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The effect of muscle fatigue and damage on endurance performance

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THE EFFECT OF MUSCLE FATIGUE AND DAMAGE ON ENDURANCE PERFORMANCE

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Thesis submitted for the Degree of Doctor of Philosophy of
Bangor University

School of Sport, Health and Exercise Sciences
Bangor University

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SUMMARY

Locomotor muscle fatigue develops during endurance events and it is traditionally thought to limit performance despite no direct experimental evidence in favour of this hypothesis. Similarly, muscle damage is known to limiting strength and power performance, but there was no evidence that it can reduce endurance exercise performance. The aims of this thesis are to investigate the effects of locomotor muscle fatigue and damage on endurance performance and the physiological and perceptual response to exercise.

In the first study, we tested the hypothesis that exercise-induced muscle damage can decrease performance during a 30 min running time trial. A significant relative 4%decrease in running performance was showed in subjects with exercise-induced muscle damage.

In the second study locomotor muscle fatigue was induced with an eccentric exercise protocol to test its detrimental effect on time to exhaustion during high-intensity constant-power cycling independently of metabolic stress. A significant 15% drop in lower limb strength (compared to baseline) determined a shortening of the time to exhaustion from 750 ± 281 s to 636 ± 278 s.

The third study provides a more in depth analysis of the effects of reduced locomotor muscle force *per se* on the physiological and perceptual response to high intensity endurance exercise. Locomotor muscle fatigue entailed an increase in heart rate, breathing frequency, and perceived exertion despite no change in the metabolic requirements.

The same exercise protocol was used in the fourth study to investigate effects of locomotor muscle fatigue on incremental exercise performance and physiological/perceptual responses during cycling at different intensities. Although no changes occurred in the physiological responses to incremental exercise, the 13% decrease in locomotor muscle force determined a 2% decrease in the peak power output (an overall difference of 21 W) and a significant increase in the perceived leg effort.

Overall, our findings show that both locomotor muscle fatigue and damage significantly and reliably impair endurance exercise performance between 10 and 30 minutes. However, the effects of increased central motor command to weaker locomotor muscles on cardiorespiratory responses seem to be dependent upon the degree of strength loss and the intensity of exercise. Finally, perception of effort is augmented by locomotor muscle fatigue and damage, and provides the most plausible explanation for the curtailment of endurance performance.

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CHAPTER 1

GENERAL INTRODUCTION

Aims of the research program

The primary aim of our research program was to investigate the effect of muscle fatigue and damage on endurance exercise performance. Because endurance exercise performance is considered a complex phenomenon determined by several physiological and psychological factors (Shepard R, 1988) the secondary aim was to look into the effects of muscle fatigue and damage on the cardiovascular, respiratory, metabolic and perceptual response to endurance exercise.

Operational definitions

Muscle fatigue, damage and endurance performance are conceptualised and measured in many different ways in the literature, and this can often generate confusion or misunderstanding. For the purpose of this thesis, **muscle fatigue** is operationally defined as any acute exercise-induced reduction in maximal voluntary force (Gandevia, 2001) whilst **muscle damage** refers to the delayed effects of eccentric exercise including reduction in maximal voluntary contraction, delayed onset muscle soreness (DOMS), increased blood creatine kinase (CK) concentration, and muscle swelling (Warren et al., 1999, Clarkson and Hubal, 2002).

Endurance performance was measured as distance covered during a fixed-duration time trial, time to exhaustion test at a fixed workload, peak power output (PPO) during an incremental exercise test to exhaustion. On average, exercise duration ranged between 10 and 30 minutes. Therefore, this can be defined as moderate to intermediate length endurance performance(Powers S, 2001).

Muscle Fatigue

The following are thought to be the major potential sites of muscle dysfunction induced by exercise (Bigland-Ritchie, 1981) (Figure 1):

1) Central mechanisms
- excitatory input to higher motor centres
- excitatory drive to lower motor neurons
- motor neuron excitability
- neuromuscular transmission

2) Peripheral mechanisms
- Sarcolemma excitability
- Excitation-contraction coupling
- Contractile mechanism
- Metabolic energy supply/metabolic accumulation

The complex decision-making processes that provide excitatory input to higher motor centres should be highlighted (site A, Figure 1). In muscle fatigue studies, this important factor is controlled for by asking and motivating a subject to perform a maximal effort (Gandevia, 2001). However, during exercise that requires submaximal muscle contractions such as endurance performance tests, effort-related decision-making processes are likely to be the most important factor limiting performance. Thus, the motivational factors that influence effort-related decision-making processes should be taken into account (see section *"Motivational factors in endurance exercise performance"*). Exercise physiologists often underestimate these aspects. In fact, when whole body exercise is considered, the first ring of the chain of command for muscular contraction is represented by the "Psyche" (Figure 2) and the positive influence of motivational factors (such as a competitive situation) on endurance performance has been shown (Wilmore, 1968).

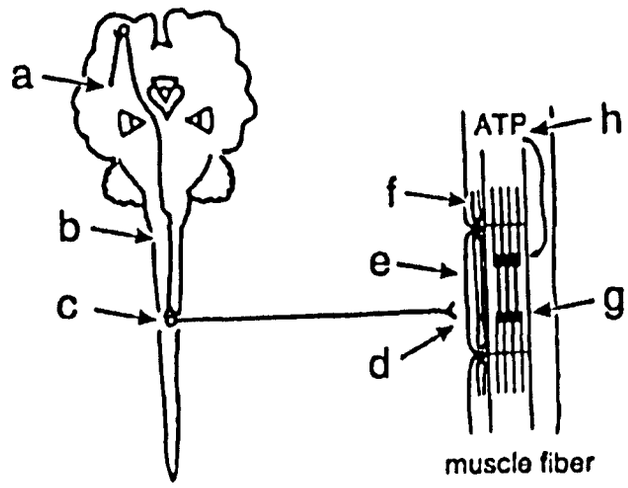

Figure 1.

Potential sites of fatigue (a) excitatory input to the motor cortex, (b) excitatory drive to lower motoneuron, (c) motoneuron excitability, (d) neuromuscular transmission, (e) sarcolemma excitability, (f) excitation-contraction coupling, (g) contractile mechanism, (h) metabolic energy supply. From (Bigland-Ritchie, 1981)

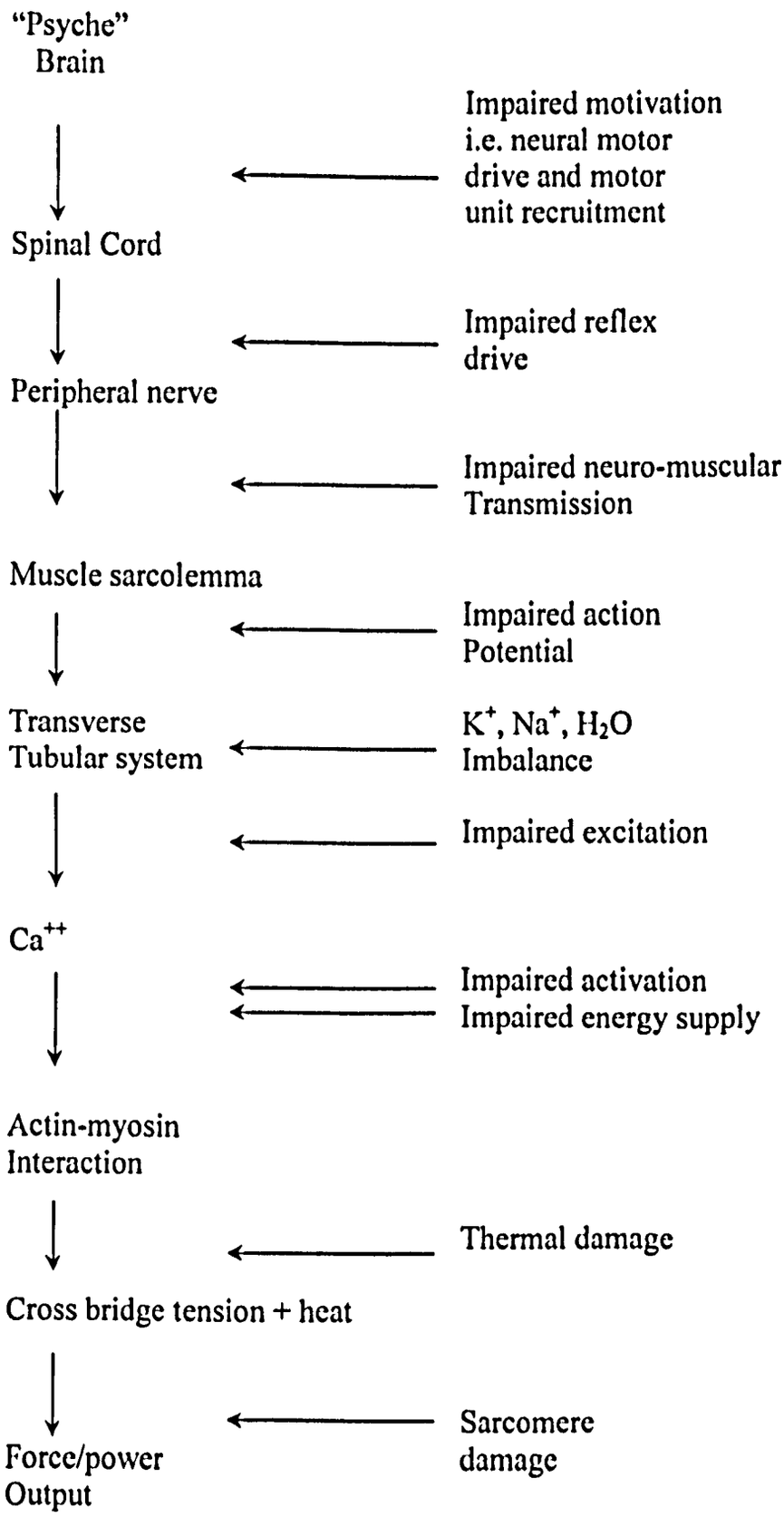
"Psyche"
Brain
Impaired motivation
i.e. neural motor
drive and motor
unit recruitment
Spinal Cord
Impaired reflex
drive
Peripheral nerve
Impaired neuro-muscular
Transmission
Muscle sarcolemma
Impaired action
Potential
Transverse
Tubular system
K+, Na+, H2O
Imbalance
Impaired excitation
Ca++
Impaired activation
Impaired energy supply
Actin-myosin
Interaction
Thermal damage
Cross bridge tension + heat
Sarcomere
damage
Force/power
Output

Figure 2.

Figure (previous page). Chain of command for muscular contraction and the possible mechanisms underlying fatigue. "Psyche" is highlighted because the first ring of this chain. Adapted from (Edwards, 1983a).

Central (Gandevia, 2001, Taylor and Gandevia, 2008) and peripheral (Allen et al., 2008, Fitts, 1994, Fitts, 2006, Fitts, 2008) aspects can be the cause of fatigue either separately or combined together. In the schema in figure 2 the possible mechanisms underlying fatigue at the different sites are summarised.

Central fatigue occurs when the Central Nervous System (CNS) can not voluntarily recruit maximally all the motor units to produce force and this is attributable to failure of processes at spinal and/or supraspinal level (Gandevia, 2001, Taylor and Gandevia, 2008). To test whether fatigue is localised at supraspinal or spinal level, potentiated twitch force is assessed respectively via transcranial magnetic stimulation or using the twitch interpolation technique. In fact, when a superimposed twitch can elicit a further increase in force during a maximal voluntary contraction, central fatigue is present. What are thought to be the possible causes of central fatigue can be summarized as: a disturbances in brain neurotransmitters (Davis and Bailey, 1997, Meeusen et al., 2006), depletion of brain glycogen (Dalsgaard et al., 2002), increase in core and brain temperature (Nybo and Secher, 2004), inhibitory supraspinal reflexes originating from fatigued respiratory muscles (Kayser, 2003), input from different classes of muscle receptors acting at motoneuronal level (Gandevia, 2001), and more recently it has been hypothesized that even inhibitory sensory feedback from fatigued locomotor muscles can decrease central neural drive during submaximal dynamic contraction (Amann and Dempsey, 2008b).

In spite of the certainty reached upon the supraspinal and spinal mechanisms of fatigue during maximal static contraction and the

inhibitory function of afferent inputs from contracting muscles and changes in the intrinsic properties of the motoneurons, more complex and less understood issues play a role during submaximal static and dynamic exercises (Taylor and Gandevia, 2008).

On the other hand, peripheral fatigue is the intrinsic inability of the skeletal muscle to produce force. Indeed, the capacity to produce maximal force is not limited by a failure of the CNS but rather owing to changes that occur in the muscle cells. The main causes of peripheral fatigue can be ascribed to biochemical changes due to exercise (such as decrease in pH, increase in Pi and Lactate), occurring in the muscle and responsible for the failure in excitation-contraction (E-C) coupling (Fitts, 1994, Fitts, 2008). Furthermore, in a recent review on the cellular mechanisms of muscle fatigue, it has been highlighted the possible role that changes in muscle fibre excitability, Ca^{2+} sensitivity and sarcoplasmic reticulum Ca^{2+} release, and production of reactive oxygen species (ROS) can have in the development of peripheral fatigue(Allen et al., 2008).
Moreover, eccentric exercise, especially when unaccustomed, can determine functional and structural changes in muscle fibres (Allen, 2001) that causes muscle fatigue. This is clearly showed in animal models (Warren et al., 2001). Indeed, the mechanisms determining the strength loss after eccentric exercise can be grouped in three main categories: 1) physical disruption/alteration of force-generating/transmitting structures, 2) failure to activate force-generating structures, and 3) loss of force-generating/transmitting structures. For our purposes categories 2), in particular a failure in Excitation-Contraction coupling (E-C coupling), and 1), physical disruption, are the mechanisms involved in determining the reduction of force immediately and up to 2 days after eccentric contractions. This evidence is supported also by the occurrence of low frequency fatigue (LFF), defined as long-lasting form of reduction in force at low frequencies of stimulation (Edwards et al., 1977), and indeed caused by a failure of E-C coupling (Edwards et al., 1977).

Muscle damage

Despite the study by Hough in 1902 represents one of the first studies on muscle damage, only during the last three decades there has been an increasing interest on this phenomenon (Clarkson and Hubal, 2002).

It is well known that eccentric contractions, especially when unaccustomed, can cause exercise-induced muscle damage. This phenomenon is characterized by structural/morphological and functional changes in the muscle fibres evidenced mainly by myofibrillar disruptions (Figure 3) and reduced strength caused by E-C coupling failure (Figure 5) (Warren et al., 2001). Additionally, a loss of protein begins few days after the eccentric contractions (Warren et al., 2001). The fast twitch fibres are the most prone to the changes (Proske and Morgan, 2001).

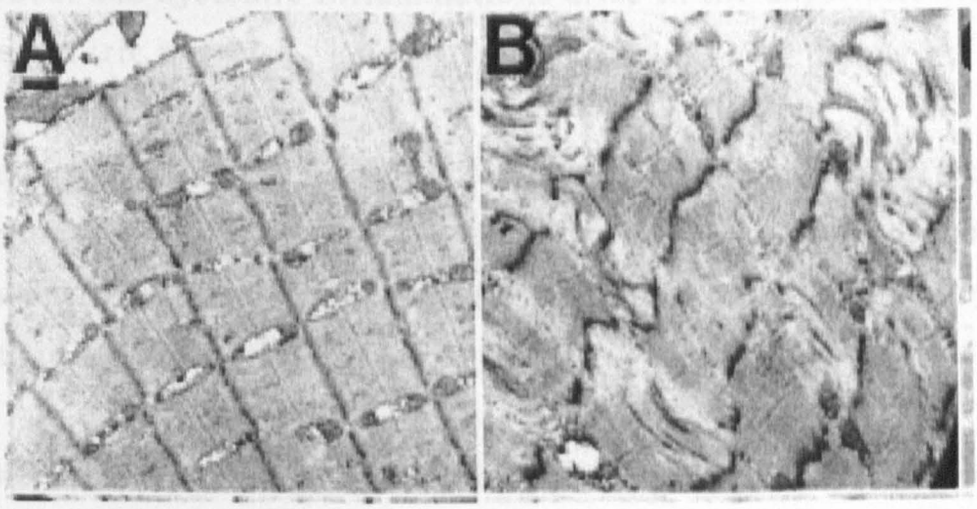

Figure 3

Longitudinal sections of human vastus lateralis before (A) and 2 (B) days 10 sets of 10 repetitions of eccentric quadriceps exercise. Note in B the myofilament disorganization and extensive damage. Magnification ×10,000 in all panels. Calibration bar in A is 500 nm and refers to all panels. From (Hortobagyi et al., 1998)

The causes of the disruption occurring to the muscle are not completely clear and some hypotheses have been postulated. The most

likely are the popping sarcomere hypothesis (Morgan and Proske, 2004) and the calpain hypothesis (Belcastro et al., 1998).
In addition to the decreased muscle strength, exercise-induced muscle damage is accompanied by typical signs that start to develop during the 24 hours following the eccentric contractions (Clarkson and Hubal, 2002, Peake et al., 2005, Proske and Morgan, 2001, Vickers, 2001, Warren et al., 2001) and can be summarised as follows (see also figures 6 and 7):

- DOMS that begins usually 24 hours after the eccentric exercise and peaks usually within 48 hours;
- muscle swelling that occurs mainly to muscles that rarely undergo several eccentric contractions (i.e. biceps of the upper limb compared to quadriceps of the lower limb);
- higher than normal CK concentration is present in the blood;
- local and systemic inflammations arise in the days following eccentric exercise.

For the purposes of this study we decided to investigate the effect of muscle damage 48 hours after the eccentric exercise when soreness is usually at its peak and the decreased strength is still significantly lower than baseline values.

A further functional alteration that occurs in presence of exercise-induced muscle damage is a shift in optimum length after eccentric contractions (Morgan and Allen, 1999, Proske and Morgan, 2001). As shown in figure 4 , the peak tension seems not to be affected but it occurs at longer lengths when the muscle is damaged.

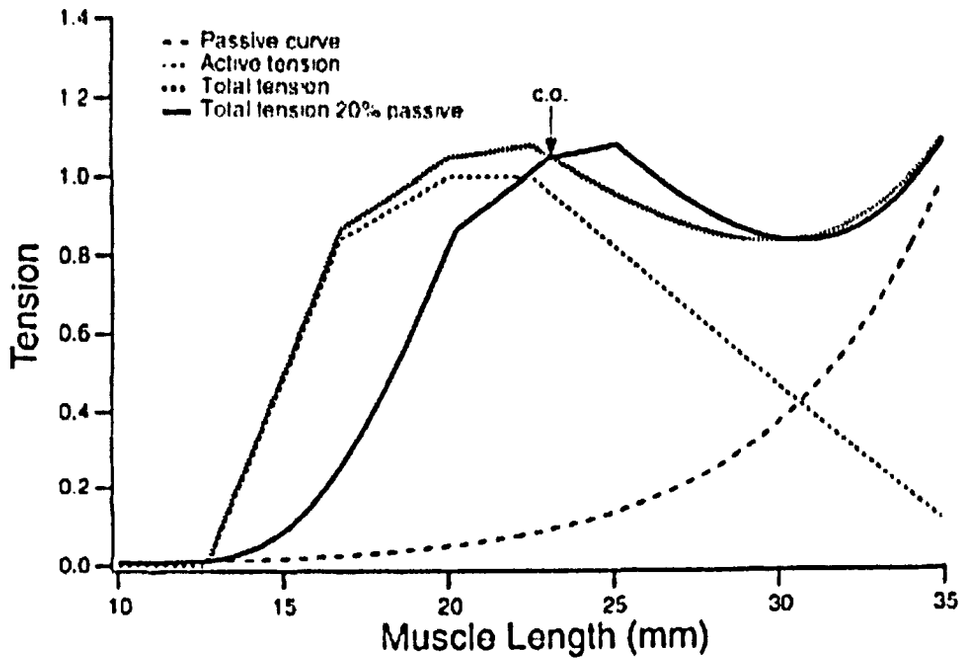

Figure 4

Model of a muscle with some sarcomeres disrupted. Length-tension curves for a simulated muscle of 10,000 sarcomeres/fiber. Note that the peak tension is unaffected but that at short lengths passive sarcomeres reduced tension, whereas at longer lengths they caused increased tension; i.e., there was a crossover (c.o.) of the total tension and the total tension, 20% passive curves.

