed for the initial-sprint score (men: 114.1–116.3 $J.kgLBM^{-1}$; women: 116.4–118.3 $J.kgLBM^{-1}$; $P = 0.81$). Furthermore, there was no effect of sex on the impact of HY on performance. In both environments, the total work accumulated over the series and the percent decrement in mechanical work (Figure 9B and Table 5) were similar (P = NS) for the men and the women.

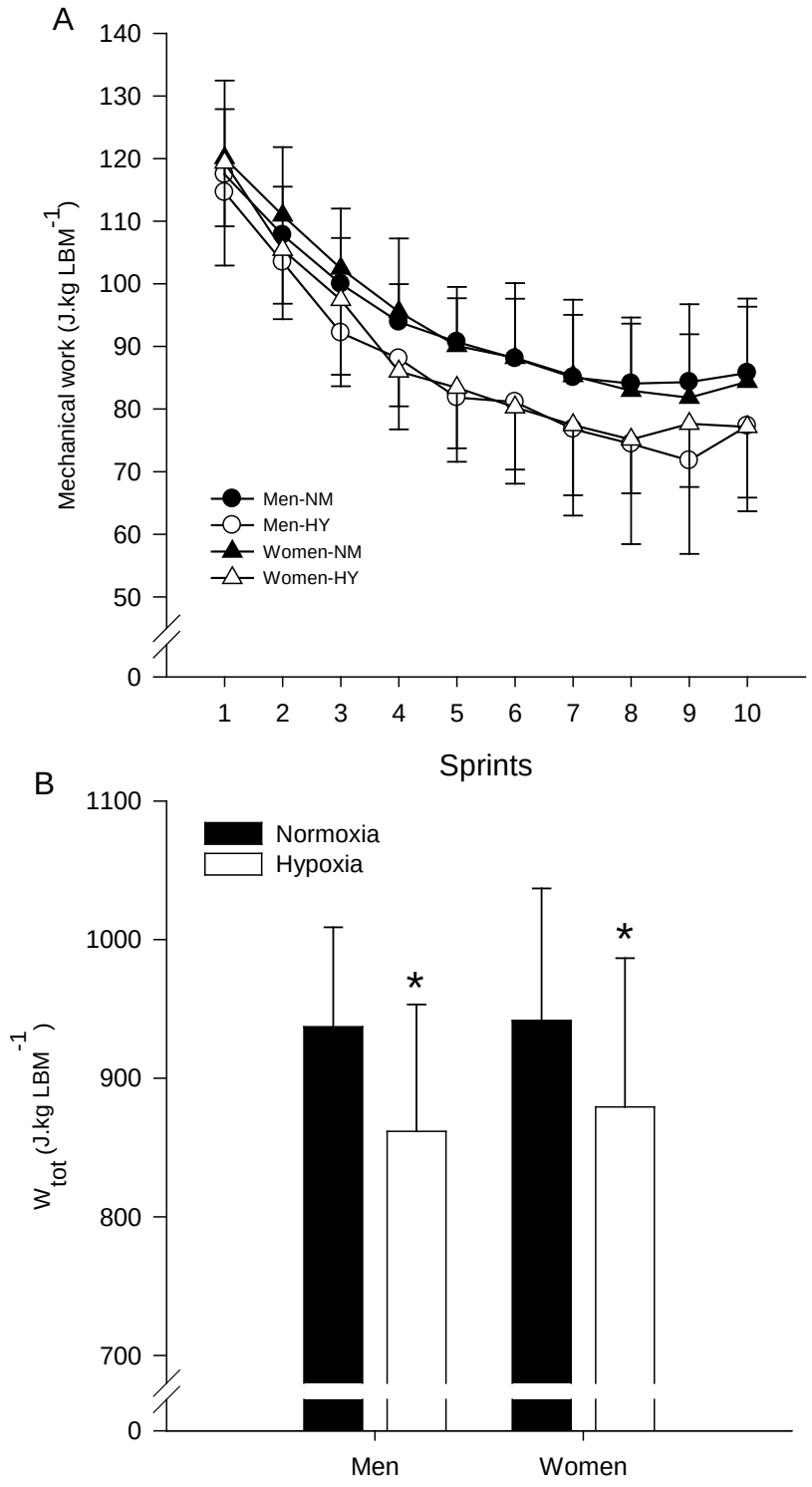

Figure 9. Mechanical work (panel A) and total work (panel B) performed during the repeated sprints in men and women in both normoxia and hypoxia. Main effect of sprint: $P < 0.05$; Main effect of condition: $P < 0.05$ (* significantly different from normoxia); Main effect of sex: NS; Interaction effects: NS.

Table 5: Mechanical scores for men (n = 10) and women (n = 10) during the repeated sprints in normoxia and hypoxia.

Groups		Initial mechanical work, J.kgLBM^{-1}	Total work, J.kgLBM^{-1}	Percent decrement, %
Men	Normoxia	116.7 ± 11.5	937.6 ± 71.3	26.9 ± 10.3
	Hypoxia	114.6 ± 11.7	861.6 ± 111.5*	32.2 ± 9.1*
Women	Normoxia	119.4 ± 10.7	941.6 ± 95.2	29.7 ± 12.1
	Hypoxia	117.5 ± 9.8	879.3 ± 107.3*	35.7 ± 9.0*

LBM = lean body mass. * Different from Normoxia (main effect of condition: $P < 0.05$).