Muscle fatigue and damage after eccentric exercise

Eccentric exercise refers to active contractions whilst the muscle is lengthening, and we used it as experimental treatment to induce both muscle fatigue and damage. The distinction is based on some differences in the mechanisms causing loss of muscle strength, and on the presence of well-known signs and symptoms of muscle damage. The reduction in force is the common aspect of muscle fatigue and damage and is caused by any change/failure occurring in the chain of events leading to muscular contraction (Edwards, 1983a).

The peripheral mechanisms underlying the negative effect of eccentric exercise on muscle strength are well known, and they are represented in Figure 5 (Warren et al., 2001). In summary, in the first 5 days after injury, Excitation-Contraction coupling (E-C coupling) failure is thought to account for most of the strength loss with an additional contribution from disruption at sarcomere level. By day 5 after injury, the contribution of E-C coupling failure is diminishing, and a gradual loss of contractile protein accounts for an increasingly larger proportion of the strength loss. The force loss still measurable two weeks after injury is entirely due to decreased contractile protein content.

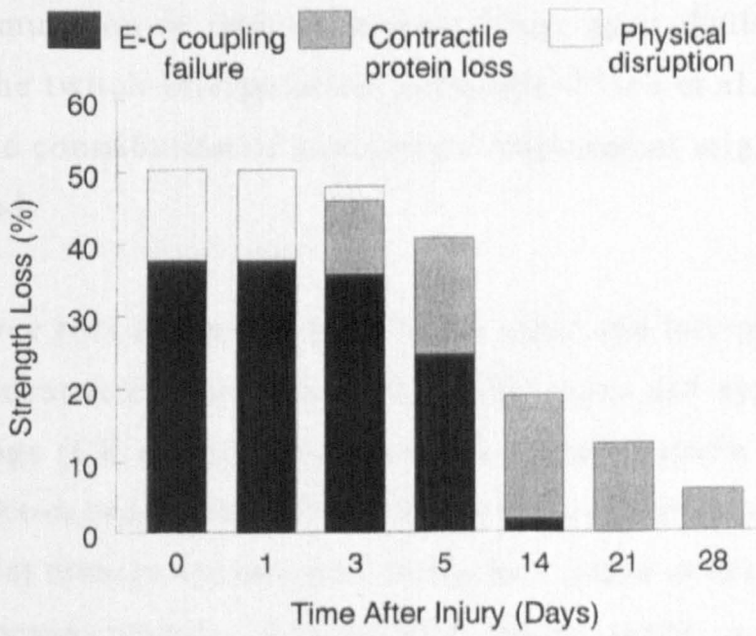

Figure 5

Estimated contributions of E-C coupling failure, decreased contractile protein content, and physical disruptions and/or alteration of force-bearing elements to the strength loss observed after muscle damaging exercise. From.(Warren et al., 2001)

In line with our operational definition of muscle fatigue, the E-C coupling failure observed in the first few hours after eccentric exercise has been considered a form of low-frequency fatigue (LFF). This type of muscle fatigue is defined as a long-lasting reduction in force at low frequencies of stimulation (Edwards et al., 1977), and it is more pronounced after eccentric compared to isometric or concentric muscle contractions (Rijkelijkhuizen et al., 2003). Importantly, this force loss is more relevant to submaximal tasks such as endurance exercise when stimulation frequency is low (Jones, 1996).

Recent studies by Gandevia and colleagues (Prasartwuth et al., 2005) have also demonstrated some impairment of central mechanisms after eccentric exercise using trans-cranial magnetic stimulation and the twitch interpolation technique. Central mechanisms seem to contribute to 19% of force loss after eccentric exercise. However, recent studies of electrostimulation on isolated muscle fibers have challenged the validity of the twitch-interpolation technique (Place et al., 2008b). Therefore the contribution of supraspinal impairment might have been overestimated.

Although force loss is present both in the early and late phase following eccentric exercise, the two typical signs and symptoms of muscle damage (CK and DOMS) present a typical pattern (Figures 6 and 7) (Clarkson and Hubal, 2002). Early losses in muscle function are "silent" whilst muscle dysfunction in the late phase is accompanied by DOMS that begins usually 24 hours after the eccentric exercise and peaks usually within 48-72 hours. Higher than normal CK concentration is present in the blood 24-72 hours after the eccentric exercise up to 7 or more days. The presence of these two signs and symptoms of muscle damage is one of the main factors differentiating muscle damage from muscle fatigue in our studies.

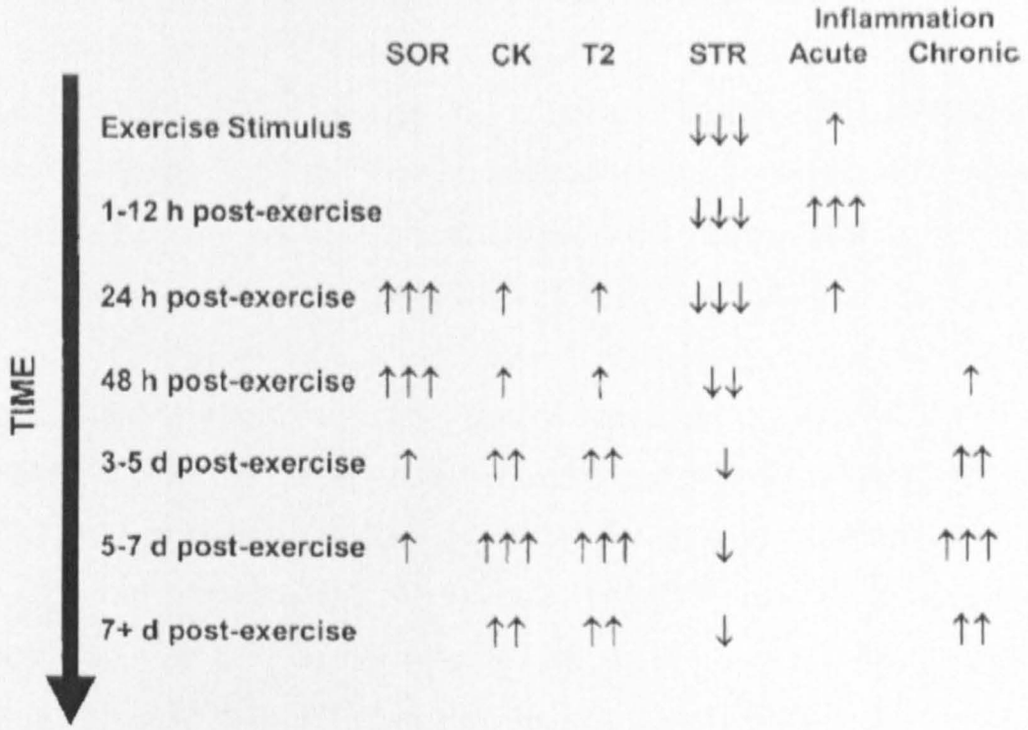

Figure 6

Time course of changes after maximal eccentric exercise. *One arrow,* minor increase/decrease; *two arrow,* moderate increase/decrease; *three arrow,* large increase/decrease. SOR, soreness; CK, creatine kinase; T2, signal intensity via magnetic resonance imaging; STR, strength. From (Clarkson and Hubal, 2002).

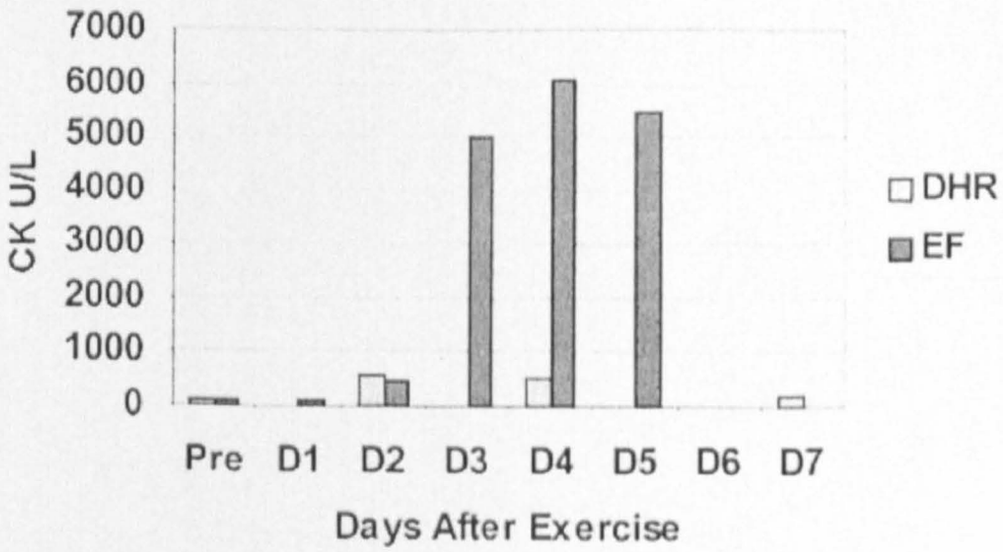

Figure 7

Plasma creatine kinase (CK) activity after downhill running (DHR) and elbow flexion maximal eccentric contractions (EF).
From (Clarkson and Hubal, 2002).

Muscle fatigue and damage after endurance exercise

There is plenty of evidence that muscle fatigue occurs during and after many different types of endurance exercise (Amann and Dempsey, 2008b, Millet and Lepers, 2004, Sargeant, 2007). Prolonged running, cycling, and cross country skiing of different duration (from 30 minutes to several hours) and intensities (from 55 to 80% of VO2max) can induce strength losses of the locomotor muscles (Millet and Lepers, 2004). Force, assessed during maximal voluntary contractions performed within a few minutes after the end of endurance tasks both in race and laboratory conditions, is reduced within a range that varies from 8 to 34% of pre-exercise values. Other authors (Amann and Dempsey, 2008b, Amann et al., 2008c, Romer et al., 2007a) showed that also cycling exercises of shorter duration and higher intensities (more than 90% of VO2 max) performed until exhaustion or as time trials can induce a strength loss up to the 14% of pre-exercise values. Table 1 summarizes all the studies in which strength losses were quantified after a bout of endurance exercise. As reviewed by Millet and Lepers (Millet and Lepers, 2004), fatigue at the end of the endurance exercises may be central and/or peripheral in origin.

Table 1

Decrease of maximal knee extensor muscles force after different endurance exercises/activities. Adapted and updated from (Millet and Lepers, 2004).

References	Exercise	Time	Intensity/ Distance	MVIC loss (%)
Davies & Thompson (1986)	Running - L	240 min	65%VO_2max	25
Nicol et al. (1991)	Running - R		42 km	26
Glace et al. (1998)	Running - L	120 min	68% VO_2max	18
Lepers et al. (2000)	Running - L	120 min	28 km	19
Millet et al. (2002)	Running - R	511 min	65 km	30
Millet et al. (2003)	Running - R	188 min	30 km	24
Place et al. (2004)	Running - L	300 min	52 km-55%VO_2max	28
Gauche et (2006)	Running - R	413 min	55 km	37
Petersen et al (2007)	Running - R	154 min	42 km	22
Sahlin & Seger (1995)	Cycling - L	85 min	75%VO_2max *	34
Booth et al. (1997)	Cycling - L	72 min	75%VO_2max *	28
Bentley et al. (2000)	Cycling - L	30 min	80%VO2max	13
Lepers et al. (2000)	Cycling - L	120 min	70%VO_2max	13
Lepers et al. (2001)	Cycling - L	30 min	80%VO_2max	13
Lepers et al. (2002)	Cycling - L	300 min	55%VO_2max	18
Millet et al. (2002)	Cycling - R	280 min	140 km	9
Sandiford et al. (2004)	Cycling - L	90 min	50%VO_2max	21
Leppik et al. (2004)	Cycling - L	72 min	74%VO_2max*	26
Sarre et al. (2005)	Cycling - L	60 min	65%VO_2max	14
Vallier et al. (2005)	Cycling - L	180 min	60%VO_2max	16
Presland et al. (2005)	Cycling - L	69 min	70%VO_2max *	29
Sandiford et al. (2005)	Cycling - L		Incremental exercise *	12
Romer (2006)	Cycling - L	13.4 min**	> 90% VO_2max*	24***
Romer (2006)	Cycling - L	13.2 min**	92% VO_2max*	35***
Amann (2006)	Cycling - L		5 Km	14
Amann (2006)	Cycling - L	8.1 min**	> 90% VO_2max*	9
Romer (2007)	Cycling - L	13.2 min**	> 90% VO_2max*	28***
Amann (2007)	Cycling - L	11 min	81% PPO*	14
Amann (2008)	Cycling - L	10 min	83% PPO*	10
Lepers et al. (2008)	Cycling - L	30 min	75% VO_2max*	11
Theurel & Lepers (2008)	Cycling - L	30 min	Variable	12
Viitasalo et al. (1982)	X-C skiing - R	330 min	85 km [$]	10
Millet et al. (2003)	X-C skiing - R	160 min	42 km	8

* Exercise performed until exhaustion , ** Average time, *** Maximal force assessed by nerve stimulation [$] Strength losses were measured 1-2 h after the race was finished.R : race condition, L : laboratory condition, PPO : Peak Power Output. MVIC: maximal voluntary isometric contraction. X-C: cross country.

Further evidence regarding the role of endurance exercise in contributing to the development of muscle fatigue, comes from investigations in which the ability of the muscles to produce power has been examined after a bout of endurance cycling exercise (Beelen and Sargeant, 1991, Sargeant and Dolan, 1987, Sargeant, 2007). Indeed, the maximal power that can be generated at the cycle ergometer during a 20-second Short Term Power Output was progressively reduced when assessed immediately after high intensity cycling (Sargeant and Dolan, 1987). Bouts of different durations (30 sec, 1 min and 3 min) performed at 98% $\dot{V}O_2max$ determined a reduction in the maximal power up to 33% of baseline. Interestingly, when high intensity cycling was executed for 6 minutes there was only an additional 5% loss in maximal power. On the contrary, in the same study had been shown that a minimum of 6 min cycling above 70-80% of $\dot{V}O_2max$ was necessary to elicit a significant drop in PPO (Sargeant and Dolan, 1987). Furthermore, in a different study (Beelen and Sargeant, 1991) a significant 25% reduction in maximal power at 120 RPM was observed after a fatiguing protocol of 6 min cycling at 90% of $\dot{V}O_2max$. Interestingly, power loss was less prominent at 90 and 105 RPM, and not significant at 60 RPM. In these studies participants were stopped at a fixed time and were not exhausted. We are not aware of any study that have investigated the level of loss of muscle power-generating capacity at the end of a time to exhaustion trial.

Marathon running is characterized by the repetition of several stretch-shortening cycles (Komi, 2000). Therefore, it is not surprising that high blood CK concentration, injury to muscle fibres, decreased muscle strength and DOMS have been found at the end of marathon and ultra-marathon runs (Armstrong, 1986, Clarkson and Hubal, 2002). These findings clearly demonstrate the occurrence of significant muscle damage after prolonged running exercise.

Furthermore, there is experimental evidence showing that a distance longer than 10 km is very likely required to produce muscle damage (Overgaard et al., 2004). In fact, a significant increase in intracellular Ca^{2+} content, which is considered an initiator in the development of muscle damage (Armstrong, 1986), has been found in some active subjects after a 10 km run.

Perception of effort

The perceptual aspect of muscle fatigue is represented by the disproportionate increase in perceived effort (when a target force is maintained) (Taylor and Gandevia, 2008) but also by its possible role in the impairment in performance (Enoka and Stuart, 1992).
Therefore, it seems useful for the purposes of this Thesis to briefly address the main aspects and determinants of perception of effort.

Perception of effort, also known as perceived exertion or sense of effort, refers to the conscious sensation of how hard, heavy, and strenuous a physical task is. This perception depends mainly on feelings of effort in the active limbs, and the sensation of heavy breathing (respiratory effort) (Borg, 1998a, Noble and Robertson, 1996).
The most common instruments used to measure perception of effort are the ratings of perceived exertion scale (RPE) and the category-ratio scale (CR-10) (Borg, 1998a, Noble and Robertson, 1996).

A well established aspect is that perceived exertion increases as workload increases and reflects the relative exercise intensity which depends on the absolute workload and individual exercise capacity. Furthermore, perceived exertion is known to increase over time during exercise at a fixed workload and this phenomenon represents a clear symptom of fatigue.(Borg, 1998a, Noble and Robertson, 1996, Enoka and Stuart, 1992, Taylor and Gandevia, 2008).

Many other physiological factors can affect perceived exertion: the most common are represented by nutritional (e.g. muscle glycogen, caffeine), environmental (e.g. altitude, ambient temperature) factors. A not trivial role is also played by pain (muscular, at the joints or tendons) that may be experienced throughout the exercise. Moreover, psycho-social factors such as personality, mood, somatic perception, locus of control, self efficacy, or even the presence and gender of another person have an effect on perceived exertion (Borg, 1998a, Noble and Robertson, 1996).

The neurophysiology of perceived exertion can be helpful to answer the question about why a person experiences the subjective feeling of effort.
The significant correlations between RPE and several markers of physiological strain during exercise support the assumption that perception of effort results from the integration of different afferent sensory inputs (e.g. proprioception, pain, thermal discomfort, etc) to the CNS (Noble and Robertson, 1996). However, correlation may not represent a cause-and-effect relationship and any other variable that increases with workload might be related to RPE. Indeed, interventions (such as epidural anaesthesia) aimed to block the afferent inputs (from muscle spindles and Golgi tendon organs, or type III and IV afferent fibres) to the CNS do not have an effect on RPE (Fernandes et al., 1990, Kjaer et al., 1999).
A more likely explanation is that perception of effort is centrally-generated by forwarding neural signals (corollary discharges) from motor to sensory areas of the cerebral cortex. The magnitude of the central command to the active muscles (locomotor and respiratory muscles) is perceived as effort (Enoka and Stuart, 1992, Noble and Robertson, 1996, Gandevia and McCloskey, 1977). This view is supported by experimental studies in which peripheral neuromuscular function is reduced (e.g. using curare) (Galbo et al., 1987, Gallagher et al., 2001, Innes et al., 1992). In this conditions the higher perceived

effort is thought to reflect the increased central neural drive necessary to exercise at the same workload with weaker muscles.

Effects of MUSCLE FATIGUE on physiological and perceptual responses to endurance exercise

The effects of muscle fatigue on physiological and perceptual responses to endurance exercise have never been thoroughly investigated before. Neuromuscular blocking agents such as curare (that can partly paralyze the working muscles) have been used to study the role of central motor command in the regulation of cardiorespiratory responses to aerobic exercise (Asmussen et al., 1965, Galbo et al., 1987, Gallagher et al., 2001). Significant changes in heart rate (HR), blood pressure regulation, ventilation and ratings of perceived exertion (RPE) have been observed after curarization (Asmussen et al., 1965, Galbo et al., 1987, Gallagher et al., 2001). However, neuromuscular blockade can not be used a valid model of locomotor muscle fatigue for several reasons. First of all, curare is a systemic drug which causes weakness in all skeletal muscles including respiratory muscles (Johansen et al., 1964). This "side effect" of curarization can have a significant effect on respiratory pattern as demonstrated by respiratory muscle fatigue studies (Romer and Polkey, 2008). Furthermore, even mild curarization causes severe strength losses (50% or more) and can not be assumed that the smaller dysfunction caused by naturally-occurring muscle fatigue would have the same significant effect on the cardiorespiratory and perceptual responses to endurance exercise. Finally, contrary to muscle fatigue, neuromuscular blockades affect primarily type I fibres, and the compensatory recruitment of type II fibres can result in higher metabolic stress and increased oxygen uptake due to a different exercise pattern (Asmussen et al., 1965, Galbo et al., 1987).