Arterial O_2 saturation

Mean data for S_pO_2 at rest, exposure and over the sprints for men and women in NM and HY are displayed in Table 9. At rest, there was a little variation among subjects and conditions (average: 98.5 ± 1.1%, $P > 0.05$) with all subjects within the normal range. S_pO_2 was profoundly affected by the condition, with lower values recorded in HY than in NM (average of both sexes: 18.6 ± 6.3%, $P < 0.05$). In addition, the pattern of arterial desaturation across sprints varied between conditions (interaction sprint x condition: $P < 0.05$), with significant reductions occurring in sprints 2–10 (overall decrement: 9.1%, $P < 0.05$) in NM, and in sprints 1–10 (overall decrement: 15.1%, $P < 0.05$) in HY. Men and women were equally affected by HY; the final S_pO_2 was 78.3 ± 5.3% in men and 74.4 ± 8.5% in women ($P > 0.05$).

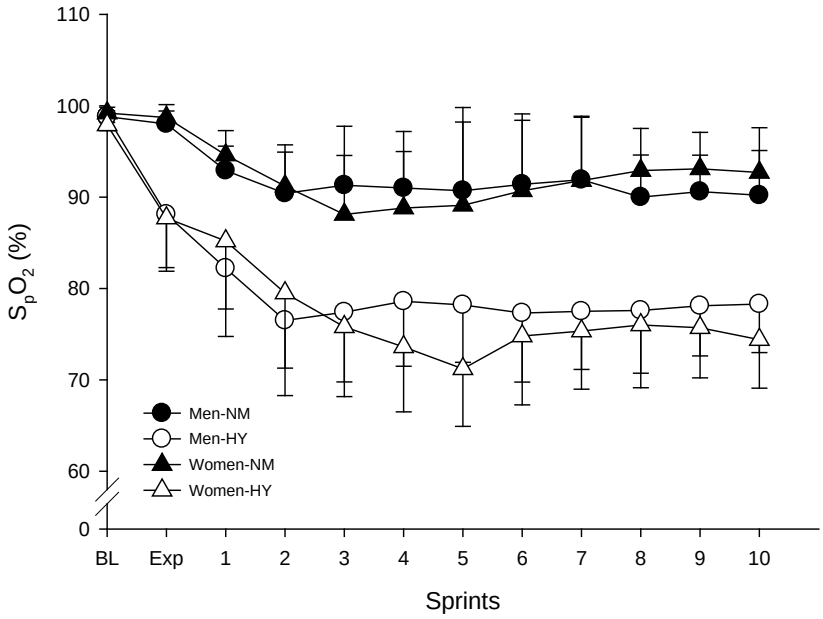

Figure 10. Arterial O_2 saturation (S_pO_2) at baseline (BL), exposure (EXP), and over the sprints in men and women in both normoxia and hypoxia. Main effect of sprint: $P < 0.05$; Main effect of condition: $P < 0.05$; Main effect of sex: NS; Interaction sprint x condition: $P < 0.05$ (please refer to *Results* for additional information).

NIRS measurements

The average concentration changes in muscle and cerebral NIRS signals across sprints for the men and the women in normoxia and hypoxia are displayed in Table 6 and Figure 11 and Table 7 and Figure 12, respectively.

Muscle analysis.

In NM, regional muscle oxygenation decreased rapidly during the first sprint (↓ $\Delta[O_2Hb]$, ↑ $\Delta[HHb]$, and ↓ $\Delta[Hb_{diff}]$). From sprints 2–10, $\Delta[Hb_{diff}]$ remained unchanged, yet $\Delta[THb]$ rose significantly, indicating a reduction in the rate of muscle deoxygenation in subsequent sprints. In HY, both the pattern and the magnitude of the deoxygenation were very similar to NM. However, in each condition, muscle $\Delta[Hb_{diff}]$ differed between the sexes: the magnitude of the sprint-induced muscle deoxygenation was significantly less for the women compared with the men (average: 63.7%, $P < 0.05$; Figure 11B).

Cerebral analysis.

In NM, regional cerebral oxygenation increased rapidly in sprints 1–2 (⇑ $\Delta[O_2Hb]$, ↔ $\Delta[HHb]$, and ↑ $\Delta[THb]$) for the men and the women, indicating cerebral vasodilatation during initial sprints. Thereafter, the NIRS signals fluctuated without showing clear evidence of deoxygenation between sprints 3 and 10 (from ↔ to ↑ $\Delta[O_2Hb]$ and ↑ $\Delta[HHb]$), while regional blood volume displayed a slight, although not significant, increase from sprint 1 to 10 ($P = 0.32$) in both sexes. For the men and the women cerebral oxygenation was markedly reduced in HY (↔ $\Delta[O_2Hb]$, ↑ $\Delta[HHb]$) across every sprint. The effect of HY on $\Delta[Hb_{diff}]$ was identical in these men and women matched for initial-sprint work.

Furthermore, $\Delta[Hb_{diff}]$ correlated positively with changes in mechanical work over the sprints in both men (NM: $R^2 = 0.84$; HY: $R^2 = 0.95$; $P < 0.05$) and in women (NM: $R^2 = 0.91$; HY: $R^2 = 0.94$; $P < 0.05$).