Another agent used to understand the cardiorespiratory responses to aerobic exercise is epidural anaesthesia (Fernandes et al., 1990, Innes et al., 1992, Kjaer et al., 1999, Kjaer et al., 1996). This experimental treatment has the advantage to induce strength loss only in the locomotor muscles. However, its main effect is to block sensory inputs from the working musculature. Because afferent feedback from locomotor muscles is known to alter cardiorespiratory regulation during exercise (Waldrop, 1996), this effect makes epidural anaesthesia completely useless as a model of muscle fatigue.

More recently, Amann and Dempsey have used exhaustive cycling at a fixed power output to induce locomotor muscle fatigue(Amann and Dempsey, 2008b). Again, such experimental treatment does not permit proper assessment of the effects of reduced locomotor muscle force *per se* on the cardiorespiratory and perceptual responses to aerobic exercise. In fact, exhaustive exercise significantly disturbs both muscle and blood biochemistry with consequent increased afferent feedback driving changes in cardiovascular and ventilatory responses (Waldrop, 1996). Furthermore, exhaustive exercise is likely to induce respiratory muscle fatigue, another confounding factor when investigating the physiological and perceptual responses to endurance exercise. In fact, respiratory muscle fatigue can alter breathing pattern and the sensation of dyspnea (Romer and Polkey, 2008).The choice of using time trials to measure performance after treatment also makes the assessment of physiological and perceptual responses more problematic (Amann et al., 2008a).

After considering all of the above shortcomings of previous methods, we decided to induce locomotor muscle fatigue using the eccentric exercise protocol developed by some authors (Skurvydas et al., 2000). This consists of 100 drop jumps from a 40 cm high platform down to 90° knee angle before jumping upward as high as possible. Between each drop jump a 20 s rest period is observed to allow for recovery, through oxidative phosphorylation, of the ATP and phosphocreatine

expended during each drop jump. As expected, this protocol induces significant LFF and reduced maximal voluntary strength. This is primarily due to impaired calcium release from the sarcoplasmic reticulum (Allen, 2001, Warren et al., 2001) with no concomitant accumulation of fatigue-related metabolites (Nielsen et al., 2005). This is an important feature of this experimental treatment because it enabled us to isolate the physiological and perceptual effects of reduced locomotor muscle force *per se* without the confounding effects of increased afferent feedback from type III and IV muscle afferents. In pilot studies we have also established that, as expected, this protocol does not induce respiratory muscle fatigue.

Effects of MUSCLE DAMAGE on physiological and perceptual responses to endurance exercise

The effects of muscle damage on the physiological responses to endurance exercise are better known than those of fatigue. However, some discrepancies exist in the literature. Some authors (Gleeson et al., 1998, Gleeson et al., 1995) studied the effects of DOMS caused by bench stepping on the cardio-respiratory and metabolic response to 15 min of submaximal cycling at 80% of $\dot{V}O_2max$. Except for no change in oxygen uptake, other parameters such as ventilation, breathing frequency, $\dot{V}_E/\dot{V}O_2$, heart rate, blood lactate and cortisol were significantly higher 48 hours after exercise-induced muscle damage (EIMD) when compared to baseline measure(Gleeson et al., 1995). In a different study (Gleeson et al., 1998), the same authors showed that during an incremental exercise to exhaustion on a cycle ergometer there were no significant differences in $\dot{V}O_2$, $\dot{V}e$, heart rate, and respiratory exchange ratio (RER) when the response to exercise performed 48 hours after EIMD was compared to baseline. The only difference detected was a higher lactate in EIMD group both during

exercise and two minutes after exhaustion. A similar peak heart rate and $\dot{V}O_2$ were detected during the incremental exercise.
As for cycling, contrasting results have been found in studies using running as endurance exercise. Muscle damage caused by downhill running gave different responses: Hamill et al. (Hamill J, 1991) found similar VO_2 and HR comparing baseline to running exercise at 80% of $\dot{V}O_2$ peak 48 hours after damage. These results contrast with those of Braun and Dutto (Braun and Dutto, 2003) and Chen et al. (Chen et al., 2007) who found significant differences in $\dot{V}O_2$, $\dot{V}_E$, RER, HR, and Lactate comparing baseline values to those 48 hours after EIMD during running at different percentages of $\dot{V}O_2$ peak (65, 75, and 85%). When DOMS was induced by series of submaximal resistance exercises (Scott et al., 2003), it did not cause a different response of $\dot{V}O_2$, RER, Lactate to 30 min running at 67% of $\dot{V}O_2$ max 30 hours after EIMD but differences were found for HR. Similarly, muscle damage caused by eccentric maximal voluntary contractions did not affect $\dot{V}O_2$, $\dot{V}_E$, RER, Bf and HR during two different running intensities carried out 24, 48, 77 and 96 hours post EIMD (Paschalis et al., 2005). The only study on the effect of DOMS on physiological responses at the onset of exercise (Hotta et al., 2006) showed a significant increase of Ve despite the same $\dot{V}O_2$, HR and blood pressure, during the first 20 seconds voluntary knee extension exercise both two and seven days after EIMD.

In terms of perceptual responses, Gleeson (Gleeson et al., 1995) showed significant increase of RPE in response to cycling at 80% $\dot{V}O_2$max two days after 30 min bench stepping. On the contrary, 30 min downhill running did not have any effect on RPE during different intensities of submaximal running two days after the damaging protocol, despite significant increased cardio-respiratory responses (Braun and Dutto, 2003). In a similar study, other authors (Chen et al., 2007) failed to detect a significantly different RPE 1, 2 and 3 days after the downhill running. Finally, RPE was found higher than at

baseline during 30 min running at 67% of $\dot{V}O_2max$ 24 hours after a series of submaximal exercise to induce soreness (Scott et al., 2003). Thus, contrasting results exist even for the perceptual response to exercise.

Effects of MUSCLE FATIGUE on endurance exercise performance

The traditional physiological model of endurance performance tested in the laboratory during exercise at constant or progressive workload assumes that, in well-motivated subjects, exercise terminates when the neuromuscular system is no longer able to produce the force/power required, a point known as exhaustion or task failure. For example, when the task consists of pedalling on a cycle ergometer, exhaustion/task failure might be defined as the failure to maintain a minimum cadence. Similarly, exhaustion/task failure can be defined as the inability to produce the target force during a submaximal isometric contraction. In other words, it is assumed that muscle fatigue is the direct cause of exhaustion (Allen et al., 2008, Jones et al., 2008, Sejersted and Sjogaard, 2000). This assumption has been depicted by Allen et al. in the most recent review of muscle fatigue(Allen et al., 2008).

Although this assumption may be valid during isometric exercise requiring force production of > 50% MVC (Bigland-Ritchie and Woods, 1984), we are not aware of any study testing this assumption during endurance exercise. This is not trivial if we consider that aerobic exercise at $\dot{V}O_2max$ requires less than 20% of an isometric MVC (Lollgen et al., 1980), and the evidence showing that during submaximal isometric contractions below 30% of MVC exercise is terminated despite an ample force reserve (Place et al., 2008a, Taylor and Gandevia, 2008) Figure 8. Interestingly, exhaustion during these submaximal isometric contractions is associated with high perception of effort (see section "*Motivational factors in endurance exercise performance*"). Overall, it can not be assumed that locomotor muscle

fatigue reduces endurance exercise performance; this effect needs to be investigated using well-designed experimental studies.

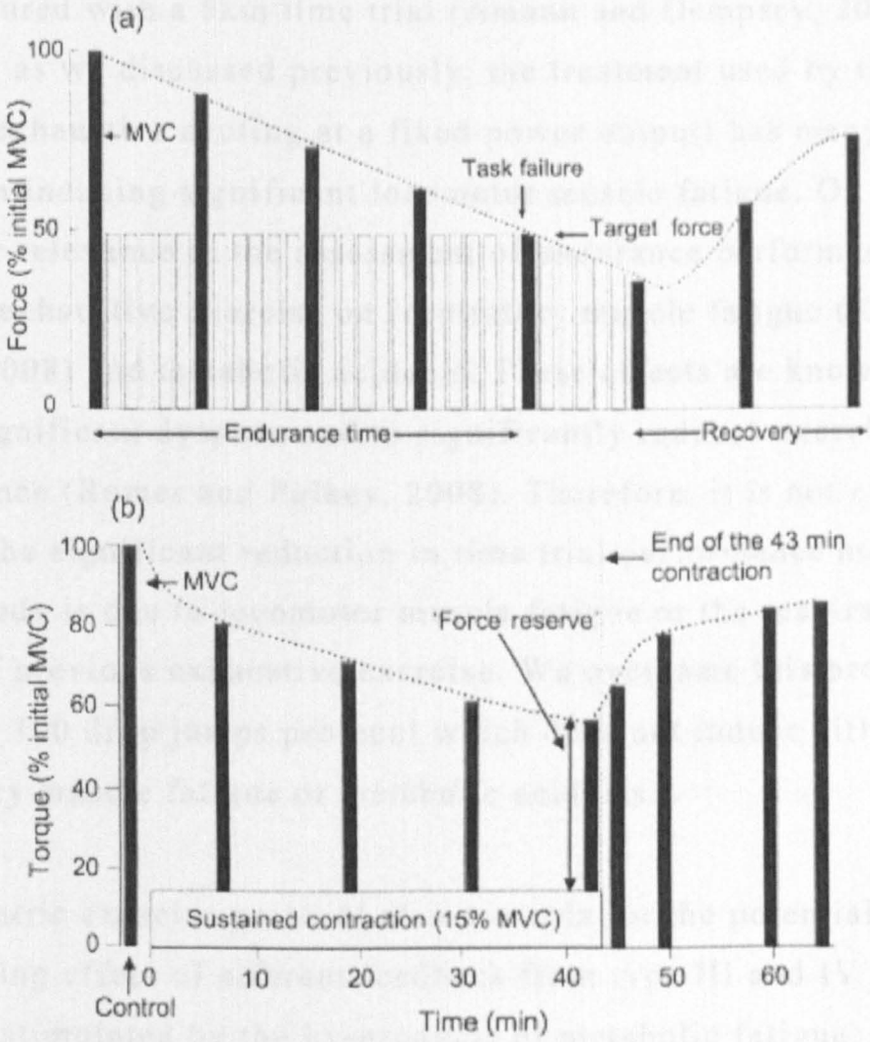

Figure 8

An illustration of the role of central fatigue during fatiguing contractions performed by humans. The repeated intermittent contractions (a) resulted in very limited central fatigue, whereas continuous low intensity contraction (b) induced large neural adaptations. Maximal voluntary contractions (MVC; black bars) were performed during the course of both tasks. The dotted lines indicate the time-course of the decrease in maximal force-generating capacity during exercise. (a) Redrawn with permission from Bigland-Ritchie and Woods; (b) redrawn with permission from Søgaard et al. From (Place et al., 2008a)

Few months before the publication of our 2nd and 3rd studies, Amann and Dempsey published an experimental study on the effect of locomotor muscle fatigue on endurance cycling performance which was measured with a 5km time trial (Amann and Dempsey, 2008b). However, as we discussed previously, the treatment used by these authors (exhaustive cycling at a fixed power output) has many effects other than inducing significant locomotor muscle fatigue. Of particular relevance to the assessment of endurance performance is the effect of exhaustive exercise on respiratory muscle fatigue (Romer and Polkey, 2008) and metabolic acidosis. These effects are known to induce significant dyspnea and to significantly reduces exercise performance (Romer and Polkey, 2008). Therefore, it is not clear whether the significant reduction in time trial performance measured in this study is due to locomotor muscle fatigue or the respiratory effects of previous exhaustive exercise. We overcame this problem by using our 100 drop jumps protocol which does not induce either respiratory muscle fatigue or metabolic acidosis.

Our eccentric exercise protocol also controls for the potential confounding effect of afferent feedback from type III and IV muscle afferents stimulated by the by-products of metabolic fatigue. In fact, the innovative models of endurance performance proposed by Noakes and colleagues (Noakes et al., 2005, St Clair Gibson and Noakes, 2004) and Amann and Dempsey (Amann, 2007, Amann and Dempsey, 2008b, Amann and Dempsey, 2008a, Dempsey et al., 2008) assume that the CNS regulates exercise performance on the basis of sensory afferent feedback from locomotor muscles and other organs. Using the experimental treatment employed by Amann and Dempsey it is not possible to differentiate between the performance effect of this inhibitory afferent feedback and the effect of reduced locomotor muscle force *per se*.

Effects of MUSCLE DAMAGE on endurance exercise performance

Despite one of the first studies on muscle damage had been published in 1902, to date there still is lack of evidence regarding the likely negative effect of exercise-induced muscle damage on endurance exercise performance in humans. Byrne et al.(Byrne et al., 2004) have reviewed the effects of exercise-induced muscle damage on athletic performance and have clearly showed the detrimental effect it has on power-generating ability, vertical jump, and sprinting performance. However, endurance time during an incremental cycling exercise to exhaustion did not seem to be affected by exercise-induced muscle damage (Gleeson et al., 1998).

Different results have been found in more recent studies on the effect of muscle damage on endurance performance in mice. The running time to fatigue after 150 min of downhill or uphill running has been investigated at 24 (Carmichael et al., 2005, Carmichael et al., 2006), 48 (Carmichael et al., 2005, Davis et al., 2007) and 72 (Davis et al., 2007) hours following two different protocols. Downhill running compared to uphill running always caused a significant decrease in performance. Downhill running had a detrimental significant effect on voluntary wheel running activity as well. These authors have shown that changes in Brain IL-1β following eccentric exercise is related to a decreased performance. They also manipulated experimentally the level of brain IL-1β and showed a cause-effect relation between this cytokine and decreased exercise performance. These findings suggest that the negative effect of EIMD on endurance performance may be mediated by central factors rather than just muscle dysfunction.

After publication of Study 1 of this Thesis, some authors extended our findings by demonstrating that EIMD significantly reduces performance during a cycling time to exhaustion test lasting approximately 7 – 8 minutes (Davies et al., 2008) and even during a 5-min all-out effort (Twist and Eston, 2009).

Motivational factors in endurance exercise performance

We have previously stressed the importance of considering motivational factors when investigating endurance exercise performance. For the purpose of this thesis, we decided to adopt the theoretical framework originally proposed by Brehm and Self (Brehm and Self, 1989)and further developed by Wright (Wright, 2008). This theory called Motivational Intensity Theory applies to any motivated behaviour (Brehm and Self, 1989) and assumes that the effort a person invests in a task is determined by two factors: task difficulty and potential motivation. This is defined as the maximum effort that people are willing to exert in order to succeed in the task (Wright and Kirby, 2001). The level at which potential motivation is set is determined by various psychological factors, namely need, incentive value, and behavioural instrumentality (Wright, 1996). This theory predicts that an individual would engage in task when success is worth the effort and it is seen as possible. When task is perceived as impossible or excessively difficult, people disengage from the task. The rationale for using this motivation theory in the study of the effects of muscle fatigue and damage on endurance performance is provided by studies of task "failure" during submaximal isometric contractions below 30% of MVC exercise (Place et al., 2008a, Taylor and Gandevia, 2008). In these studies, MVC was measured immediately after exhaustion and found to be well over the force required by the exercise task (Figure 8). Given the high RPE levels measured at exhaustion, the most plausible alternative explanation is that subjects voluntarily disengaged from the task when effort reached their level of potential motivation. These data clearly fits with Motivational Intensity Theory, and a similar psychobiological mechanism may explain the effects of locomotor muscle fatigue and damage on endurance exercise performance.

Structure of the thesis

After this General Introduction, the four experimental studies comprising our research program will be described in chronological order. Study 2 and 3 have been combined in a single chapter. All three experimental chapters are written as stand-alone papers.
Study 1 is on the effects of muscle damage on endurance running performance and the physiological and perceptual responses to exercise. This study has already been published:
Marcora SM, **Bosio A.** Effect of exercise-induced muscle damage on endurance running performance in humans. Scand J Med Sci Sports. 2007 Dec;17(6):662-71.
The primary aim of Study 2 was to investigate the effect of muscle fatigue on high intensity cycling performance. The effects of muscle fatigue on the physiological and perceptual responses to high intensity cycling exercise have been further investigated in Study 3. Studies 2 and 3 have been already published as a single paper:
Marcora SM, **Bosio A**, de Morree HM. Locomotor muscle fatigue increases cardiorespiratory responses and reduces performance during intense cycling exercise independently from metabolic stress. Am J Physiol Regul Integr Comp Physiol. 2008 Mar;294(3):R874-83.
Finally, in Study 4 we investigated the effect of muscle fatigue on aerobic capacity and the physiological and perceptual response to incremental exercise. Study 4 has been submitted to publication:
Bosio A, Marcora SM. Effects of locomotor muscle fatigue on physiological responses , exertional symptom intensity, and performance during incremental cycling exercise. European Journal of Applied Physiology. Under revision.
All cited references are included in a single list at the end of the thesis. Figures and tables are numbered as the original published/submitted papers. Similarly, abbreviations are defined at their first appearance within each chapter of the thesis. As all the manuscript included in this thesis are independent but linked, at times there is a necessary overlap between chapters.

CHAPTER 2

EFFECT OF EXERCISE-INDUCED MUSCLE DAMAGE ON ENDURANCE RUNNING PERFORMANCE IN HUMANS

INTRODUCTION

Unaccustomed exercise, especially one characterised by intense eccentric muscle contractions, induces significant damage to the sarcomere, sarcoplasmic reticulum, t-tubules and sarcolemma (Proske and Allen, 2005, Friden and Lieber, 2001). It is well established that these structural alterations translate functionally in a prolonged reduction of strength in the affected muscles (Proske and Allen, 2005, Friden and Lieber, 2001). More recent studies have demonstrated that exercise-induced muscle damage (EIMD) has a negative impact on measures of athletic performance requiring muscle power (Byrne and Eston, 2002b, Byrne and Eston, 2002a, Byrne et al., 2001, Twist and Eston, 2005).

The effect of EIMD on endurance running performance is, however, unclear. In mice, Carmichael et al. (Carmichael et al., 2005, Carmichael et al., 2006) demonstrated a significant reduction in running time to exhaustion 24-48 h after muscle-damaging exercise. On the other hand, with the exception of one study (Braun and Dutto, 2003), available evidence in humans suggests that EIMD does not negatively affect running economy, cardiorespiratory responses, and energy metabolism during standardized submaximal running (Hamill et al., 1991, Paschalis et al., 2005, Scott et al., 2003). However, none of these studies included a direct measure of endurance performance such as time to exhaustion at a fixed workload or time trials (Jeukendrup et al., 1996). This is surprising considering that significant muscle damage occurs during prolonged running and considerable effort has been made to develop effective interventions to prevent it (Hikida et al., 1983, Santos et al., 2004, Knitter et al., 2000, Mastaloudis et al., 2006, Warhol et al., 1985, Dawson et al., 2002). Therefore, the main aim of our study was to directly test the hypothesis that EIMD impairs endurance performance in moderately trained runners.

Although the majority of human studies on running economy argue against the above hypothesis, it is important to consider the important

role of the sense of effort in limiting exercise tolerance (Jones and Killian, 2000). Indeed, several well controlled human experiments employing force matching tasks have demonstrated a significant increase in the sense of effort after muscle damaging exercise (Carson et al., 2002, Proske et al., 2004). Importantly, a significant increase in ratings of perceived exertion (RPE) has also been measured during submaximal running despite no major effects of EIMD on the physiological responses to aerobic exercise (Scott et al., 2003). Therefore, we hypothesised that the negative effect of EIMD on endurance running performance is mediated by increased perception of effort rather than significant alterations in running economy, cardiorespiratory responses, and energy metabolism.

MATERIALS AND METHODS

Subjects

Thirty subjects (24 males and 6 females) were recruited among sport science students of the University of Wales-Bangor and athletes from the local running and triathlon clubs. Their main baseline characteristics are shown in Table 1. The main inclusion criteria for participation in the present study were adult age and a history of distance running for at least 30 min twice a week in the previous 6 months. A medical questionnaire was administered to exclude subjects with conditions contraindicating maximal exercise. The study protocol was approved by the Ethics Committee of the School of Sport, Health and Exercise Sciences (SSHES) of the University of Wales-Bangor. All participants were informed of the purpose and procedures of the study, related benefits and risks and had to give their signed informed consent before taking part.