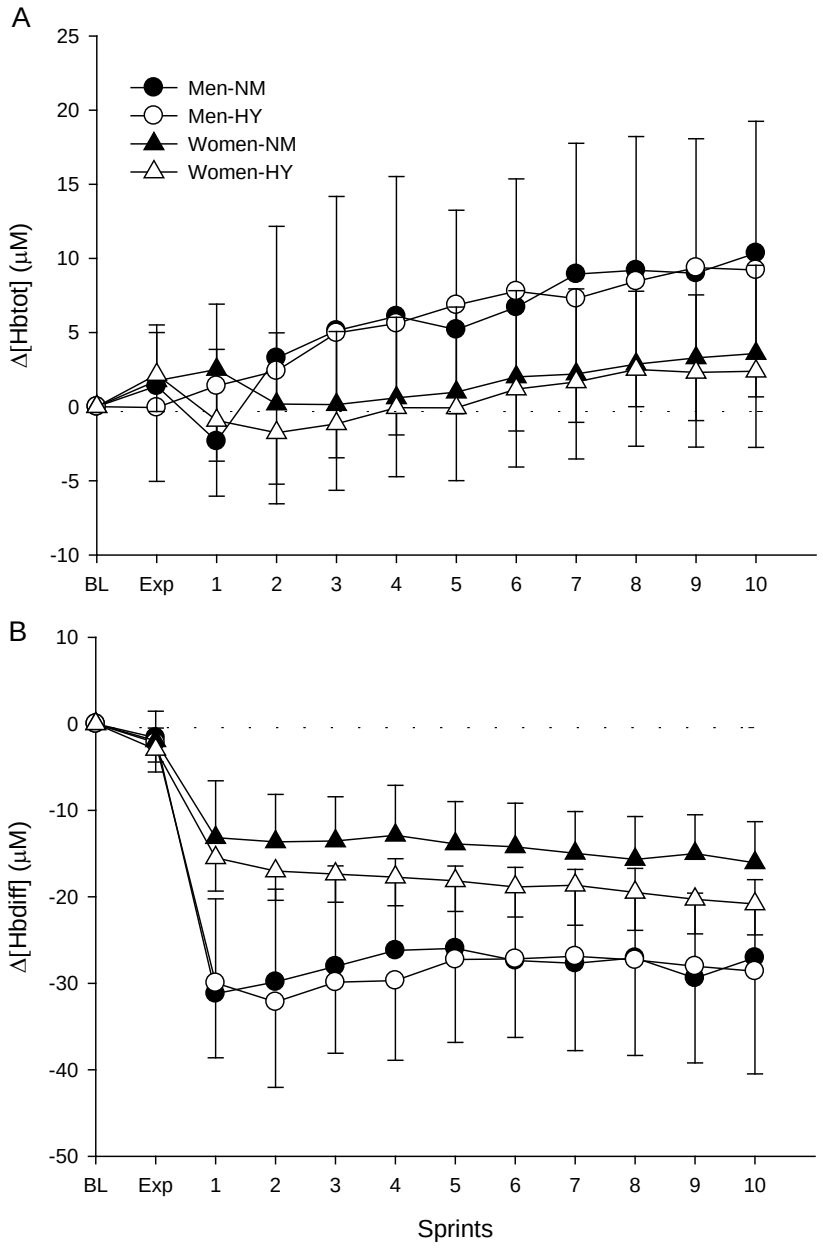

Figure 11. Muscle NIRS concentration changes from resting baseline (BL) during exposure (EXP), and throughout the sprints for men and women in normoxia and hypoxia. THb: total haemoglobin; Hb_{diff}: haemoglobin difference. Brackets indicate concentration. Main effect of sprint for [THb]: $P < 0.05$ (sprints 8–10 significantly different from baseline); Main effect of condition for [THb]: NS; Main effect of sex for [THb]: $P < 0.05$ (men > women); Interaction effects: NS. Main effect of sprint for [Hb_{diff}]: $P < 0.05$ (sprints 1–10 significantly different from baseline); Main effect of condition for [Hb_{diff}]: NS; Main effect of sex for [Hb_{diff}]: $P < 0.05$ (men < women); Interaction effects: NS.

Table 6. Muscle NIRS concentration changes during the repeated sprints for men and women in normoxia and hypoxia.

Variable		Exp	Sprints									
			1	2	3	4	5	6	7	8	9	10
$\Delta[O_2Hb]$	Men	Normoxia	0.6±3.0	-14.8±6.8	-13.3±6.5	-11.5±6.7	-10.1±6.6	-9.4±6.7	-9.3±6.5	-9.4±6.8	-7.7±8.8	-10.2±6.1	-7.1±9.6
		Hypoxia	-1.1±2.7	-14.3±4.9	-14.8±5.1	-12.5±5.0	-12.0±5.1	-10.2±4.6	-9.7±4.8	-9.7±5.3	-9.4±5.4	-9.3±5.7	-9.7±6.0
	Women	Normoxia	0.1+2.1	-4.1±8.8	-5.7±7.6	-5.8±7.4	-5.6±7.7	-5.6±7.9	-5.2±8.8	-5.3±8.7	-5.0±8.9	-4.7±9.0	-4.8±9.6
		Hypoxia	-0.1±1.5	-8.4±1.1	-9.5±0.9	-9.4±1.7	-9.1±1.9	-9.2±2.0	-9.0±2.2	-8.6-±2.9	-8.9±2.2	-9.4±2.0	-9.4±2.1
Δ[HHb]	Men	Normoxia	1.9±2.3	12.7±8.5	16.6±11.0	16.6±10.8	16.1±9.6	14.5±8.9	16.0±9.3	18.3±10.1	16.9±9.8	19.2±9.2	17.5±10.8
		Hypoxia	1.1±3.3	15.7±8.4	17.3±10.5	17.4±10.2	17.6±10.4	17.1±10.5	17.5±10.8	17.1±11.0	17.9±11.1	18.7±11.5	18.9±12.0
	Women	Normoxia	1.7±1.6	6.6±5.2	5.9±4.8	5.9±4.8	6.2±5.1	6.6±5.0	7.2±5.1	7.5±5.2	7.9±5.1	8.0±5.1	8.4±5.3
		Hypoxia	2.3±1.4	7.8±2.9	8.0±3.3	8.4±3.8	9.1±3.8	9.6±3.9	10.5±4.2	10.5±4.3	11.5±4.3	11.6±4.2	11.9±4.2

Values are micromolar changes from resting baseline. O_2Hb: oxy-haemoglobin; HHb: deoxy-haemoglobin. Brackets indicate concentration.
Main effect of sex for [HHb]: $P < 0.05$ (men > women); Interaction effects: NS

Table 7. Cerebral NIRS concentration changes during the repeated sprints for men and women in normoxia and hypoxia.

Variable			Exp	Sprints									
				1	2	3	4	5	6	7	8	9	10
Δ[O_2Hb]	Men	Normoxia	2.1±3.0	7.5±6.5	4.1±2.7	3.4±3.3	1.7±3.3	1.7±4.0	2.0±4.0	2.4±4.3	3.1±4.6	5.0±3.9	5.3±4.6
		Hypoxia	-0.3±2.1	2.9±5.3	-2.0±2.7	-3.3±2.8	-3.5±4.9	-3.3±5.4	-2.7±5.9	-1.8±6.4	-0.9±7.3	-0.3±7.7	0.6±8.9
	Women	Normoxia	0.7±2.2	7.8±6.9	5.8±6.5	3.9±6.0	3.6±5.7	4.4±3.4	6.0±4.0	6.4±4.2	7.0±5.6	7.4±5.6	8.5±7.2
		Hypoxia	1.3±3.5	4.3±9.5	0.5±10.7	-1.8±8.4	-2.8±8.4	-3.8±6.7	-3.4±8.3	-3.8±11.5	-3.5±10.1	-3.0±11.0	-3.5±8.4
Δ[HHb]	Men	Normoxia	1.1±1.2	1.2±4.5	2.6±4.0	3.5±4.0	4.0±4.0	4.3±3.8	5.5±3.9	6.2±3.5	7.0±4.0	8.1±2.4	8.6±2.7
		Hypoxia	2.8±2.7	6.3±1.9	7.5±3.3	8.4±4.0	9.2±4.0	9.7±4.8	10.6±5.4	11.7±6.0	12.1±5.8	12.9±6.2	13.2±6.4
	Women	Normoxia	1.6±0.7	3.0±2.7	3.0±2.0	3.5±2.2	3.9±2.1	3.7±2.2	4.6±2.2	4.8±1.9	4.9±2.2	4.0±3.9	5.0±2.6
		Hypoxia	4.2±2.2	6.3±3.2	7.4±2.9	7.9±4.0	8.7±3.8	9.1±4.8	9.5±3.8	9.5±3.8	9.4±4.7	9.7±4.1	10.4±5.4

Values are micromolar changes from resting baseline. O_2Hb: oxy-haemoglobin; HHb: deoxy-haemoglobin. Brackets indicate concentration. Main effect of sex: NS; Interaction effects: NS.

Rating of perceived exertion

The RPE scores were similar for the men and the women during the sprints in both NM and HY ($P > 0.05$). The RPE values progressed from initial values of 6 ± 1 to 19 ± 1 after the final sprint. No significant interaction effect of sex and sprint was observed on RPE.

Discussion

The current study aimed to compare the ability to repeat maximal performance during short cycling sprints by men and women who were matched for mechanical work. The repeated-sprint ability was similar for the work-matched men and women. Accordingly, there were no differences in the changes in S_pO_2, RPE, and cerebral NIRS data during the sprints. Despite these similar performance characteristics, the pattern of muscle deoxygenation differed for the men and women: the women exhibited smaller changes in muscle [Hb_{diff}] than men during the sprints.

Technical considerations

Studies of NIRS must acknowledge that prefrontal cortex oxygenation is a regional measurement that may not be reflective of global cerebral oxygenation. Nevertheless, Subudhi and colleagues (2009) demonstrated that the deoxygenation occurring during whole-body, strenuous exercise is not confined to the prefrontal cortex but seems widespread to motor areas as well. Furthermore, the deoxygenation of the prefrontal cortex has been purported to contribute to performance decrement on varied occasions (Amann et al., 2007; Shibuya, Tanaka, Kuboyama, Murai et al., 2004; Shibuya, Tanaka, Kuboyama, & Ogaki, 2004; Subudhi et al., 2007), and in particular during repeated cycling sprints (Smith & Billaut 2010). However, since the differential pathlength factors were only estimated, it must be reminded that absolute NIRS measurements and, therefore, actual tissue oxygenation values remain unknown.

As far as the muscle tissue is concerned, no post-exercise, vascular occlusion was performed in the current study because Chance et al. (1992) reported little additional

deoxygenation (< 2%) during supra-systolic cuff occlusion of the vastus lateralis muscle immediately following strenuous exercise. We also chose not to perform arterial occlusion to stay consistent with previous studies that have investigated muscle oxygenation trends during repeated sprints (Buchheit, et al., 2009; Racinais,et al., 2007; Smith & Billaut, 2010). Nevertheless, if a leg cuff ischemia technique had allowed obtaining a low-oxygenation reference point it would have been similar in both conditions, and thus, would not have affected the conclusions of the current study.

Repeated-sprint performance was similar in work-matched men and women

Men and women who were matched for mechanical work of the initial sprint had similar performance decrements during ten cycling sprints performed in NM. These results are consistent with previous observations (Billaut, Giacomoni, & Falgairette, 2003a; Brooks et al., 1990b; Falgairette, et al., 2004b), but they may appear contradictory to other studies in which women have been found to be less fatigable than men (Billaut & Smith, 2009; Bishop, Edge, Dawson, Goodman, 2003; Yanagiya, et al., 2003a). For instance, men (who were taller and heavier) presented higher absolute and relative work than women during five 6-s cycle sprints repeated every 30 s, but experienced a greater work decrement than women (14% vs. 11%, respectively; $P < 0.05$) (Bishop, Edge, et al., 2003). Similar results were observed during twenty 5-s cycle sprints separated by 25 s of rest (Billaut & Smith, 2009); while both absolute and relative work were higher in men, the fatigue index was less for the women than the men (19% vs. 30%, respectively; $P < 0.05$). A higher decrement in mechanical output during repeated sprints in men, as compared with women, has previously been interpreted as a sign of a greater fatigue in the men (Billaut & Smith, 2009; Esbjörnsson-Liljedahl, et al., 2002; Esbjornsson-Liljedahl, et al., 1999a; Yanagiya, et al., 2003a). Recently however, on the basis of other research (Bishop, Lawrence, & Spencer 2003; Bishop & Spencer, 2004b) we have questioned the influence of the initial-sprint mechanical score on the fatigue characteristics of men vs. women (Billaut & Bishop, 2009), and have

suggested that the sexes should be matched for mechanical work completed during the first sprint of a series when attempting to compare performance and physiological responses. In fact, it is now well established that the higher the initial-sprint score, the greater the performance decrement in subsequent sprints (Bishop, Lawrence, & Spencer 2003; Bishop & Spencer, 2004b).