Based on an in-house reliability study (see time trial section for further details) of the same endurance performance test employed in the present study, we calculated that a total of 38 subjects were needed for detecting the smallest worthwhile change (0.5%) for

competitive distance running events of similar duration (Hopkins and Hewson, 2001). This calculation assumes a Type I error of 5% (two-tailed) and a Type II error of 20%. For expediency, we decided to recruit a total of 30 subjects which is adequate to detect the smallest worthwhile change when accepting a Type I error of 10%.

Table 2

Subjects baseline characteristics

Variable	**EIMD (n = 15)**	**Control (n = 15)**	***P***
Sex (males/females)	12/3	12/3	1.000
Age (yrs)	31 ± 9	31 ± 9	0.921
Stature (cm)	175 ± 6	175 ± 9	1.000
Body mass (kg)	71.0 ± 8.5	72.7 ± 9.6	0.610
Body fat (%)	12.4 ± 5.5	13.7 ± 6.3	0.564
VO_{2max} (mL/kg/min)	55.0 ± 6.0	53.3 ± 6.1	0.434
Max heart rate (bpm)	187 ± 11	187 ± 11	0.997
Time trial performance (m)	6781 ± 772	6545 ± 891	0.445
Running sessions per week	4.4 ± 1.6	4.2 ± 1.4	0.716
Average session duration (min)	51 ± 20	45 ± 18	0.371
Total running time per week (min)	236 ± 145	195 ± 122	0.414
Resistance training (# subjects)	3	3	1.000
Plyometric training (# subjects)	1	0	1.000

Unless otherwise noted, values are means ± SD. Baseline between group differences were examined using multiple independent t tests for continuous variables and Fisher's exact probability tests for categorical variables. EIMD = exercise-induced muscle damage, VO_{2max} = maximal oxygen consumption

Study design

A single blind, randomised, controlled, pretest-posttest design was used for the present study (Figure 1). The research assistants administering the time trials were unaware of subject treatment allocation to avoid experimenter bias on our primary outcome variable. However, subjects were obviously aware of treatment allocation. Therefore, this study should be considered single-blinded. Subjects came to the SSHES Physiology Laboratory three times. During the first visit, body size and composition, and maximal oxygen consumption (VO_{2max}) were measured. After a minimum of 24 h, subjects came for the second visit (pretest). On this occasion, four indirect markers of muscle damage were measured in the following order: delayed-onset muscle soreness (DOMS), creatine kinase (CK), mid-thigh circumference, and knee extensors strength. After 5 min rest, subjects physiological and perceptual responses were monitored during a standardized constant speed run at submaximal intensity. Ten minutes after this standardized submaximal run, subjects performed the 30 min time trial. Once the time trial was completed, subjects were matched for sex and randomly assigned to experimental treatment, i.e. EIMD or control. Forty-eight hours later, subjects returned to the Laboratory for the third and last visit (posttest) and repeated in the same order the tests administered in the pretest. We decided to schedule the posttest 48h after the second visit to ensure full recovery from the pretest time trial in both groups and, at the same time, ensure full development of symptoms and signs of muscle damage (which are known to peak 24 to 72h after exercise) in the EIMD group.

Subjects were asked to avoid smoking, alcohol, tea, coffee, and to drink, on average, 2.5 L of water in the 24 h preceding each visit. They were also instructed to have a light meal at least 3 h before reporting to the Laboratory and to maintain their usual diet throughout the study. All visits were scheduled at the same time of the day, and environmental conditions in the Laboratory were always between 20 and 21.5° C (temperature), and 35 and 45 % (humidity). In the 24 h before Visit 1, subjects were asked to avoid strenuous exercise.

Subjects were also instructed to refrain from any kind of exercise and anti-inflammatory/analgesic agents between Visit 2 and 3.

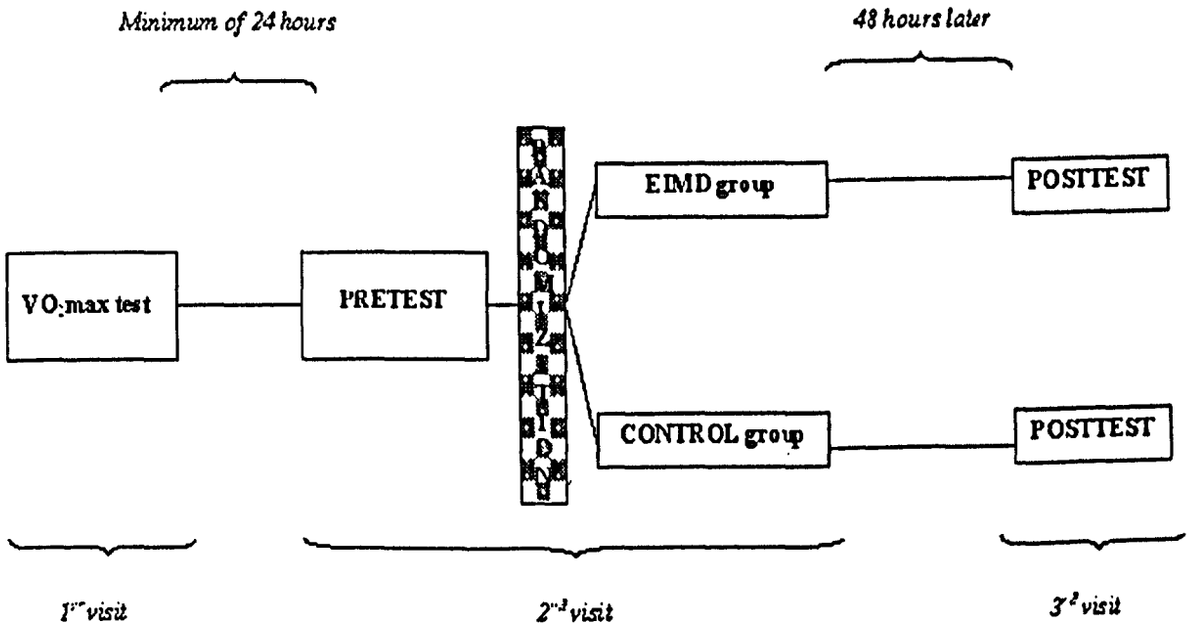

Figure 9

Schematic diagram of the experimental design. VO_{2max} = maximal oxygen consumption.

Experimental treatment

Ten minutes after the pretest time trial, a protocol consisting of 100 drop jumps was used to induce muscle damage in the legs of subjects allocated to the EIMD group. Subjects were asked to step on a 35 cm bench alternating the left and right leg. Once they were standing on the bench, subjects dropped on the floor with both feet and squatted down to about 90° knee angle before jumping in place as high as possible. This was repeated continuously 10 times and then followed by 1 min rest. This set of 10 repetitions was repeated 10 times. Throughout the entire protocol, subjects maintained their hands on the hips. Repeated and intense stretch-shortening cycles are known to induce significant muscle damage (Komi, 2000), and a similar protocol has been effectively used by previous investigators (Twist and Eston, 2005). Subjects in the control group did not perform any muscle-damaging exercise.

Markers of muscle damage

Delayed-onset leg muscle soreness was subjectively assessed using the 7-point Likert scale developed by Vickers (Vickers, 2001). Subjects were asked to rate the overall level of DOMS felt in both legs (i.e., buttocks, groin, thighs, hamstrings, calves, and shins) during the past 12 waking hours according to the following verbal anchors:

0 A complete absence of soreness;

1 A light pain felt only when touched / a vague ache;

2 A moderate pain felt only when touched / a slight persistent pain;

3 A light pain when walking up or down stairs;

4 A light pain when walking on a flat surface / painful;

5 A moderate pain, stiffness or weakness when walking / very painful;

6 A severe pain that limits my ability to move.

A 30 µl sample of whole fresh blood was taken from a finger tip and immediately pipetted on the appropriate strip for analysis of CK using a colorimetric assay (Reflotron, Boehringer Mannheim, Germany).

Mid-thigh circumference, defined as the middle point between the groin fold and the upper patella, was measured on the dominant leg while standing to assess swelling of the thigh using an inextensible anthropometric tape (Lufkin, Cooper Industries, Houston, Texas). A permanent mark was used to ensure mid-thigh circumference was taken in the same location at both pre and posttest. The average of three measurements was used for statistical analysis. Bilateral voluntary isometric strength of the knee extensors was measured with subjects seated in a rigid, straight-backed chair with a 90 degree knee and hip angle. After three submaximal warm-up and familiarisation trials (25%, 50%, 75% of maximal effort), subjects were asked three times to push maximally for 5 s against pads placed just proximal to their ankle joints and inextensibly attached to a load cell (Model No. 615, Tedea Huntleigh-Vishay, California) connected to an A/D converter for data recording and analysis (Bridge Amp, Powerlab/16SP, Power Lab Chart V 4.2.3, Adi Instruments Pty LTD, Australia). Between all six trials 1 min rest was observed. During the maximal trials, strong verbal encouragement was given. Peak force produced during each of the three maximal trials was recorded and the best score noted for statistical analysis.

Steady-state physiological and perceptual responses to standardized submaximal running

Cardiovascular, respiratory, metabolic and perceptual responses were monitored during a standardized constant speed run at submaximal intensity on a motor-driven treadmill (PPS 55 sport-I, Woodway GMBH, Germany) set at 1% inclination to reproduce the energetic cost of running outdoor on a flat surface (Jones and Doust, 1996). After 5 min warm-up at 50% of VO_{2max}, subjects ran for 10 min at a speed corresponding to 70% of their VO_{2max} (EIMD 11.6 ± 1.4 km/h, Control 11.2 ± 1.4 km/h) as predicted by the ACSM metabolic equation for running. During these 10 min, tidal volume (VT), breathing frequency (BF), minute ventilation (VE), VO_2, carbon dioxide production (VCO_2), and respiratory exchange ratio (RER) were measured breath

by breath using an automated metabolic gas analysis system (600Ergo Test, ZAN Messgeräte, Oberthulba, Germany). This device was calibrated before each test using certified gases of known concentration (11.5% O_2 and 5.1% CO_2) and a 3.0 L calibration syringe (Series 5530, Hans Rudolph Inc., Kansas City, Missouri). Heart rate (HR) was also measured continuously by telemetry (Model S810, Polar, Kempele, Finland). Overall ratings of perceived exertion (RPE) were obtained every 2 min using the 15-point Borg RPE scale following standard instructions and anchoring during the VO_{2max} test (Borg, 1998a). For all these measures, the average of the last 6 min of exercise was considered for statistical analysis to ensure steady-state and more reproducible results. Immediately after the end of the 10 min run, a 5 µl sample of whole fresh blood was taken from the finger tip and analyzed using a portable blood lactate concentration (La) analyzer (Lactate Pro LT-1710, Arkray, Shiga, Japan).

Time trial

Subjects were required to run as far as possible in 30 min on the Woodway motor-driven treadmill set at 1% inclination. The treadmill was regularly checked for accuracy of speed, inclination, and distance measured. Feedback on elapsed time was available, but subjects could not see the treadmill's speedometer and the HR monitor display which were covered with cardboard and thick white tape. The time trial started with subjects standing on the treadmill belt while speed was increased up to 9.0 km/h. After this speed was reached, subjects were free to increase or decrease running speed at their will using the + and - buttons on the right side of the treadmill. Once the 30 min were elapsed, subjects stopped running immediately and placed their feet on the platforms at the sides of the belt while distance ran in the time trial was recorded. This was our operational definition of endurance running performance. A fan was placed in a standardised position in front of the subject during the entire duration of the trial and he/she was allowed to drink water ad libitum. Every 3 min, speed, HR and RPE were recorded as described above and used for statistical

analysis. Strong verbal encouragement was provided by a research assistant unaware of subject treatment allocation to avoid experimenter bias on endurance running performance. Furthermore, cash prizes for best performance in the pretest, best posttest performance in the EIMD group, and best posttest performance in the control group were given to motivate maximal effort during all time trials. In a preliminary in-house reliability study conducted in a similar group of 10 male runners tested twice without a habituation trial, this 30 min time trial demonstrated good reliability with a test-retest correlation coefficient of 0.91 and a coefficient of variation of 3.8%.

Other measures

Maximal oxygen consumption was measured with the Zan automated metabolic gas analysis system while running on the Woodway motor-driven treadmill following the modified Åstrand protocol described by Pollock et al. (Pollock et al., 1978). Briefly, subjects ran at a fixed self-selected speed between 8 and 12.9 km/h. After 3 min at ground level, inclination was increased by 2.5% every 2 min until exhaustion. Stature, body mass, and body fat percentage were assessed by mean of a wall-mounted stadiometer (Model 26SM, Seca, Hamburg, Germany) and a standing bioelectrical impedance analyzer (TBF-305, Tanita Corporation, Tokyo, Japan) using the proprietary sex-specific equations for athletes.

Statistical analysis

Unless otherwise noted, data are presented as mean ± SD. Baseline differences between the EIMD and control group were examined using multiple independent *t* tests for continuous variables and Fisher's exact probability tests for categorical variables. Multiple two-way (group x test) ANOVAs with repeated measures on the test factor were used to assess the effect of experimental treatment on indirect markers of muscle damage, the physiological and perceptual responses to standardized submaximal running, and distance ran in the time trial.

Because both factors (group and test) have only two levels, a significant interaction was followed-up by data plotting and visual exploration. In case of a non significant interaction, only the main effect of test was considered. Multiple three-way (group x test x time) ANOVAs with repeated measures on the test and time factors were used to assess the effect of experimental treatment on pacing strategy, i.e. speed, HR and RPE recorded every 3 min during the 30 min time trial (10 time points). A significant second order interaction (group x test x time) was followed-up by tests of simple interactions, i.e. two-way (group x test) ANOVAs with repeated measures on the test factor at each time point. If the second order interaction was not significant, only the first order group x test interaction (i.e. the effect of experimental treatment on average time trial speed, HR and RPE) was considered and followed-up as previously described. Relevant assumptions were checked and appropriate corrections employed if necessary. Significance was set at 0.05 (two-tailed) for all analyses. A *P* level between 0.05 and 0.10 was considered a trend.

As suggested by Hopkins et al. (Hopkins et al., 1999) when analysing sport performance studies, we calculated the 95% confidence limit of the between group difference in relative change between the pretest and posttest. Furthermore, we assessed individual changes in endurance running performance in response to experimental treatment. This assessment was conducted using the reliable change index (RCI) with a correction for observed practice effect (Heaton et al., 2001). The RCI of time trial performance was calculated as follows using the pretest and posttest data of subjects in the control group.

The test–retest reliability coefficient (r) was computed and the standard error of measurement (SEm) calculated by

$SEm=SD1(\sqrt{[1-r]})$

where SD1 is the SD of the baseline score. The standard error of the difference (SEdiff), i.e. the spread of distribution of change scores that would be expected if no change occurred, was calculated by

$SEdiff=\sqrt{[2(SEm)^2]}$.

The 90% confidence interval of the reliable change was established by

multiplying the SEdiff by ±1.645 SD. These upper and lower limits were corrected for observed practice effect, the mean difference between the posttest and the prestest scores. Thus, the RCI was calculated as

RCI = (SEdiff)(±1.64 SD) + observed practice effect. For each participant, a change score (posttest – pretest) representing the difference in the distance ran in the time trial was calculated. If this score fell outside the RCI, a reliable (i.e., unlikely to occur by chance) change in time trial performance was considered to have occurred.

RESULTS

Our recruitment and treatment allocation procedures were successful in forming two groups (EIMD and control) of moderately trained runners with similar baseline characteristics (Table 2). Although training load and performance level varied greatly among our subjects, the variances within each group were equal (all Levene's Tests $P > 0.05$).

The 100 drop jumps protocol was effective in inducing significant alterations in three out of four indirect markers of muscle damage (Table 3). In fact, there was an increase in DOMS and CK and a concomitant 12% decrease in knee extensors strength in the EIMD group with no changes in the control group (all group x test interactions, $P < 0.007$). However, no significant changes in mid-thigh circumference were measured in either the EIMD or control group (main effect of test, $P = 0.351$). Subjects in the EIMD group reported significant soreness, ache and pain not only in the thigh muscles, but also in the buttock and calf muscles.

Table 3.

Effects of experimental treatment on markers of muscle damage

Variable	Group	Pretest	Posttest	*P*
DOMS (0-6)	EIMD	0.4 ± 0.5	4.5 ± 1.1	< 0.001
	Control	0.5 ± 1.1	1.1 ± 1.2	
CK (IU/L)	EIMD	159 ± 107	332 ± 269	0.006
	Control	159 ± 102	163 ± 115	
Knee extensors strength (N)	EIMD	681 ± 139	601 ± 136	0.001
	Control	686 ± 149	691 ± 185	
Mid-thigh circumference (cm)	EIMD	54.0 ± 3.3	54.0 ± 3.4	0.828
	Control	54.8 ± 3.8	54.9 ± 3.8	

Each group includes 15 subjects. Values are means ± SD. *P* values refer to the group x test interaction of two-way ANOVA with repeated measures on the test factor. Main effects of test are reported in the Results section when appropriate. EIMD = Exercise-induced muscle damage, DOMS = delayed-onset muscle soreness, CK = creatine kinase.

Experimental treatment did not significantly affect the physiological responses to standardized submaximal running (Table 4). In fact, there were no significant changes in either the EIMD or control group in HR (main effect of test, $P = 0.976$), VT (main effect of test, $P = 0.240$), VO_2 (main effect of test, $P = 0.667$), VCO_2 (main effect of test, $P = 0.501$), and La (main effect of test, $P = 0.925$). Breathing frequency (main effect of test, $P = 0.004$), VE (main effect of test, $P = 0.030$) and respiratory exchange ratio (main effect of test, $P = 0.005$) increased significantly in both groups. In spite of no major physiological changes, there was a trend for an increase in RPE in the EIMD group compared to a slight decrease in the control group (group x test interaction, $P = 0.076$) (Table 4).

Table 4.

Effects of experimental treatment on steady-state physiological and perceptual responses to running at 70% of maximal oxygen consumption

Variable	Group	Pretest	Posttest	*P*
Heart Rate (bpm)	EIMD	151 ± 18	152 ± 18	0.262
	Control	149 ± 14	148 ± 13	
VT (L)	EIMD	1.87 ± 0.32	1.86 ± 0.31	0.451
	Control	2.04 ± 0.56	1.97 ± 0.46	
BF (breaths/min)	EIMD	36.4 ± 7.5	38.7 ± 7.9	0.848
	Control	33.8 ± 6.4	35.7 ± 6.2	
VE (L/min)	EIMD	67.3 ± 15.2	70.5 ± 13.8	0.526
	Control	67.0 ± 15.9	68.8 ± 13.3	
VO_2 (L/min)	EIMD	2.72 ± 0.46	2.71 ± 0.41	0.963
	Control	2.69 ± 0.61	2.68 ± 0.47	
VCO_2 (L/min)	EIMD	2.68 ± 0.53	2.70 ± 0.44	0.867
	Control	2.60 ± 0.61	2.63 ± 0.49	
RER	EIMD	0.982 ± 0.050	0.997 ± 0.048	0.950
	Control	0.965 ± 0.036	0.981 ± 0.027	
La (mmol/L)	EIMD	2.02 ± 1.36	2.12 ± 1.16	0.306
	Control	1.86 ± 0.79	1.73 ± 0.71	
RPE (6-20)	EIMD	12.5 ± 0.9	13.2 ± 1.2	0.076
	Control	11.8 ± 1.8	11.7 ± 2.1	

Each group includes 15 subjects. Values are means ± SD. *P* values refer to the group x test interaction of two-way ANOVA with repeated measures on the test factor. Main effects of test are reported in the Results section when appropriate. EIMD = Exercise-induced-muscle damage. VT = tidal volume, BF = breathing frequency, VE = minute ventilation, VO_2 = oxygen consumption, VCO_2 = carbon dioxide production, RER = respiratory exchange ratio, La = blood lactate concentration, RPE = ratings of perceived exertion.