While controlling for the effects of the initial-sprint performance, the current study demonstrated that women were equally affected by HY than men. Each sex displayed a similar degree of decline of mechanical work from NM values during every sprint (Figure 9) after we induced similar changes in S_pO_2 in the two sexes (Table 9). Although this is in contrast with our initial hypothesis of greater sprint endurance in women, it supports the general consensus that the initial-sprint performance is a major determinant of repeated-sprint ability, and suggests that it may have been a methodological confound in previous investigations of sex differences (Billaut & Smith, 2009; Bishop et al., 2003; Yanagiya et al., 2003). Overall, the current data are in good agreement with the finding that the ability to generate high power outputs consecutively is impaired in an hypoxic environment (Balsom et al., 1994; Smith & Billaut, 2010), and add to the current knowledge that, as for men (Billaut & Smith, 2010; Smith & Billaut, 2010), arterial O_2 saturation limits repeated-sprint ability in women.

Arterial O_2 saturation was similar in work-matched men and women

The S_pO_2 response indicated the perturbations of the O_2 transport system that occurred during the sprints in HY. The work-matched men and women exhibited a similar arterial desaturation during the sprints in both conditions, which suggests that they likely experienced similar magnitudes of O_2 delivery impairment. Therefore, the results showed that men and women exposed to similar levels of reduced F_IO_2 are likely to exhibit similar levels of systemic adjustments. This metabolic similarity is likely to explain the similar fatigue development observed during the sprints in HY. These results support previous findings that women displayed

similar S_pO_2 changes than men during twenty 6-s sprints performed in NM (Billaut & Smith, 2009). Knowing that arterial saturation may be considered as a determinant of repeated-sprint ability (Billaut & Smith, 2009; Billaut & Smith, 2010; Smith & Billaut, 2010) and that S_pO_2 is correlated with cerebral oxygenation (Smith & Billaut, 2010), similar patterns and magnitudes of blood desaturation in these work-matched subjects are likely to induce similar tissue oxygenation perturbations.

Cerebral oxygenation was similar in work-matched men and women

Several studies have insinuated the importance of O_2 metabolism in underpinning the sex-specific fatigue (C.S. Fulco, et al., 2001; Fulco, et al., 1999; Hunter, et al., 2006; Hunter, et al., 2004b; S.K. Hunter & R.M. Enoka, 2001; Saito, et al., 2008), yet the conclusions remained speculative since tissue oxygenation was not measured. To our best knowledge, the current study was the first to investigate the oxygenation of the prefrontal cortex of men and women in both NM and HY. The deoxygenation of the prefrontal cortex occurred earlier and to a larger extent in HY than in NM, as previously noted (Smith & Billaut, 2010), but more importantly, these changes were nearly identical in these men and women matched for initial-sprint work. All subjects exhibited a fall in cerebral oxygenation when performing the HY trial, and this fall was strongly correlated with the exacerbated reduction in mechanical work in HY, as compared with NM. This strengthens the multiple observations that the oxygenation of the brain and the ability to perform work at supramaximal intensity (Nielsen, et al., 1999; Shibuya, Tanaka, Kuboyama, Murai, et al., 2004) and repeatedly (Shibuya, Tanaka, Kuboyama, & Ogaki, 2004; Smith & Billaut, 2010) are correlated.

Although it is impossible to ascertain what caused the reduction in cerebral oxygenation in the current study, it is interesting to note that men and women who experienced identical perturbations at the systemic level (*i.e.*, changes in S_pO_2) exhibited identical perturbations at the

cerebral level. Consequently, the reduction in O_2 availability likely contributed to the cerebral deoxygenation observed in the current conditions.

Muscle oxygenation differed for work-matched men and women

Recently, Smith and Billaut (2010) demonstrated for the first time that HY has little impact on muscle oxygenation changes during repeated sprints, which landed support to the idea that muscle O_2 extraction is not considered as a limiting factor of repeated-sprint ability. In the current study, muscle oxygenation decreased during the first sprint and remained constant thereafter. Since NIRS measurements have been correlated with changes in intracellular O_2 tension (Tran, et al., 1999) and venous O_2 saturation (Esaki, et al., 2005), the plateau in oxygenation may be interpreted as evidence of maximal O_2 extraction in both men and women (Esaki, et al., 2005; Tran, et al., 1999). As shown previously in men (Smith & Billaut, 2010), the reduced O_2 availability in HY (Table 9) did not alter muscle O_2 uptake in women.

That being said, the magnitude of decrease in muscle oxygenation was significantly less for the women. The lesser O_2 used by the women was not due to the women developing lower mechanical work: the total work performed during the entire series was similar for the two sexes in both NM and HY (Figure 9), and they had similar rates of rise in RPE. The difference in the magnitude of muscle deoxygenation during the sprints was also not due to recording conditions because there was no difference between the two sexes in the muscle $\Delta[Hb_{diff}]$ obtained at rest and during the exposition period to the gas mixture. Therefore, the lesser O_2 used by the women, combined with the same power capacity during the sprints, could have been due a greater efficiency of the oxidative metabolism in women. These data, however, do not explain how the women were able to develop the same mechanical work across the ten sprints with a lower muscle O_2 uptake.

To date, most of the existing information related to sex-based differences in muscle metabolism has come from biopsy studies. Women have been reported to exhibit a greater distribution of type I fibres and lower distribution of type IIX fibres than men (Brooke & Engel, 1969; Miller, MacDougall, Tarnopolsky, & Sale, 1993; Simoneau & Bouchard, 1989; Simoneau et al., 1985), although this is not a consensus (for review see (Billaut & Bishop, 2009)). This is consistent with the finding that women, compared with men, have been found to exhibit a lower decrease in ATP and PCr concentrations after five 6-s sprints repeated every 30 s (Bishop, Edge, et al., 2003). The current finding of greater oxidative metabolism efficiency in women is also supported by the results of Fulco and colleagues (2001) showing a lack of effect of HY on women's muscle performance (whereas men were affected), which is indicative of a relatively high oxidative capacity in women. It is therefore possible that women were able to make a better use of any O_2 available to produce work, in support of the proposed metabolic basis for sex differences in fatigue resistance (Fulco, et al., 2001; Kent-Braun, Doyle, & Towse, 2002; Russ & Kent-Braun, 2003; Russ, Lanza, Rothman, & Kent-Braun, 2005).

In summary, men and women who were matched for mechanical work displayed similar arterial O_2 desaturation and cerebral deoxygenation and thereby fatigue during repeated sprints performed in HY. These results indicate that work-matched men and women experienced similar systemic and cerebral adjustments during the sprints. However, the muscle deoxygenation that is typically observed during such activity differed between the sexes. Consequently, the men and women achieved similar performance with varying strategies for using O_2 at the muscle level. This provides new evidence strengthening the hypothesis of sex differences in metabolic pathway utilization.

-CHAPTER 4-

CONCLUSION

4.1 Summary and conclusions

Compared with endurance exercise, research investigating factors limiting muscle performance during RSE is very limited. Alterations in the excitation-contraction coupling and/or in the contractile apparatus (*i.e.*, muscle fibres) have traditionally been regarded as the primary causes of muscle fatigue (Glaister, 2005, Spencer et al., 2005). During RSE, muscle metabolism is stretched to its limits and, consequently, power decrements observed during this mode of exercise have mainly been ascribed to metabolic changes within the muscle fibre itself (*i.e.*, peripheral fatigue). Due to the paucity of data regarding central factors of fatigue during RSE, several authors have explored this hypothesis and found that in most cases muscle recruitment is lowered during RSE, which contributes to the decline in mechanical performance (Billaut &Smith, 2010; Mendez-Villenueva et al., 2007; Mendez-Villenueva et al., 2008; Racinais 2007). However, these studies could not describe the mechanisms behind these neuromuscular adjustments. The series of studies purposed in this thesis was designed to investigate the effects of cerebral and muscle tissue oxygenation on RSA in men and women by using a hypoxia paradigm. This was motivated by the fact that basic physiology studies reported an association between O2 availability and neuronal activity (Amann et al., 2007; Bigland-Ritchie et al., 1986; Dousset et al., 2001; Nybo & Rasmussen, 2007).

These studies used young male and female team-sport athletes who performed a RSE (10 x 6-s sprints interspersed with 30 s of rest) in normoxia (F_IO_2 = 0.21) and hypoxia (F_IO_2 = 0.13). The subjects were blind to the condition, and were asked to perform maximally in every sprint. To our best knowledge, these studies are the first to investigate oxygenation of the prefrontal cortex and the muscle in such exercise, and to relate these perturbations to the ability to repeat maximal

performance. In both conditions mechanical work performed was reduced. However, subjects were more affected in hypoxia. Indeed, both men and women exhibited impaired performance in simulated altitude, which paralleled the decline in arterial O_2 saturation. Similar performance data had already been obtained by Balsom et al. (1994) in men. Therefore, our data add to the literature that this phenomenon is also present in women and is of similar magnitude. By controlling for the effects of the initial-sprint performance on RSA (*i.e.*, identical performance of men in both normoxia and hypoxia, and identical performance of men and women), we were able to show that 1) the factors impairing RSA are related to O_2 availability, and 2) men and women use different combinations of central (cerebral) and peripheral (muscle) adjustments to cope with fatigue.

The main finding from Study 1 and 2 was that hypoxia profoundly affected the pattern and magnitude of cerebral oxygenation in young healthy athletes. This may have contributed to performance impairment in hypoxia. On the other hand, cerebral oxygenation changes were not considered to limit performance in normoxia. This suggests that the increase in metabolic demand stemming from increased neuronal activation during RSE was adequately met with a sufficientO_2 availability and blood flow during exercise in normoxia. Additionally, the progressive increase in Δ[HHb] observed during the final sprints may indicate that, through a homeostatic regulation of blood flow, cerebral oxygen availability (delivery↑Δ[O_2Hb] and extraction:↑Δ[HHb]) is well maintained in men and women. Of interest is that arterial saturation was similar in the two studies, which may suggest the action of other unmeasured systemic variables such as O_2 and CO_2 tensions, ventilation, and blood pressure in the regulation of cerebral oxygenation (Nybo & Rasmussen, 2007; Ogoh & Ainslie, 2009)). However, this was not the case in hypoxia. From the above observations rises the question of how impaired cerebral oxygenation explain the reduction in RSA in acute hypoxia?