Experimental treatment had a clear significant effect on endurance running performance (group x test interaction, $P = 0.012$). In fact, subjects in the EIMD group ran a shorter distance in the posttest (6631 ± 839 m) than in the pretest (6781 ± 772 m). In the control group there was, on the contrary, a slight increase in distance ran in the time trial (pretest 6545 ± 891 m, posttest 6652 ± 880 m). These changes translate in a mean -4.0% difference in endurance running performance between the EIMD and control group, with a 95% confidence limit of -7.0 and -1.0%.

This significant effect of EIMD on endurance running performance was confirmed by the analysis of individual changes using the RCI method (Figure 2). The upper and lower limits of the RCI corrected for the practice effect (+108 m) were +393 m and -178 m respectively. In the EIMD group seven subjects had a reliable decrement in distance ran in the time trial compared to only one in the control group. This difference was statistically significant as revealed by Fisher's exact probability test ($P = 0.035$). Only one subject in the EIMD group had a reliable improvement in time trial performance with the remaining subjects in both groups showing no reliable changes ($P = 1.000$).

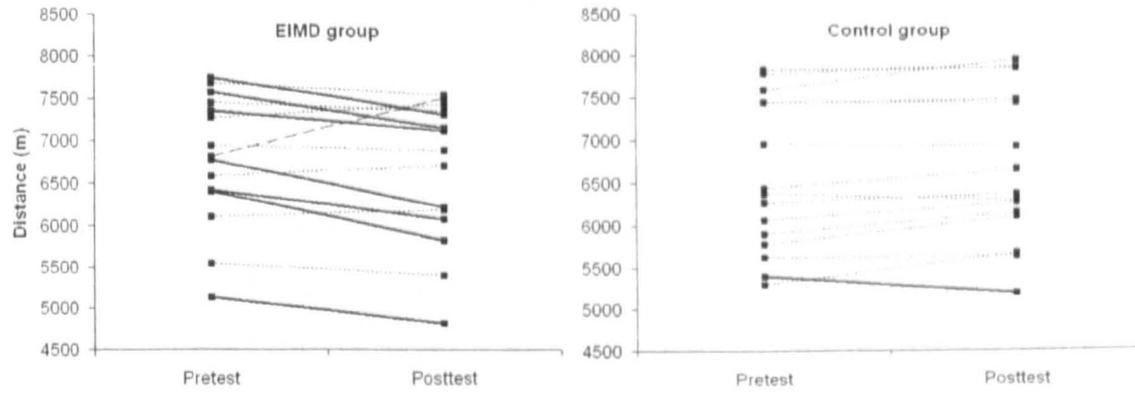

Figure 10

Individual changes in time trial performance. The bold line represents a reliable decrease, the dashed line represents a reliable increase, and the dotted line represents a non reliable change. See Materials and Methods and Results sections for the details on the reliable change index (RCI) calculations.

The in-depth analysis of speed, heart rate and RPE recorded every 3 min during the 30 min time trial tests did not reveal any significant group x test x time interaction (Figure 3). This finding implies that EIMD did not affect pacing strategy. Analysis of the first order group x test interactions shows, however, that there was a significant effect of experimental treatment on average time trial speed with a small decrease in the EIMD group (pretest 13.9 ± 1.7 km/h, posttest 13.6 ± 1.7 km/h) and an increase of similar magnitude in the control group (pretest 13.4 ± 1.8 km/h, post 13.6 ± 1.8 km/h) (group x test interaction, $P = 0.022$) despite no significant effect on HR (group x test interaction, $P = 0.771$) and RPE (group x test interaction, $P = 0.305$). In fact, average HR during the time trial did not change significantly in either the EIMD group (pretest 172 ± 17 bpm, posttest 171 ± 15 bpm) or the control group (pretest 172 ± 9 bpm, posttest 171 ± 11 bpm) (main effect of test, $P = 0.252$). Similarly, average time trial RPE did not show any significant change in the EIMD group (pretest 15.6 ± 0.9, posttest 15.8 ± 1.2) or the control group (pretest 14.8 ± 2.1, posttest 14.7 ± 1.9) (main effect of test, $P = 0.874$). In other words, subjects with EIMD perceived the same effort throughout the time trial despite a significant reduction in their average running speed.

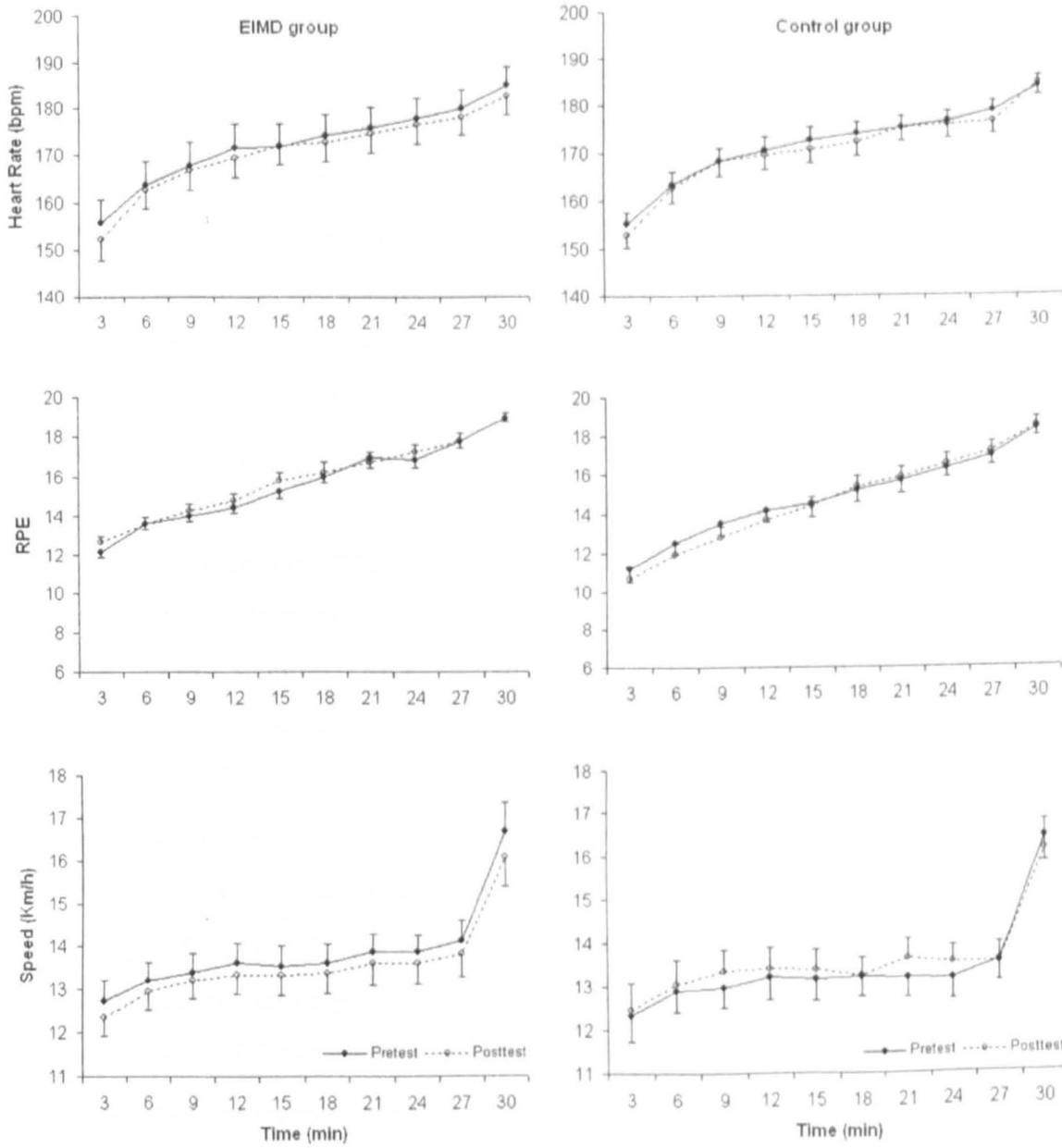

Figure 11

Effects of experimental treatment on heart rate (HR), ratings of perceived exertion (RPE) and speed measured every 3 min during the 30 min time trial tests. Values are means ± SEM. No significant second order group x test x time interaction by three-way ANOVA with repeated measures on the test and time factors was found for HR ($P = 0.368$), RPE ($P = 0.316$), or speed ($P = 0.758$). First order group x test interactions are reported in the Results section.

DISCUSSION

This is the first study to demonstrate a significant effect of EIMD on endurance running performance in humans. Our results are in agreement with the studies of Carmichael and colleagues in mice (Carmichael et al., 2005, Carmichael et al., 2006). In their investigations the detrimental effect of EIMD was even more pronounced with a 65% reduction in running time to exhaustion. Bearing in mind obvious species differences, this might be due to the more severe muscle-damaging exercise protocol (130-150 min of downhill running) and the different endurance performance test employed (long time to exhaustion vs. shorter time trial). Albeit smaller, the negative effect (-4.0%) of our 100 drop jumps protocol on distance ran in 30 min is highly relevant to human performance. In fact, the likely range of the true effect of this experimental treatment on the average subject (95% confidence limit) is between -7.0 and -1.0% which is higher than the smallest worthwhile change in performance (0.5%) in competitive distance running events of similar duration (Hopkins and Hewson, 2001). Furthermore, the analysis of individual changes shows that the risk of having a significant decrease in endurance running performance is significantly higher in subjects suffering from EIMD relative to subjects in the control group. It is important to consider, however, that highly trained runners might be less susceptible to EIMD compared to our moderately trained runners because of the repeated bout effect (McHugh, 2003), and this possibility should be investigated before the results of our study can be confidently applied to high level athletes. Nonetheless, Figure 2 suggests that within our EIMD group those who were more trained were not less susceptible to the detrimental effect of muscle-damaging exercise than those less trained.

As hypothesised, a decrease in running economy was not to blame for the negative effect of EIMD on endurance performance. In fact, steady-state VO_2 while running at a constant speed corresponding to 70% of VO_2max was not affected by EIMD. This finding is in

agreement with the results of Hamill et al. (Hamill et al., 1991) (80% of VO_{2max}), Paschalis et al. (Paschalis et al., 2005) (55 and 75% of VO_{2max}), and Scott et al. (Scott et al., 2003) (~70% VO_2max). Braun and Dutto (Braun and Dutto, 2003), on the contrary, measured a significant increase in steady-state VO_2 while running at 65, 75 and 85% of VO_{2max}. The reason for this discrepancy does not seem to be more severe EIMD as our 100 drop jumps protocol induced similar levels of DOMS compared to the 30 min downhill running protocol utilised by Braun and Dutto (Braun and Dutto, 2003). However, comparisons are difficult because Braun and Dutto (Braun and Dutto, 2003) used a different DOMS scale and did not report strength changes in the affected muscles, one of the best methods to quantify eccentric contraction-induced injury in humans (Warren et al., 1999). Furthermore, we could not measure any influence of EIMD on other physiological responses to standardized submaximal running. Similarly to Paschalis et al. (Paschalis et al., 2005), Hamill et al. (Hamill et al., 1991) and Scott et al. (Scott et al., 2003), we failed to demonstrate any major effect of EIMD on HR, ventilatory response, RER and La during aerobic exercise. Again, these results contrast with the ones reported by Braun and Dutto (Braun and Dutto, 2003) who found a significant increase in HR, VE, RER and La during standardized submaximal running in subjects suffering from EIMD. Taken as a whole, the results of our study and previous investigations suggest that impaired endurance running performance in subjects with EIMD is not mediated by adverse effects on the cardiorespiratory and metabolic responses to aerobic exercise.

As hypothesised, the detrimental effect of EIMD on endurance running performance seems to be mediated by increased perception of effort. This conclusion is supported by three separate findings of our study. The first is the trend ($P = 0.076$) for an increase in RPE during submaximal running at 70% of VO_{2max} in the EIMD group compared to the control group (Table 2). This effect has been previously reported by Scott and colleagues (Scott et al., 2003) who found a significant increase in RPE ($P < 0.05$) in subjects with EIMD running at similar

exercise intensity for 30 min. The second finding supporting a significant effect of EIMD on the sense of effort in our study is the reduction (P = 0.022) in average speed during the 30 min time trial in the EIMD group while running at the same average RPE (P = 0.305) (Figure 3). During the time trial, the only cues subjects had to voluntary regulate their running pace were perceived exertion and time elapsed. Therefore, it is not surprising that all subjects ran each time trial at a pace which gradually led them to an average final RPE of 18-19, the maximum level most subjects are willing to tolerate (Noakes, 2004). We assume that subjects with EIMD compensated the increased sense of effort with a slower running speed so that their RPE was maintained within tolerable limits. Finally, we conducted an unplanned exploratory statistical analysis and found a significant correlation between the increase in RPE during submaximal running and the decrease in endurance performance (N=30, r = -0.56, P = 0.002).

Importantly, our findings are in agreement with the results of psychophysical studies investigating the effects of EIMD on the ability to subjectively estimate force production (Proske et al., 2004, Carson et al., 2002). In most conditions, humans estimate force based on their sense of effort and when asked to match with the damaged arm the force produced by the control arm, subjects underestimate force production. Conversely, when subjects are asked to match with the control arm the force produced by the damaged arm, they overestimate force production. This means that subjects with EIMD perceive higher effort when producing the same force, and produce less force for the same perceived effort. Similarly, subjects in our EIMD group reported higher RPE when running at the same submaximal speed, and ran at slower speed for the same RPE during the time trial. Future larger studies should confirm our findings and investigate the mechanisms of increased perception of effort during running in subjects with EIMD.

Possible mechanisns include a higher central motor command necessary to produce the same speed with weaker leg muscles (Jones

and Killian, 2000, Proske et al., 2004), the contribution of leg muscle pain to overall RPE (Borg et al., 1985, Proske et al., 2004), and alterations in glycogen metabolism and availability (Asp et al., 1998) (Baldwin et al., 2003). However, the unaltered steady-state RER and La measured in the EIMD group during the standardized submaximal run argues against the relevance of this mechanism to our study. It also possible that EIMD has a direct effect on the brain (Carson et al., 2002, Prasartwuth et al., 2005). Interestingly, interleukin-1β (IL-1β) causes symptoms of fatigue in human subjects (Rinehart et al., 1997, Omdal and Gunnarsson, 2005) and Carmichael and colleagues measured a high concentration of this inflammatory cytokine in the cortex and cerebellum of mice with impaired endurance running performance because of EIMD (Carmichael et al., 2005). In a follow-up study, the same group demonstrated that blocking the activity of IL-1β reduces the negative effect of EIMD on endurance running performance, and that artificially increasing brain IL-1β mimics the effect of EIMD on running time to exhaustion (Carmichael et al., 2006). These studies strongly suggest that the brain inflammatory response to EIMD might cause central fatigue during prolonged exercise (Davis & Bailey, 1997). This interesting hypothesis warrants future investigations in humans.

PERSPECTIVES

The present study clearly demonstrates that, in humans, EIMD has a negative impact not only on measures of athletic performance requiring muscle strength and power (Byrne et al., 2004), but also on endurance running performance. This effect seems not to be mediated by alterations in running economy, exercise metabolism and cardiorespiratory strain. The study suggests that the sense of effort *per se* could be mediating this effect. Further larger studies are needed to confirm this finding, and to understand the peripheral and central mechanisms of increased perceived exertion during dynamic exercise in subjects with EIMD.

From a practical point of view, the results of our study warn against the inclusion of muscle damaging exercise (e.g. plyometric training, downhill running, and very long running sessions) in the days preceding an important endurance running competition. Although we did not measure directly the effect of muscle damage that develops during marathon and ultramarathon races (Armstrong, 1986), our results suggest that interventions aimed at preventing/reducing such muscle damage might improve performance in these endurance events. Future studies should test this interesting hypothesis.

CHAPTER 3

LOCOMOTOR MUSCLE FATIGUE INCREASES CARDIORESPIRATORY RESPONSES AND REDUCES PERFORMANCE DURING INTENSE CYCLING EXERCISE INDEPENDENTLY FROM METABOLIC STRESS

INTRODUCTION

Significant locomotor muscle fatigue, defined as an exercise-induced reduction in maximal voluntary force produced with the locomotor muscles (Gandevia, 2001), occurs during sustained exercise (Millet and Lepers, 2004, Presland et al., 2005, Amann et al., 2006, Amann et al., 2007, Romer et al., 2007a, Amann and Dempsey, 2007a) and is commonly considered the ultimate factor limiting exercise performance (Edwards, 1983b, Sejersted and Sjogaard, 2000, McKenna and Hargreaves, 2007). Accordingly, studies on the determinants of exercise performance have focused on various mechanisms contributing to central and/or peripheral fatigue during prolonged exercise such as metabolic and ionic changes within the locomotor muscles, insufficient oxygen delivery, and hyperthermia (McKenna and Hargreaves, 2007). However, in spite of its importance, the fundamental assumption that reduced locomotor muscle force has a negative effect on exercise performance has never been tested experimentally and remains one of the important unknowns in exercise physiology (Dempsey et al., 2006b). Another related and still unanswered research question is whether the increased central motor command required to exercise at the same workload with fatigued locomotor muscles has a significant influence on the cardiorespiratory responses to sustained exercise (Syabbalo et al., 1994, Norton et al., 1999, Spengler et al., 2000, Dempsey et al., 2006a).

The main challenge in testing these hypotheses experimentally is to isolate the reduction in locomotor muscle force (and consequent increase in central motor command) from other physiological effects of fatiguing exercise which may independently affect cardiorespiratory responses and performance during prolonged exercise. Of particular concern is the metabolic stress usually associated with muscle fatigue during high-intensity exercise (Sargeant, 2007). Indeed, accumulation of various metabolites such as lactic acid is known to stimulate group IV and some group III muscle afferents which generate reflexes (the metabo-reflex) that can significantly affect the cardiorespiratory

responses to sustained exercise independently from increased central motor command (Mateika and Duffin, 1995, Smith et al., 2006). Furthermore, in conditions ranging from hyperoxia to moderate hypoxia, stimulation of these small sensory neurons by fatigue-related metabolites might limit exercise performance by generating a painful sensation of leg discomfort (and/or a not yet identified inhibitory supraspinal reflex) that forces the subject to reduce exercise intensity or terminate exercise well before locomotor muscle fatigue becomes a limiting factor (Amann and Dempsey, 2007b, Amann and Dempsey, 2007a). Indeed, the proponents of this conscious and/or subconscious regulatory feedback mechanism believe that its function is to protect the locomotor muscles from excessive peripheral fatigue (Amann and Dempsey, 2007b, Amann and Dempsey, 2007a).

The aim of the present investigation was to control for these confounding effects of metabolic stress and test the hypotheses that a reduction in locomotor muscle force *per se* causes i) a significant increase in central motor command with consequent alterations in the cardiorespiratory responses to high-intensity constant-power cycling, despite unaltered metabolic requirements and ii) a reduction in time to exhaustion, a sensitive measure of exercise performance (Amann et al., 2008b). In order to test these hypotheses, we used an unusual fatiguing exercise protocol as experimental treatment to induce a significant and prolonged reduction in locomotor muscle force without accumulation of muscle metabolites (Nielsen et al., 2005, Skurvydas et al., 2000). Indeed, the excitation-contraction coupling failure induced by this eccentric exercise protocol is caused by structural alterations rather than metabolic stress (Nielsen et al., 2005, Proske and Morgan, 2001).

MATERIALS AND METHODS

Subjects

This investigation consisted of two separate studies. For the first study, we recruited ten healthy male subjects undergoing regular aerobic exercise for recreational or competitive purposes (age 23 ± 4 years; stature 176 ± 7 cm; body mass 79 ± 14 kg; peak power output 346 ± 56 W; VO_{2peak} 51 ± 8 ml kg^{-1} min^{-1}). For the second study we recruited a similar population of 14 subjects (age 26 ± 5 years; stature 179 ± 5 cm; body mass 79 ± 9 kg; peak power output 334 ± 56 W; VO_{2peak} 51 ± 7 ml kg^{-1} min^{-1}). Only one subject participated in both studies. The experimental protocols were approved by the Ethics Committee of the School of Sport, Health and Exercise Sciences and conformed to the standards set by the *Declaration of Helsinki*.