Neuronal activity, especially α-motor neuron activity, has been observed to be dramatically influenced by O_2 availability (Amann, et al., 2007; Bigland-Ritchie, Furbush & Woods, 1986; Szubski, Burtscher & Loscher, 2006b), which plays a pivotal role in the homeostatic preservation of central and peripheral nervous system functionality. To date however, data in exercising humans were limited. Our results in figure 8 support such a phenomenon during RSE, since the lower cerebral oxygenation related to the largest decline in EMG activity ($R^2 = 0.83$). This lends support to the effect of oxygen availability on cerebral down-regulation of motor neuron excitability (Amann and Kayser 2009) and summarized in figure 13, which highlights the possible mechanisms of fatigue attributed to oxygen availability during exercise. Interestingly, and in contrast with our hypothesis, there was no difference between these men and women matched for mechanical work. This shows that the systemic (similar S_pO_2) and cerebral (and therefore neuronal) adjustments to a hypoxic stress may be similar in men and women, and strengthen previous findings that arterial O_2 saturation is a powerful determinant of cerebral autoregulation. In summary, the studies conducted in the current thesis presented data demonstrating that altering the O_2 availability has a direct effect on muscle recruitment and thereby performance in RSE. It is of concern, however, that surface EMG activity used as a sole determinant of central neural drive is limited (Amann, et al., 2007; Enoka & Stuart, 1992).

In these studies we also measured oxygenation at the muscle level. Muscle deoxygenation occurred quickly at the onset of the exercise and eventually reached a plateau during the final sprints. This pattern is consistent with other studies using similar sprinting and cycling protocols (Buchheit, et al., 2009; Racinais, et al., 2007). A similar trend was observed in the vastus lateralis muscle during 30 and 45 s Wingate sprints (Bhambhani, Maikala, & Esmail, 2001). This seems to indicate a similar pattern of deoxygenation in the active muscles during anaerobic exercises. Since NIRS measurement have been correlated with changes in intracellular O_2 tension and venous O_2 saturation (Esaki, et al., 2005), the plateau in oxygenation may be interpreted as

evidence of maximal O_2 extraction (Esaki, et al., 2005; Tran, et al., 1999). In contrast with above-mentioned studies, we relied on the use of hypoxia to measure the influence of reduced O_2 availability, and subsequently demonstrate the influence of muscle oxygenation of RSA. Our results showed that patterns as well as the magnitude of tissue deoxygenation were nearly identical in normoxia and hypoxia (Fig. 7). Further impairment of quadriceps oxygenation during the hypoxic trial was not observed compared with the normoxic trials in both men and women. This result demonstrates that during hypoxia muscle O_2 uptake was maintained despite a reduction in oxygen availability. This is in good standing with a study comparing whole-body (cycling) and small-muscle (one-leg, knee extension) exercises that related the main factor of performance limiting capacity in acute hypoxia to systemic O_2 delivery and not muscle O_2 extraction (Calbet, et al., 2009). Therefore, the current findings demonstrate that muscle oxygenation is not a limiting factor in RSE in either men or women (Buchheit, et al., 2009; Racinais, et al., 2007).

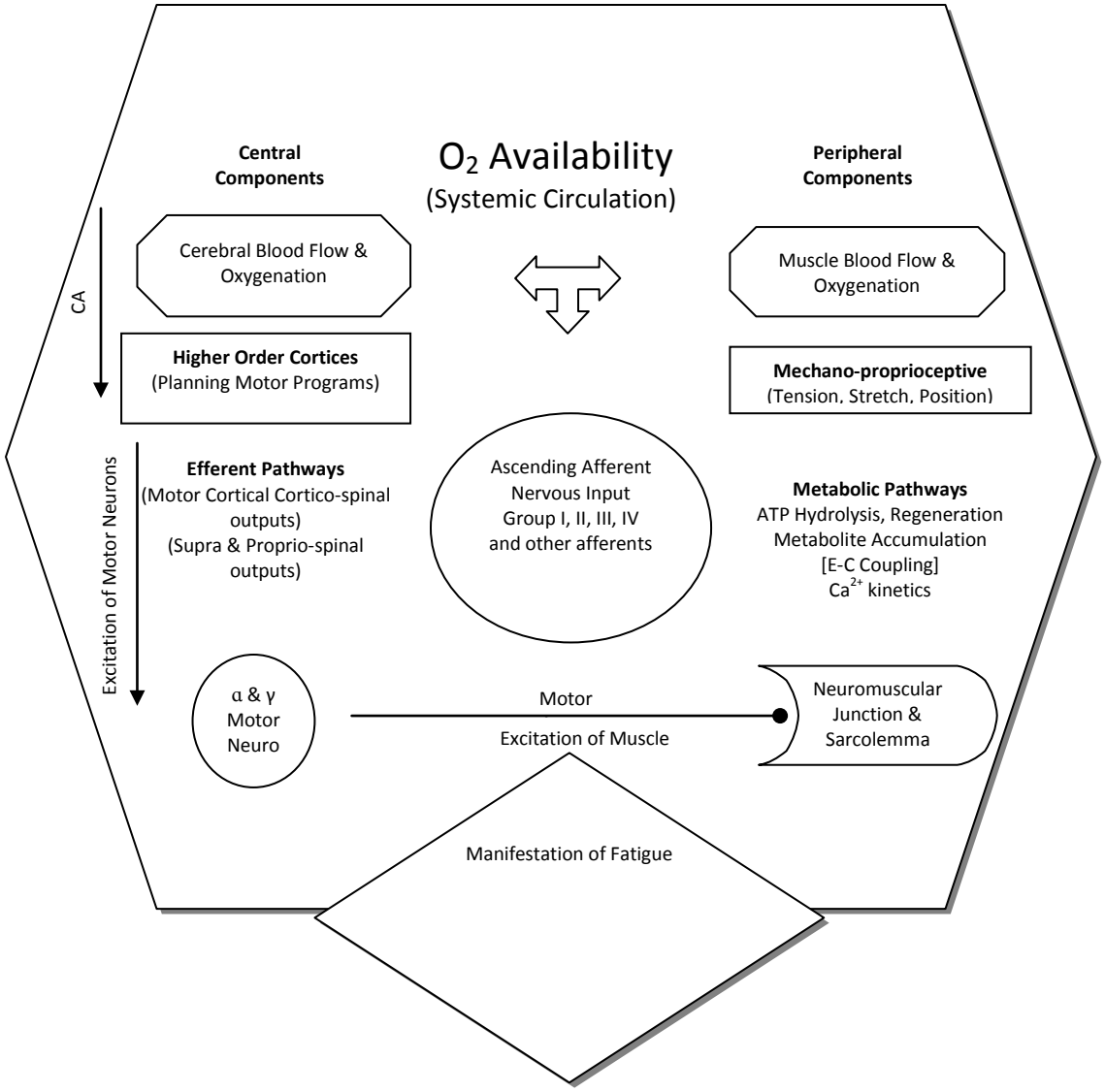

Fig. 13. Schematic for the influence of oxygen availability on the down regulation of motor neuron excitability during fatiguing exercise. **ATP**=Adenosine triphosphate;**CA**=Cerebral autoregulation; **E-C coupling** =excitation contraction coupling **.**

Muscle deoxygenation, however, was greater in men than women during both normoxic (percent difference: Δ[HHb]= 52%) and hypoxic conditions (percent difference: Δ[HHb]= 48%), although the overall response to hypoxia was similar in the sexes(Mox_{NM}=Mox_{HY}). It is not clear why women were able to perform as well as men with a lower extraction rate of O_2. Consistent with other observations (Fulco et al., 2001, Kent-Braun et a.,l 2002, Russ and Kent-Braun 2003), the finding from Study 2 could have been due a greater efficiency of the oxidative metabolism in women. In fact, it has already been hypothesised that during RSE women could make a better use of any O_2 available to produce work (Billaut and Smith 2009). It appears therefore that the same mechanical output was developed by the men and the women with a different balance between central (cerebral) and peripheral (muscle) adjustments.

To conclude, this thesis has shown that O_2 availability does influence prefrontal cortex, but not muscle, oxygenation during repeated short sprints in humans. Based on the strong relationships of cerebral deoxygenation to mechanical work and EMG activity, in both normoxia and hypoxia, it is proposed that cerebral oxygenation contributes to the impairment of performance during short all-out efforts performed in a hypoxic environment. Previous studies of RSA have clearly, and on several occasions, identified a correlation between the initial mechanical output and the magnitude of the performance reduction in subsequent sprints (Bishop, et al., 2003a; Mendez-Villanueva, et al., 2007b; Racinais, et al., 2007; Racinais, Connes, Bishop, Blonc, & Hue, 2005). Therefore, the initial mechanical output developed by the subject is a strong confounding factor when interpreting fatigue occurrence in this type of exercise. Differences in initial peak power and mechanical work production elicit a corresponding effect of metabolic and oxidative stress on the nervous and musculoskeletal systems (Mendez-Villanueva, et al., 2007b). Consequently, when investigating men versus women ability to perform repeated sprints, this difference in the initial power output should be controlled. In the studies conducted in this thesis, accurate matching was performed for each condition and sex on the basis of

mechanical work relative to lean body mass performed during the first sprint. In Study 2, men and women exhibited similar ($P > 0.05$) mechanical output in the first sprint in each condition, which demonstrates the effectiveness of our matching method. The similar patterns of performance in men and women observed in both normoxia and hypoxia conditions (Fig. 9) support the idea that equally-matched men and women exhibit similar fatigue patterns. The lack of a sex difference in the physiological parameters (see sections below) also corroborates the thought that our equally-matched-men and women displayed similar physiological perturbations during RSE.

4.2 Consideration for future research

The combined results from Study1 and 2 demonstrate that RSA is not likely to be limited by cerebral oxygenation in normoxia, however, alterations in cerebral oxygenation may contribute to performance impairment in hypoxia. Furthermore, the effect of hypoxia on RSA appears to be similar in men and women, despite a possibly higher efficiency in oxygen utilisation by the muscle in women. Although the hypoxic paradigm did not induce or reduce sex-related differences in RSA in our study, it may be possible that longer-duration exhaustive exercise may be a more relevant method to quantify oxygen-related sex differences during exercise (Russ and Kent-Braun 2003) and further comprehend the role of oxygen uptake in fatigue. Alternatively, it may be possible that the NIRS technique employed by researchers to quantify tissue oxygenation is not sensitive enough to identify the subtle adjustments of the oxidative metabolism in women.

Finally, the negative consequences of reduced oxygenation of the brain neurons have also been identified in terms of compromised fine psychomotor skills and cognitive ability (Amann & Kayser, 2009; Leon-Carrion, et al., 2008). The implications of these findings for team-sport performance in both male and female athletes warrant further research. Now that cerebral oxygenation has been identified as a determinant of RSA, the next logical step of this research

would be to investigate avenues to delay or attenuate the decline in brain oxygenation during a game played at altitude. For example, the effects of fatigue on skilled-task performance are not well described in the current literature, and it is not known whether this ability could be affected by an exercise-induced brain deoxygenation. Furthermore, the current results highlight those found by others investigating the benefits of intermittent hypoxic conditioning on arterial O_2 desaturation while exercising at a given altitude (Ainslie, Hamlin, Hellemans, Rasmussen, & Ogoh, 2008; Hamlin, Marshall, Hellemans, Ainslie, & Anglem, 2009). A maintenance of arterial O_2 saturation may delay cerebral deoxygenation during games played in a hypoxic environment and as such improve exercise performance. This exciting research, which could benefit coaches and athletes, has yet to be conducted.

-Chapter 5-

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