Experimental protocols

All volunteers visited the laboratory on three different occasions. During the first visit the study and its aims were explained, and a medical and training questionnaire was administered. Eligible subjects signed an informed consent form and anthropometric measures were taken. Then an incremental exercise test (2 min at 50 W + 50 W increments every 2 min) was performed until exhaustion [operationally defined as a pedal frequency of less than 60 revolutions per min (RPM) for more than 5 s despite strong verbal encouragement] on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) to measure peak oxygen uptake (VO_{2peak}), and peak power output which was calculated according to the equation of Kuipers et al. (Kuipers et al., 1985). The cycle ergometer was set in hyperbolic mode which allows the power output to be set independently of pedal frequency over the range of 30 to 120 RPM. Before the incremental exercise test the position on the cycle ergometer was adjusted for each subject, and settings were recorded so that they could be reproduced at each subsequent visit.

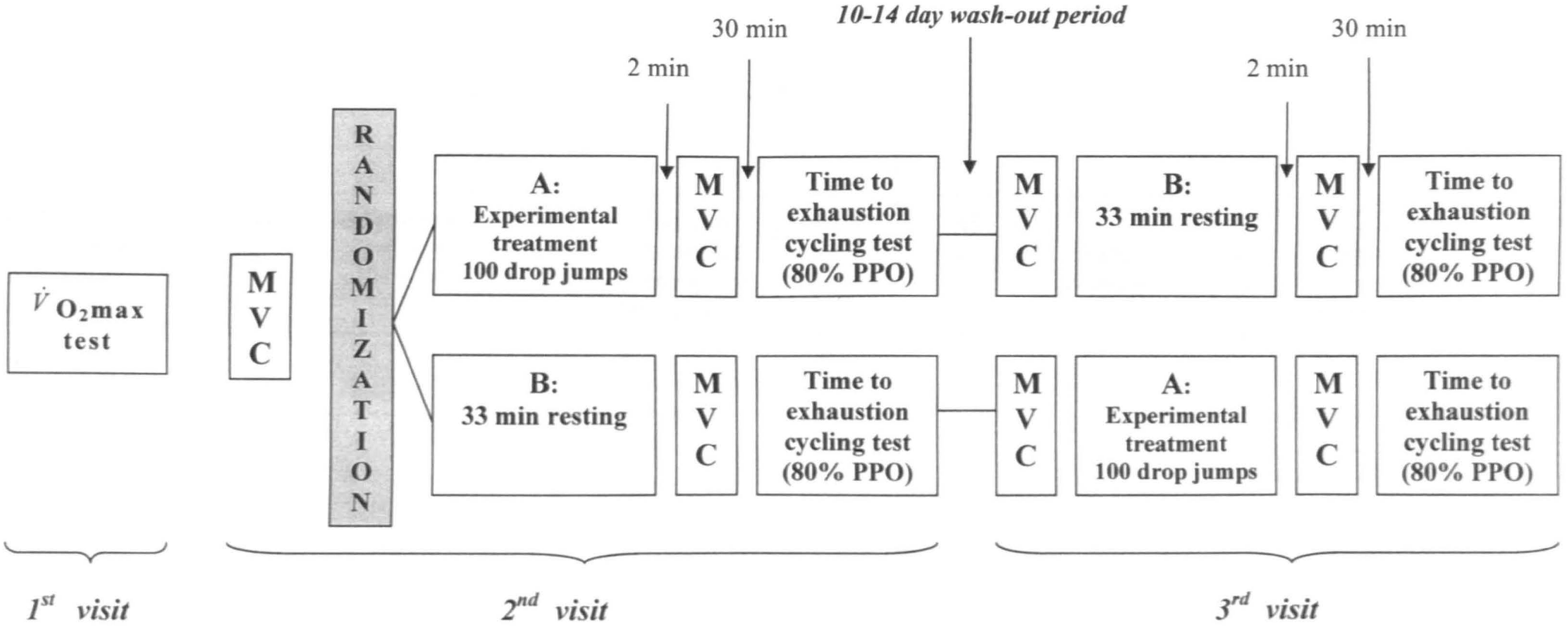

Figure 1.

Schematic diagram of the experimental design of Study 2. $\dot{V}O_2max$ = maximaloxygen consumption; MVC = bilateral Maximal Voluntary Contraction; PPO = Peak Power Output achieved during the $\dot{V}O_2max$ test; A = fatigue condition; B = control condition. Subjects were randomly assigned to either A or B condition with a 1:1 allocation ratio.

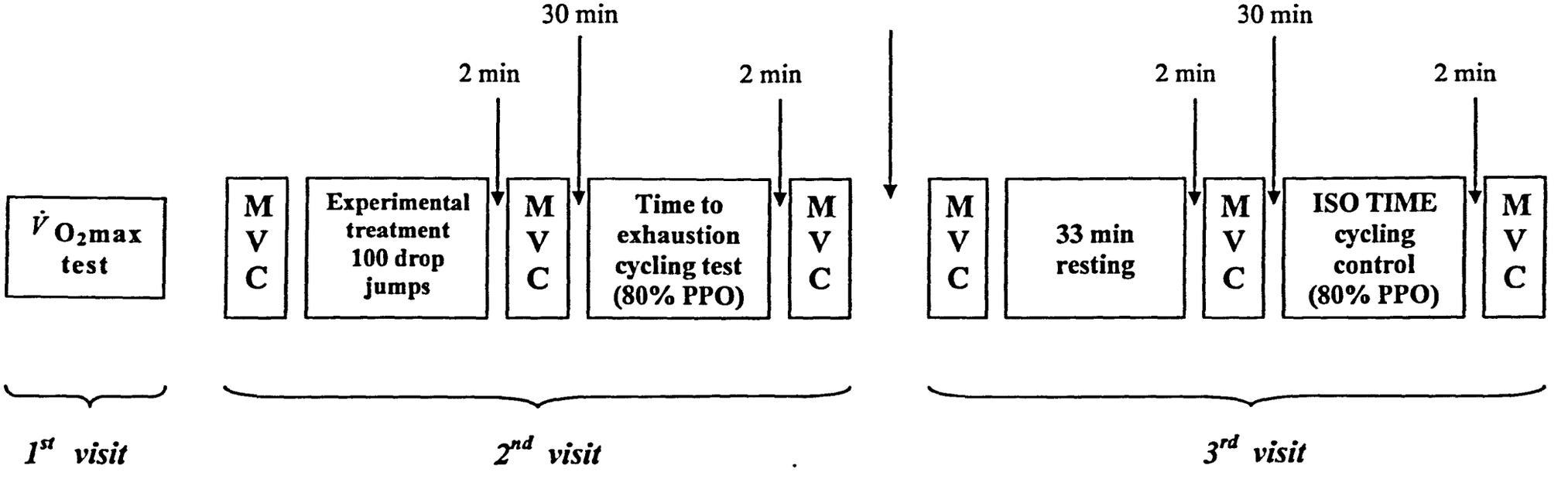

Figure 2.

Schematic diagram of the experimental design of Study 3. $\dot{V}O_2max$ = maximaloxygen consumption; MVC = bilateral Maximal Voluntary Contraction of the knee extensors; PPO = Peak Power Output achieved during the $\dot{V}O_2max$ test; ISO TIME = same time when exhaustion occurred during the second visit.

Subjects were also given standard instructions for overall rating of perceived exertion (RPE) using the 6 to 20 scale developed by Borg (Borg, 1998b). During the incremental exercise test, the low and high anchor points were established using standard procedures (Noble and Robertson, 1996).

In Study 2 (figure 12), after a minimum of 24 h, subjects reported for the second time to the laboratory where delayed-onset muscle soreness (DOMS) was assessed using a 7-point Likert scale (Vickers, 2001). Creatine kinase (CK) concentration (UI l^{-1}) was measured with a colorimetric assay (Reflotron, Boehringer Mannheim, Germany) in a 30 µl sample of whole fresh blood taken from the right earlobe. After a 5 min warm-up on the cycle ergometer at 10% of peak power output, bilateral MVC of the knee extensors, an index of locomotor muscle force, was assessed in isometric condition with subjects seated in a rigid, straight-backed chair with a 90° knee and hip angle. After three submaximal warm-up and familiarization trials (25%, 50%, 75% of maximal effort), subjects were asked three times to push maximally for 5 s against pads placed just proximal to their ankle joints and inextensibly attached to a load cell (Model No. 615, Tedea Huntleigh-Vishay, California) connected to a computerized A/D converter for data recording and analysis (Bridge Amp, Powerlab/16SP, Power Lab Chart V 4.2.3, Adi Instruments, Bella Vista, Australia). Between all six trials 1 min rest was observed. During the maximal trials, strong verbal encouragement was given. Peak force (N) produced during each of the three maximal trials was recorded and the best score noted for statistical analysis. After this baseline isometric test, subjects were randomly assigned to either the A) fatigue or B) control condition with a 1:1 allocation ratio. The fatigue condition consisted of the eccentric exercise protocol developed by Skurvydas et al. (Skurvydas et al., 2000). Subjects dropped 100 times from a 40 cm high platform down to 90° knee angle before jumping upward as high as possible. Between each drop jump there was a 20 s rest period to allow for recovery, through oxidative phosphorylation, of the ATP and phosphocreatine expended during each drop jump. Indeed, in a pilot

study (unpublished results), we measured no increase in capillary blood lactate concentration after this fatiguing exercise protocol which requires only moderate cardiovascular strain (an average of 58% of maximum HR for 33 min). Furthermore, it does not induce any respiratory muscle fatigue, another factor which might affect breathing pattern and exercise performance (Mador and Acevedo, 1991). The control condition consisted of resting comfortably for 33 min. Two minutes after completing the assigned treatment, locomotor muscle force was assessed again with three maximal trials only (pre-cycling isometric test). After this second isometric test, a 30 min rest period was prescribed to allow for further cardiorespiratory and metabolic recovery after the 100 drop jumps whilst, at the same time, controlling for the confounding effects of DOMS which usually peaks 48 h after eccentric exercise because of increased sensitivity of small muscle afferent neurons to mechanical stimuli (Taguchi et al., 2005). After this rest period, subjects began the high-intensity constant-power cycling test to exhaustion with the ergometer set in hyperbolic mode. This cycling test consisted of 3 min rest sitting on the cycle ergometer, 3 min warm-up at 10% of peak power output, and a rectangular workload corresponding to 80% of peak power output which corresponded to 90 ± 7% of VO_{2peak} measured during the preliminary incremental exercise test. Pedal frequency was freely chosen between 60-100 RPM and was recorded every minute. Time to exhaustion was measured from the start of the rectangular workload until the pedal frequency was less than 60 RPM for more than 5 s despite strong verbal encouragement which was provided by a research assistant blinded to the assigned treatment. Physiological and perceptual responses were measured throughout the cycling test. After a period of 10-14 days to wash-out the detrimental effects of muscle damage induced by the 100 drop jumps protocol, subjects reported for the third time to the laboratory. During this visit, the same procedures as during the second visit were followed except for the experimental treatment which was the opposite of the second visit (randomized counterbalanced AB/BA cross-over design).

In Study 3 (figure 13), the same procedures as in Study 2 were used apart from the following. First of all, treatment order was not randomized. During the second visit, all subjects performed the 100 drop jumps protocol before the high-intensity constant-power cycling test to exhaustion at 80% of peak power output which, in these subjects, corresponded to 86 ± 5% of VO_{2peak} measured during the preliminary incremental exercise test. During the third visit all subjects rested comfortably for 33 min. On this occasion the cycling test was stopped at the same time when exhaustion occurred during the second visit. None of the subjects reached exhaustion before the prescribed time during this second cycling test. Furthermore, 2 min after the end of the cycling test, locomotor muscle force was assessed for a third time. In this post-cycling isometric test, subjects were asked to perform only the three maximal trials.

All subjects were instructed to avoid smoking, intense exercise, alcohol, tea, and beverages containing caffeine in the 24 h preceding each visit. During the same time, they were asked to drink 40 ml of water per kg of body mass and to maintain their usual diet. They were also instructed to have a light meal at least 3 h before reporting to the laboratory. All visits were scheduled at the same time of the day, and environmental conditions in the laboratory were kept between 18-22 °C for temperature and 45-60% for humidity.

Physiological and perceptual responses to exercise

In both studies, tidal volume (l), breathing frequency (min^{-1}), ventilation ($l\ min^{-1}$), VO_2 ($l\ min^{-1}$), and carbon dioxide production (VCO_2) ($l\ min^{-1}$) were measured breath-by-breath using a computerized metabolic gas analysis systemes (Study 2: 600Ergo Test, ZAN Messgeräte GmbH, Oberthulba, Germany; Study 3: MetaLyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany) connected to an oro-(mouth) mask (7600 series, Hans Rudolph Inc, Kansas City, Missouri). These automated devices were calibrated before each test using certified gases of known concentration (11.5% O_2 and 5.1% CO_2) and a 3.0 l calibration syringe (Series 5530, Hans Rudolph Inc, Kansas

City, Missouri). All respiratory gas exchange data were averaged over 1 min periods before statistical analysis. During rest and 1 min after the end of the high-intensity constant-power cycling test, a 5 µl sample of whole fresh blood was taken from the right earlobe and analyzed for lactate concentration (mmol l^{-1}) using a portable analyzer (Lactate Pro LT-1710, Arkray, Shiga, Japan). Lactate accumulation was calculated by subtracting the resting value from the value obtained after cycling. During the final 15 s of each minute of exercise, subjects were asked to rate their perceived exertion using a 6-20 RPE scale which was displayed throughout the cycling test.
In Study 2, a bioimpedance device (Physioflow PF05L1, Manatec, Petit-Ebersviller, France) was used to measure heart rate (HR), stroke volume (SV) and cardiac output (CO). Two sets of two electrodes (Ambu Blue Sensor VL, Ambu A/S, Ballerup, Denmark), one transmitting and the other one receiving a low amperage alternating electrical current, were applied on the supraclavicular fossa at the left base of the neck and along the xiphoid. Another set of two electrodes was used to monitor a single ECG lead in the V1/V6 position. All electrode placement areas were shaved if necessary, cleaned with an alcohol pad and dried with a paper towel. Wires connected to the electrodes were fixed on the body using tape to reduce movement artifacts. Stroke volume (ml) is estimated by this computerized device from changes in transthoracic impedance during cardiac ejection according to the method described in detail by Charloux et al. (Charloux et al., 2000). Cardiac output (l min^{-1}) was calculated as

$$CO = (HR \times SVi \times BSA) / 1000$$

where BSA is body surface area (m^2) calculated according to the Haycock formula [$BSA = 0.02465 \times \text{body mass (kg)}^{0.5378} \times \text{stature (cm)}^{0.3964}$] and SVi (ml m^{-2}) = SV / BSA. Heart rate (min^{-1}) is based on the R-R interval determined from the first derivative of the ECG. These data were averaged over 1 min periods before statistical analysis. Accuracy of CO estimation has been validated against direct Fick methods (Charloux et al., 2000). Furthermore, in a group of 20 healthy men with characteristics similar to the subjects included in

this investigation, reproducibility during intense cycling was high (coefficient of variation 3.4%) (Hsu et al., 2006). Before each test, the Physioflow was autocalibrated using a procedure based on 30 consecutive heartbeats recorded whilst the participant was resting in a seated position on the cycle ergometer, anthropometric data and resting systolic and diastolic blood pressure values (mmHg) (Charloux et al., 2000). These were the averages of two separate blood pressure recordings taken before and after the Physioflow autocalibration using an automated blood pressure monitor (Tango, SunTech Medical Ltd, Morrisville, North Carolina). The Tango device was interfaced to the Physioflow by an analog cable for the ECG trigger. The size of the cuff, which was placed on the left arm of the subject, was based on individual arm girth. Blood pressure was also monitored at the end of warm-up and every 2 min during the time to exhaustion test. Mean arterial pressure (MAP) (mmHg) was calculated as

$$MAP = [(2 \times \text{diastolic pressure}) + \text{systolic pressure}] / 3$$

Total peripheral resistance (TPR) (mmHg l^{-1}) was calculated as

$$TPR = MAP / CO$$

In Study 3, HR was measured every 5 s using a telemetric monitor (Polar S610i, Polar Electro OY, Kempele, Finland). These data were averaged over 1 min periods before statistical analysis. Rectal temperature was measured continuously during the cycling test with a disposable temperature probe (Henleys Medical Supplies Ltd, Herts, UK) inserted 10 cm beyond the anal sphincter and connected to a monitor (YSI 4000A, YSI Inc., Dayton, Ohio, USA). Temperature data were recorded every minute.

Statistical analysis

Unless otherwise noted, all data are presented as mean ± SD. The effects of condition (fatigue *vs.* control) and time on locomotor muscle force (Study 2: baseline and pre-cycling; Study 3: baseline, pre-cycling and post-cycling) and on all physiological/perceptual parameters at isotime (Study 2: end of warm-up (0 min) + first 5 min of exercise; Study 3: end of warm-up + 33, 66, and 100% of total

time) were tested using fully repeated measures multivariate analyses of variance (MANOVAs). For all MANOVAs, if a significant condition x time interaction was revealed, the main effect of condition was not considered and tests of simple main effects of condition were conducted as follow-up using the Holm-Bonferroni method (Holm, 1979). In addition to the standard follow-up procedures, in Study 3 pre-cycling *vs.* post-cycling changes in locomotor muscle force were analyzed using a paired *t* test within each condition (fatigue and control). A two-way fully repeated measures MANOVA was conducted to compare the locomotor muscle fatigue induced by the 100 drop jumps protocol (baseline *vs.* pre-cycling locomotor muscle force in the fatigue condition) with the locomotor muscle fatigue induced by the high-intensity constant-power cycling test (pre-cycling *vs.* post-cycling locomotor muscle force in the control condition). For this purpose, only the interaction was considered.

With the exception of time to exhaustion which was analyzed using the Hills-Harmitage approach (Senn, 2002), when comparing two means, paired *t* tests or Wilcoxon signed ranks tests were used as appropriate. Significance was set at 0.05 (two-tailed) for all analyses, which were conducted using the Statistical Package for the Social Sciences Version 11.

RESULTS

Study 2

Effect of experimental treatment on locomotor muscle force

There were no significant baseline differences in DOMS (median/interquartile range) (fatigue 0.00/1.00, control 0.50/1.25) and [CK] (fatigue 183 ± 204 UI l^{-1}, control 151 ± 59 UI l^{-1}) between conditions. Furthermore, follow-up tests of the significant condition x time interaction ($P = 0.003$) for knee extensor MVC revealed no significant baseline difference in this parameter (fatigue 703 ± 114 N, control 694 ± 122 N). Taken together, these three markers of exercise-

induced muscle damage suggest that the 10-14 days wash-out period prescribed in both studies was appropriate. As expected, follow-up tests revealed that pre-cycling locomotor muscle force was lower in the fatigue condition (599 ± 127 N) compared to control (697 ± 111 N) (P = 0.001). None of the subjects reported muscle pain before the cycling test in the fatigue condition but some reported symptoms of leg weakness.

Effect of experimental treatment on time to exhaustion

Average self-selected pedal frequency during the high-intensity constant-power cycling test to exhaustion was not significantly different between the fatigue (77 ± 7 RPM) and control (76 ± 7 RPM) condition. As shown in the condition by condition scatter-plot (Figure 14), all but one subject had a reduction in exercise performance in the fatigue condition compared to control. On average, time to exhaustion was 636 ± 278 s after the 100 drop jumps and 750 ± 281 s in the control condition (P = 0.003). There was no significant order effect between the first and the second time to exhaustion test irrespective of condition.

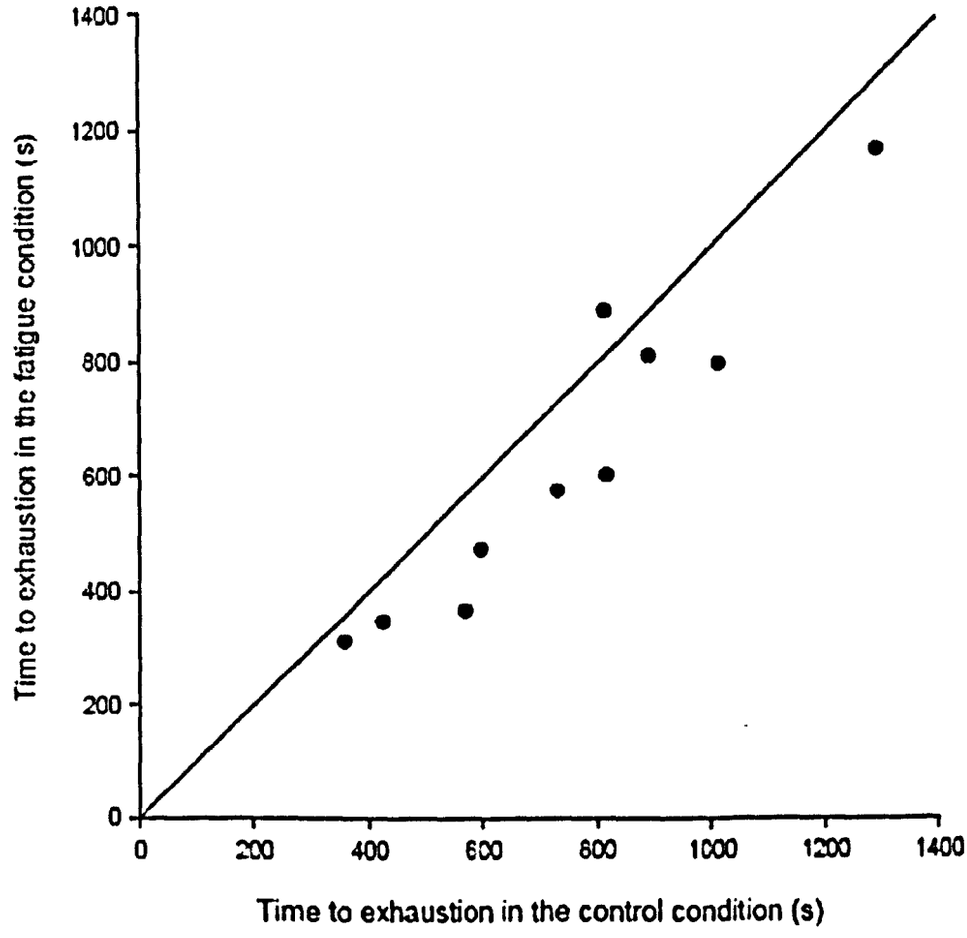

Figure 3

Effect of experimental treatment on time to exhaustion during high-intensity constant-power cycling (N = 10)

Scatterplot of time to exhaustion in the fatigue condition (100 drop jumps over 33 min) and time to exhaustion in the control condition (33 min rest). The points below the identity line represent a decreased performance in the fatigue condition compared to the control condition in individual participants.

Effects of experimental treatment on physiological and perceptual responses to exercise

All physiological and perceptual parameters showed the expected response to high-intensity constant-power cycling to exhaustion (time $P < 0.05$). No significant effects of experimental treatment on VO_2 were found during the first 5 min of exercise (isotime) or at exhaustion (Figure 15A). Similarly, lactate accumulation was not significantly different between the fatigue (8.9 ± 2.4 mmol l^{-1}) and control condition (10.0 ± 2.4 mmol l^{-1}). Even when normalized for the different duration of two cycling tests, the increase in capillary blood lactate concentration was not significantly different between the fatigue (1.0 ± 0.5 mmol l^{-1} min^{-1}) and control (0.9 ± 0.4 mmol l^{-1} min^{-1}) condition. Despite similar metabolic requirements, ventilation at isotime was significantly higher after the 100 drop jumps compared to control (condition $P = 0.016$) (Figure 15C). However, no significant difference was found at exhaustion. The hyperpnea observed during the first 5 min of exercise in the fatigue condition was due to higher breathing frequency (condition $P = 0.019$) (Figure 15D) as tidal volume was not affected by experimental treatment (Figure 15E). No significant differences between conditions were found at exhaustion in both breathing frequency and tidal volume. During the first 5 min of exercise there was a small but statistically significant increase in VCO_2 (condition $P = 0.026$) (Figure 15B). No significant difference between conditions was found at exhaustion. Transthoracic impedance analysis revealed that during the first 5 min of exercise CO was significantly increased in the fatigue condition compared to control (condition $P = 0.047$) (Figure 16D). This was a hyperdynamic circulatory response as HR (condition $P < 0.001$) (Figure 16B) rather than SV (Figure 16C) was significantly affected by experimental treatment. However, the eccentric exercise protocol did not significantly affect CO, HR and SV at exhaustion. The effect of experimental treatment on MAP during the first 5 min of exercise did not reach statistical significance (condition $P = 0.097$) (Figure 16E). The last MAP reading was taken (median/interquartile range) 29/82 s

before exhaustion in the fatigue condition and 26/76 s before exhaustion in the control condition ($P = 0.646$ by Wilcoxon signed ranks test). No significant difference in MAP was found between conditions at these times. Similarly, the 100 drop jumps protocol did not have a significant effect on TPR either at isotime or at exhaustion (Figure 16F). The small increase in RPE at isotime observed in the fatigue condition compared to control was not statistically significant (Figure 16A). Perception of effort at exhaustion was unaffected by experimental treatment.

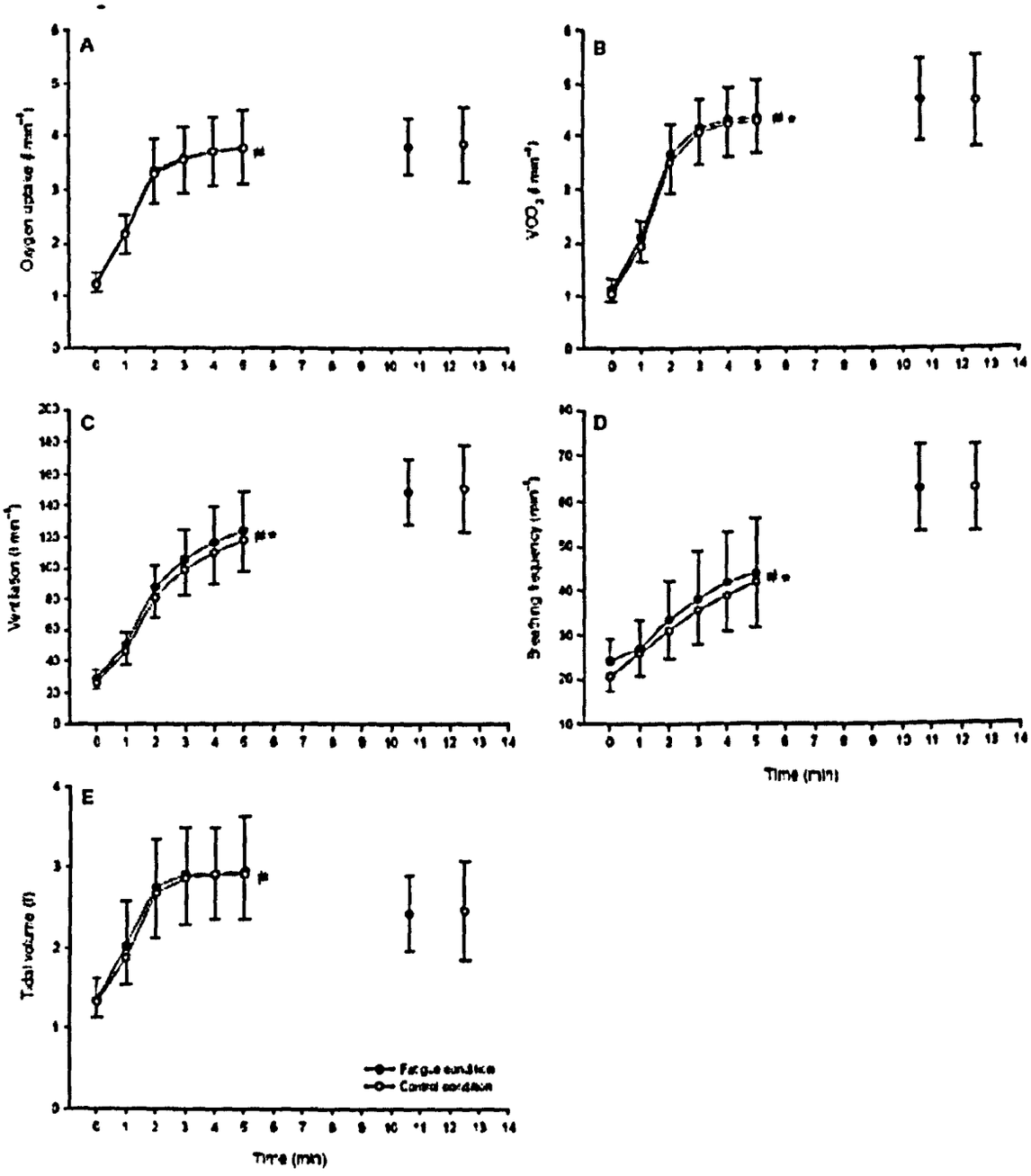

Figure 4

Figure 2. Effects of experimental treatment on metabolic and respiratory responses during high-intensity constant-power cycling (N = 10)

Fatigue condition consisted of 100 drop jumps over 33 min. Control condition consisted of 33 min rest. # Significant main effect of time ($P < 0.05$). * Significant main effect of condition ($P < 0.05$). Data are presented as mean ± SD. VCO_2 = carbon dioxide production.

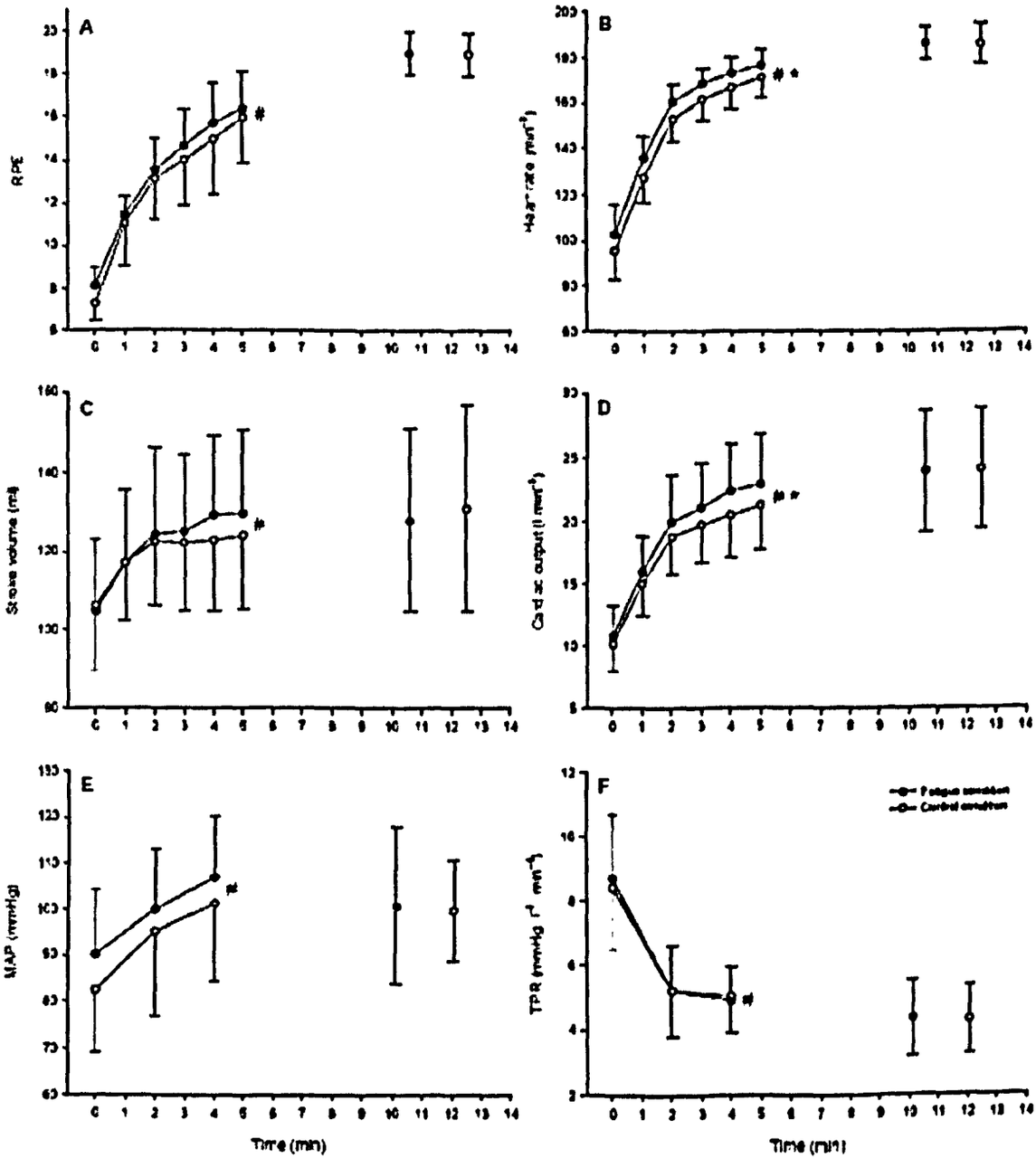

Figure 5

Effects of experimental treatment on perception of effort and cardiovascular responses during high-intensity constant-power cycling (N = 10)

Fatigue condition consisted of 100 drop jumps over 33 min. Control condition consisted of 33 min rest. # Significant main effect of time ($P < 0.05$). * Significant main effect of condition ($P < 0.05$). Data are presented as mean ± SD. RPE = rating of perceived exertion. MAP = mean arterial pressure. TPR = total peripheral resistance.

Study 3

Effects of experimental treatment and high-intensity cycling on locomotor muscle force

As in Study 2, follow-up tests of the significant condition x time interaction ($P < 0.001$) for knee extensor MVC (Figure 17) revealed no significant baseline difference but a significant simple main effect in the pre-cycling isometric test with locomotor muscle force significantly lower in the fatigue condition ($P = 0.001$). None of the subjects reported muscle pain before the cycling test in the fatigue condition but some reported symptoms of leg weakness.

In both the fatigue and control condition, subjects cycled for an average of 871 ± 280 s, and average self-selected pedal frequency was not different between conditions (fatigue 80 ± 8 RPM, control 81 ± 6 RPM). The longer duration of the cycling test in Study 3 is due to the slightly lower exercise intensity (86 ± 5% of VO_{2peak}) compared to Study 2 (90 ± 7% of VO_{2peak}). At the end of this exercise, knee extensor MVC was not significantly different between the fatigue and control condition. The two additional follow-up tests conducted between pre- and post-cycling in both conditions revealed no significant change in locomotor muscle force in the fatigue condition. However, in the control condition there was a significant decline in knee extensor MVC ($P < 0.001$). The additional two-way fully repeated measures MANOVA comparing this muscle fatigue (pre-cycling *vs.* post cycling in the control condition) with the muscle fatigue induced by the 100 drop jumps protocol (baseline *vs.* pre-cycling in the fatigue condition) revealed no significant interaction (Figure 17).

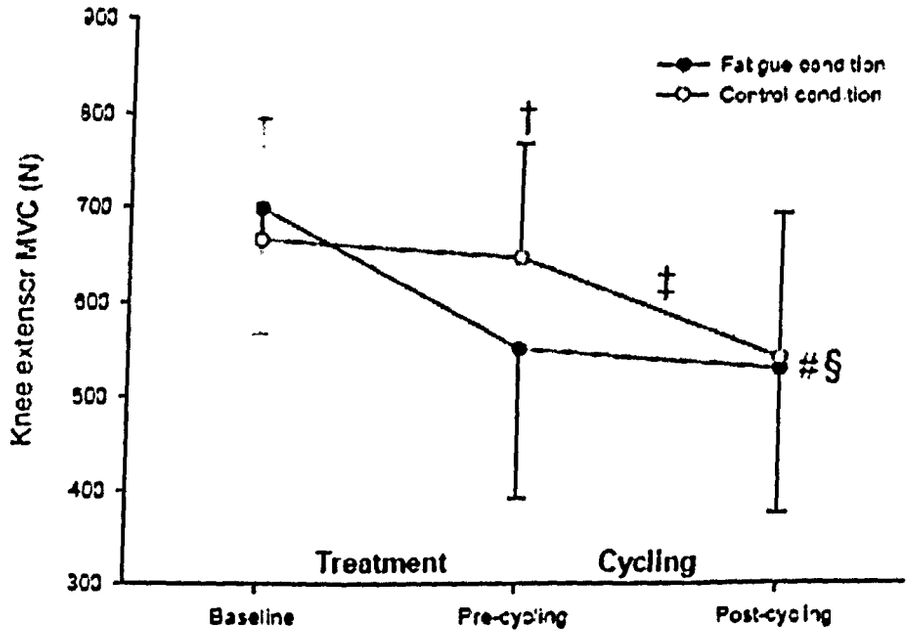

Figure 6

Effects of experimental treatment and high-intensity constant-power cycling on knee extensor MVC (N = 14)

Fatigue condition consisted of 100 drop jumps over 33 min. Control condition consisted of 33 min rest. # Significant main effect of time ($P < 0.05$). § Significant interaction ($P < 0.05$). † Significant simple main effect of condition according to Holm-Bonferroni method. ‡ Significant simple main effect of time according to Holm-Bonferroni method. Data are presented as mean ± SD. MVC = maximal voluntary contraction.

Effects of experimental treatment on physiological and perceptual responses to exercise

All physiological and perceptual parameters showed the expected response to high-intensity constant-power cycling (time $P < 0.05$). Although there was a significant interaction ($P = 0.036$), tests of simple main effects revealed no significant differences in VO_2 between fatigue and control condition at any time point (Figure 18A). Similarly, lactate accumulation was not significantly different between the fatigue (12.7 ± 2.1 mmol l^{-1}) and control condition (12.3 ± 2.4 mmol l^{-1}). As expected, there was a significant effect of experimental treatment on breathing pattern (interaction $P = 0.021$) (Figure 18D). Tests of simple main effects of condition revealed a significant difference in breathing frequency at 100% of total time ($P < 0.001$). Despite this tachypnea, ventilation was only marginally different between the fatigue condition and control (condition $P = 0.065$) (Figure 18C). This occured because there was a concurrent marginal reduction in tidal volume (condition $P = 0.064$) (Figure 18E). The small effect of experimental treatment on ventilation did not result in a significant difference in VCO_2 between the fatigue and control condition (Figure 18B). However, there was a significant effect of experimental treatment on rectal temperature which was 0.5 °C higher in the fatigue condition compared to control throughout the cycling test (condition $P < 0.001$) (Figure 18F). As in Study 2, the HR response to exercise was affected by experimental treatment (interaction $P = 0.008$). Tests of simple main effects revealed that HR was significantly higher in the fatigue condition compared to control at each time point (0%, 33%, and 66% $P < 0.001$; 100% $P = 0.002$) (Figure 19B). In this study the effect of experimental treatment on perception of effort was statistically significant with higher RPE in the fatigue condition compared to control (condition $P = 0.043$) (Figure 19A).

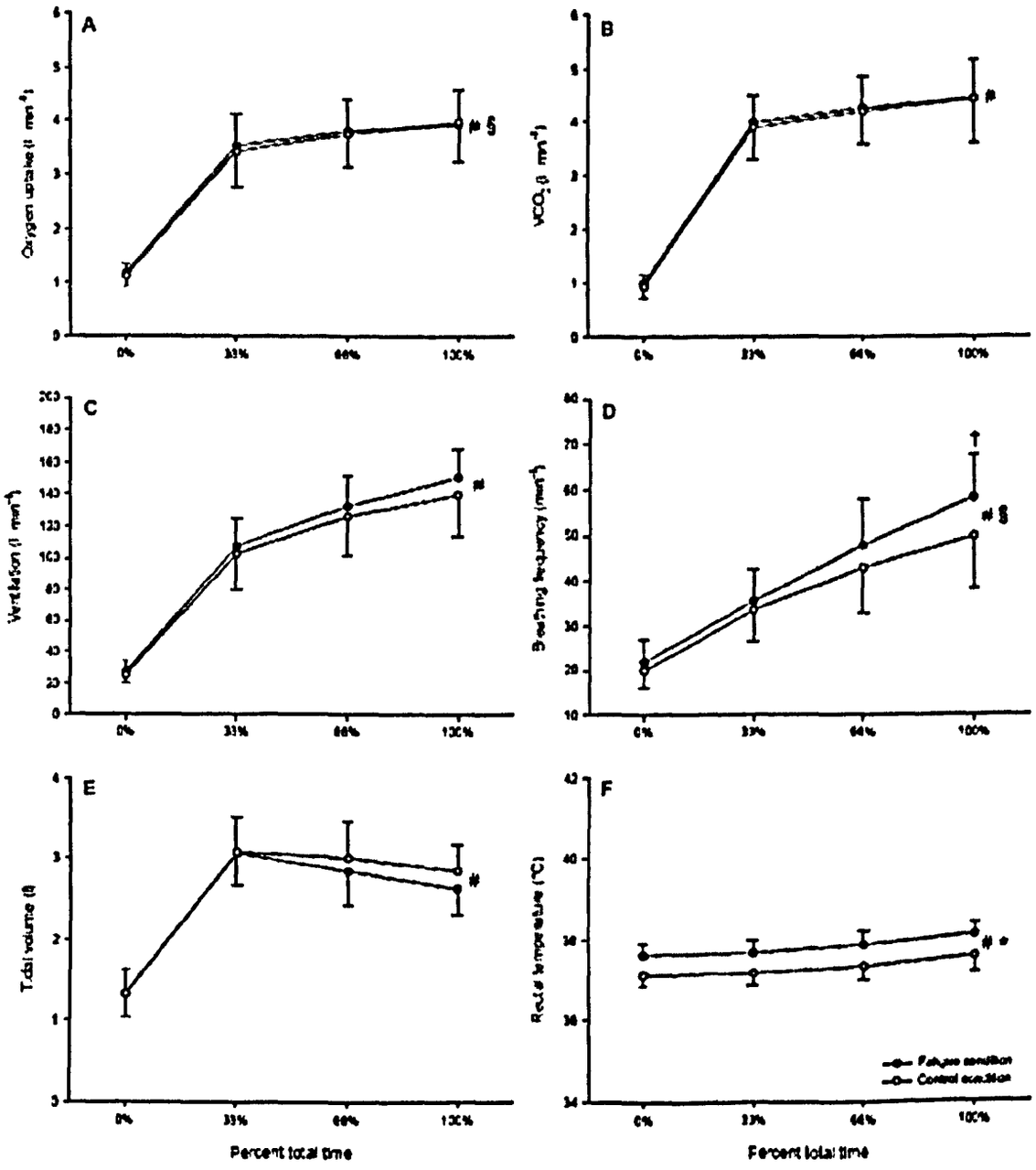

Figure 7

Effects of experimental treatment on metabolic, respiratory, and thermal responses during high-intensity constant-power cycling (N = 14)

Fatigue condition consisted of 100 drop jumps over 33 min. Control condition consisted of 33 min rest. # Significant main effect of time ($P < 0.05$). § Significant interaction ($P < 0.05$). † Significant simple main effect of condition according to Holm-Bonferroni method. Data are presented as mean ± SD. VCO_2 = carbon dioxide production.

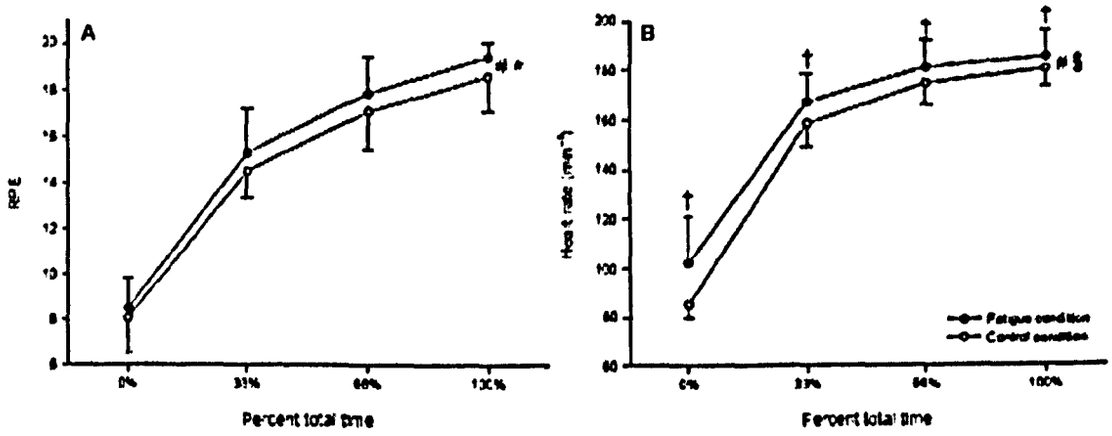

Figure 8

Effects of experimental treatment on perception of effort and heart rate during high-intensity constant-power cycling (N = 14)

Fatigue condition consisted of 100 drop jumps over 33 min. Control condition consisted of 33 min rest. # Significant main effect of time ($P < 0.05$). * Significant main effect of condition ($P < 0.05$). § Significant interaction ($P < 0.05$). † Significant simple main effect of condition according to Holm-Bonferroni method. Data are presented as mean ± SD. RPE = rating of perceived exertion.

DISCUSSION

Effects of experimental treatment and intense cycling exercise on locomotor muscle force

The 100 drop jumps protocol induced, on average, a significant 18% reduction in knee extensor MVC. This locomotor muscle fatigue is similar to that reported in previous studies using the same eccentric exercise protocol (Nielsen et al., 2005, Skurvydas et al., 2002) and it is physiologically relevant. In fact, a similar reduction in knee extensor MVC has been measured after high-intensity constant-power cycling tests to exhaustion (Amann et al., 2006, Amann et al., 2007, Romer et al., 2007a, Amann and Dempsey, 2007a) and, from a functional point of view, it is irrelevant whether excitation-contraction coupling failure is induced by structural alterations or by metabolic stress (Fitts, 2006).

Interestingly, in Study 3 the locomotor muscle fatigue induced by the 100 drop jumps (difference between baseline and pre-cycling in the fatigue condition, Figure 17) was not significantly different from the locomotor muscle fatigue induced by 14.5 min of intense cycling in the control condition (difference between pre-cycling and post-cycling, Figure 17). The same exercise intensity and duration, however, did not induce a further reduction in locomotor muscle force in the fatigue condition (difference between pre-cycling and post-cycling in Figure 17). These findings are extremely interesting if we consider that metabolic fatigue at whole-muscle or muscle group level is primarily due to mechanical dysfunction in a relatively small population of fast fatigue-sensitive fibres which occurs early during intense cycling (Sargeant, 2007), and that these muscle fibres are also the most sensitive to the fatiguing effects of eccentric exercise (Rijkelijkhuizen et al., 2003, Proske and Morgan, 2001). We therefore speculate that i) the same population of fast fatigue-sensitive fibres was fatigued during both the 100 drop jumps protocol and high-intensity constant-power cycling in the control condition, and ii) no further loss of knee extensor MVC occurred in the fatigue condition

because the fast fatigue-sensitive fibres were already fatigued by the 100 drop jumps protocol, and metabolic stress did not affect the remaining fast and slow fatigue-resistant fibres (Sargeant, 2007).

Effect of locomotor muscle fatigue on markers of central motor command

In order to cycle at high-intensity and the same constant-power with locomotor muscles weakened by the 100 drop jumps, central motor command must have been increased. This effect of experimental treatment was checked by measuring HR and RPE, two markers of central motor command commonly used in physiological studies (Smith et al., 2003, Gallagher et al., 2001, Norton et al., 1999). In agreement with our hypothesis, we found significant increases in HR in both studies, and a significant increase in RPE in Study 3. The non significant increase in RPE in Study 2 is likely due to lower statistical power (because of smaller sample size and higher variability of RPE scores below 17 (Eston and Williams, 1988)) rather than to a lack of effect. Indeed, the difference in perception of effort at isotime between the fatigue and control condition in Study 2 (Figure 16A) is similar to the one measured in Study 3 (Figure 19A). The small increases in HR and RPE measured in our investigation should be expected if a dose-response relationship exists between muscle weakness, central motor command, HR and RPE. Indeed, our eccentric exercise protocol induced an 18% reduction in force which was limited to the locomotor muscles. In curarization studies force of all skeletal muscles, not just the locomotor muscles, was reduced by 50% or more to obtain larger changes in central motor command, HR and RPE (Asmussen et al., 1965, Gallagher et al., 2001).

Effects of locomotor muscle fatigue on metabolic stress and cardiorespiratory responses to exercise

As expected from the results of a previous muscle biopsy study (Nielsen et al., 2005) and our pilot, the 100 drop jumps protocol did not affect the metabolic stress associated with high-intensity constant-

power cycling. Indeed, in both studies VO_2 and lactate accumulation did not differ between the fatigue and control condition. Furthermore, eccentric exercise does not increase the sensitivity of group III and IV muscle afferents to metabolic stimuli (Taguchi et al., 2005). Therefore, we can assume that the influence of the metabo-reflex on the cardiorespiratory responses to exercise (Mateika and Duffin, 1995, Smith et al., 2006) was similar between the fatigue and control condition. The influence of the mechano-reflex was also controlled as power output and cadence were the same in both conditions, and eccentric exercise does not alter the sensitivity of muscle spindles and Golgi tendon organs (Gregory et al., 2004, Gregory et al., 2002). Nevertheless, in Study 2 we measured a significant increase in cardiac output (which was mainly due to the increase in HR) and a trend for increased MAP in the fatigue condition with no significant changes in TPR compared to control. This hyperdynamic circulatory response in excess of metabolic requirements is similar to the one reported in studies using low doses of curare to weaken skeletal muscles (Asmussen et al., 1965, Gallagher et al., 2001). The smaller magnitude of the cardiovascular effects of our experimental treatment is likely to reflect the smaller change in central motor command induced by the 100 drop jumps protocol compared to partial neuromuscular blockade. In Study 2 we also noticed a remarkable effect of experimental treatment on breathing frequency, particularly near exhaustion (Figure 20). This tachypneic response has been described in previous studies (Spengler et al., 2000) and it is typical of high-intensity constant-power cycling (Syabbalo et al., 1994).

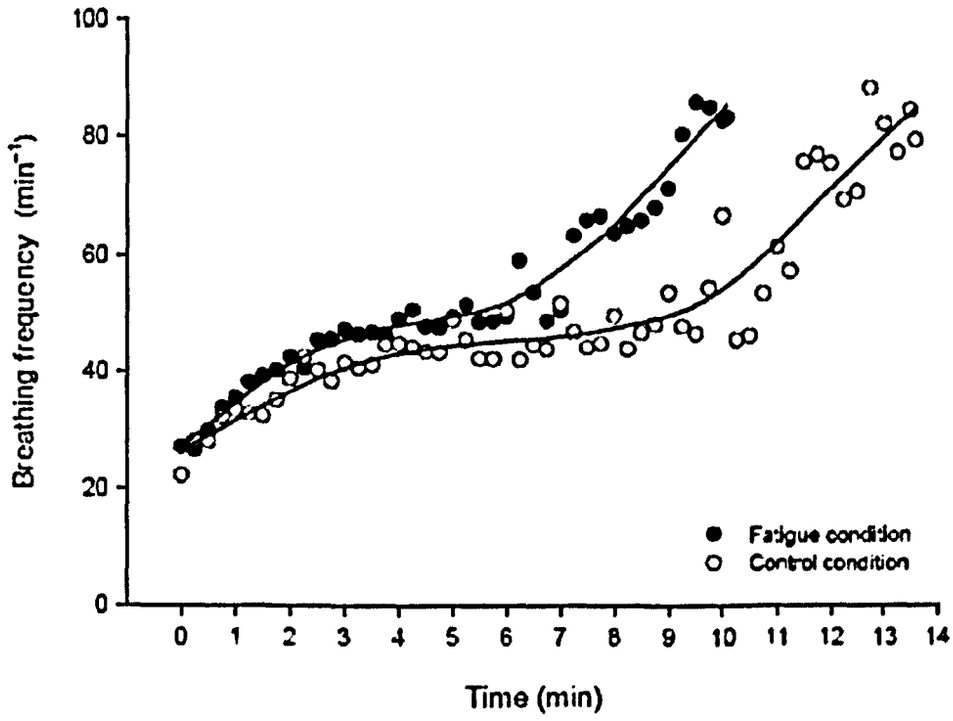

Figure 9

Tachypneic response during high-intensity constant-power cycling to exhaustion in a representative subject

In order to study this phenomenon in more detail, in Study 3 we compared ventilatory responses at the same time points and measured core body temperature, another potential mechanism for the tachypnea observed during high-intensity constant-power cycling (Dempsey et al., 2006a). As expected, the phenomenon observed in Study 2 was replicated in Study 3 where a higher breathing frequency was found in the fatigue condition compared to control particularly at the end of the cycling test (Figure 18D). Despite the 30 min rest period between the end of the experimental treatment and the beginning of the cycling tests, the heat accumulated during the 100 drop jumps protocol was not fully dissipated. For this reason, in the fatigue condition subjects started cycling with a rectal temperature 0.5 °C higher than in the control condition. The rate of heat storage was not affected by experimental treatment and, therefore, this difference in rectal temperature was maintained throughout the cycling test. Nevertheless, this effect of experimental treatment is not large enough to explain the different breathing pattern observed during exercise between the fatigue and control condition. Indeed, a difference in core body temperature of more than 1.0 °C is necessary to significantly affect respiratory responses to exercise (Mateika and Duffin, 1995). There is also evidence that the sensitivity of group III and IV muscle afferents to thermal stimuli is not altered by eccentric exercise (Taguchi et al., 2005). Therefore, it is unlikely that the small difference in core and, most likely, muscle temperature induced by the 100 drop jumps protocol mediated its striking effect on the tachypneic response to high-intensity constant-power cycling (Figure 20). Overall, the results of our study provide experimental support to previous suggestions that the increased central motor command required to exercise at a constant workload with fatigued locomotor muscles plays an important role in the complex regulation of the cardiorespiratory responses to sustained exercise (Syabbalo et al., 1994, Spengler et al., 2000, Norton et al., 1999, Dempsey et al., 2006a).

exactly the same point in time. The finding that reduced locomotor muscle force *per se* caused premature exhaustion suggests that the conscious (leg discomfort) and/or subconscious (inhibitory supraspinal reflex) effects of muscle metabolic stress on the CNS do not play a determinant role in regulating exercise performance in normoxia as hypothesized by Amann and Dempsey on the basis of correlative data (Amann and Dempsey, 2007b, Amann and Dempsey, 2007a).
So why did subjects stop cycling earlier in the fatigue condition compared to control? We propose that exhaustion occurred prematurely after the 100 drop jumps because of the higher effort required to exercise at high intensity with fatigued locomotor muscles. According to Brehm's motivational intensity theory (Brehm and Self, 1989) people engage in a task until the effort required reaches the maximum level of effort they are willing to invest in that task, the so-called potential motivation (Wright, 1996). Because of increased central motor command to the weaker locomotor muscles and higher breathing frequency (Noble and Robertson, 1996), overall perception of effort was significantly increased in the fatigue condition compared to control (Figure 19A). As a result fatigued subjects reached the maximum level of effort they were willing to invest in the exercise task (~ 19 on the 6-20 RPE scale) on average two minutes earlier than in the control condition (Figure 16A). At this time point, task disengagement, rather than task failure, occurred. In other words, we propose that exhaustion occurred because subjects were unwilling to invest further effort in keeping their cadence above 60 RPM rather than because they were physiologically unable to do so. According to this psychobiological model based on Brehm's motivational intensity theory, any physiological or psychological factor affecting overall perception of effort and/or potential motivation (Wilmore, 1968) would affect exercise performance.

Perspectives and Significance

By dissociating locomotor muscle fatigue from the metabolic stress that usually accompanies it, we demonstrated for the first time that

reduced locomotor muscle force *per se* has significant effects on cardiorespiratory responses and time to exhaustion during intense cycling exercise. These findings are in contrast with the recent proposal that exercise performance in normoxia is regulated by the CNS on the basis of afferent neural feedback related to metabolic stress in the locomotor muscles (Amann and Dempsey, 2007b, Amann and Dempsey, 2007a). However, our results confirm previous suggestions that increased central motor command to fatigued locomotor muscles has an important influence on cardiorespiratory regulation during prolonged constant-workload exercise (Syabbalo et al., 1994, Spengler et al., 2000, Norton et al., 1999, Dempsey et al., 2006a). In accordance with the largely ignored recommendation that the study of fatigue should address both perceived exertion and the decline in force that occurs during sustained exercise (Barry, 2007), we propose that further studies are necessary to investigate the sensory psychobiology of perception of effort and its important role in the regulation of exercise performance (Jones and Killian, 2000).

CHAPTER 4

EFFECTS OF LOCOMOTOR MUSCLE FATIGUE ON PHYSIOLOGICAL RESPONSES, EXERTIONAL SYMPTOM INTENSITY, AND PERFORMANCE DURING INCREMENTAL CYCLING EXERCISE

INTRODUCTION

Locomotor muscle fatigue, defined as an exercise-induced reduction in maximal voluntary force produced with the locomotor muscles (Gandevia, 2001), reduces time to exhaustion and increases cardiorespiratory responses during high-intensity constant-power cycling exercise as demonstrated in Chapter 3. Because locomotor muscle fatigue was not associated with metabolic stress in our experimental model (Nielsen et al., 2005), we proposed that these effects are mediated by the increase in central command required to cycle at the same workload with weaker locomotor muscles. In fact, corollary discharges of the central motor command stimulate the medullary cardiorespiratory centres (Waldrop, 1996) and provide sensory inputs for perception of effort during exercise (Chapter 3). According to our psychobiological model based on motivational intensity theory (Chapter 3), increased perceived exertion is the main factor mediating the negative effect of locomotor muscle fatigue on exercise performance.

An increase in central motor command may also contribute to the reduced exercise performance and exaggerated cardiorespiratory and perceptual responses observed during incremental exercise tests in patients with cardiorespiratory diseases (Hamilton et al., 1995). In fact these patients present with locomotor muscle dysfunction which includes both muscle weakness and increased fatigability (Gosker et al., 2000, Mador and Bozkanat, 2001, Wilson, 1996). However, in patients it is impossible to dissociate the effects of increased central command due to reduced locomotor muscle force from the effects of increased afferent feedback from the working muscles. In fact, these patients have both an increased metabolic stress and increased sensitivity to metabolic stimuli of type III and IV muscle afferents (Piepoli et al., 1996, Ponikowski et al., 2001, Schmidt et al., 2005). Therefore, the aim of the present study was to use the same fatiguing protocol we used previously (Chapter 3) to investigate the effects of reduced locomotor muscle force *per se* on physiological responses and

performance during incremental cycling exercise, one of the most common test used in healthy people and patients with cardiorespiratory disorders (Jones and Killian, 2000, Hamilton et al., 1995). The effects of locomotor muscle fatigue on the intensity of different exertional symptoms (leg effort, dyspnea, and leg pain) were also investigated. We hypothesised that reduced locomotor muscle force increases cardiorespiratory responses during the final stages of an incremental exercise test, and reduces peak power output because of a significant increase in leg effort and dyspnea independently of metabolic stress.

MATERIALS AND METHODS

Subjects

Twenty-four subjects (18 males and 6 females) were recruited among students at Bangor University. All subjects were involved in regular aerobic activities and a medical questionnaire was administered before testing to exclude subjects with conditions contraindicating maximal exercise. The study protocol was approved by the Ethics Committee of the School of Sport, Health and Exercise Sciences (SSHES), Bangor University. All participants were informed of the procedures of the study, related benefits and risks, and had to give their signed informed consent before taking part.

Study design

A two-group, randomized, controlled, pretest–posttest design was used for the present study (Figure 21). Subjects came to SSHES Physiology Laboratory three times.

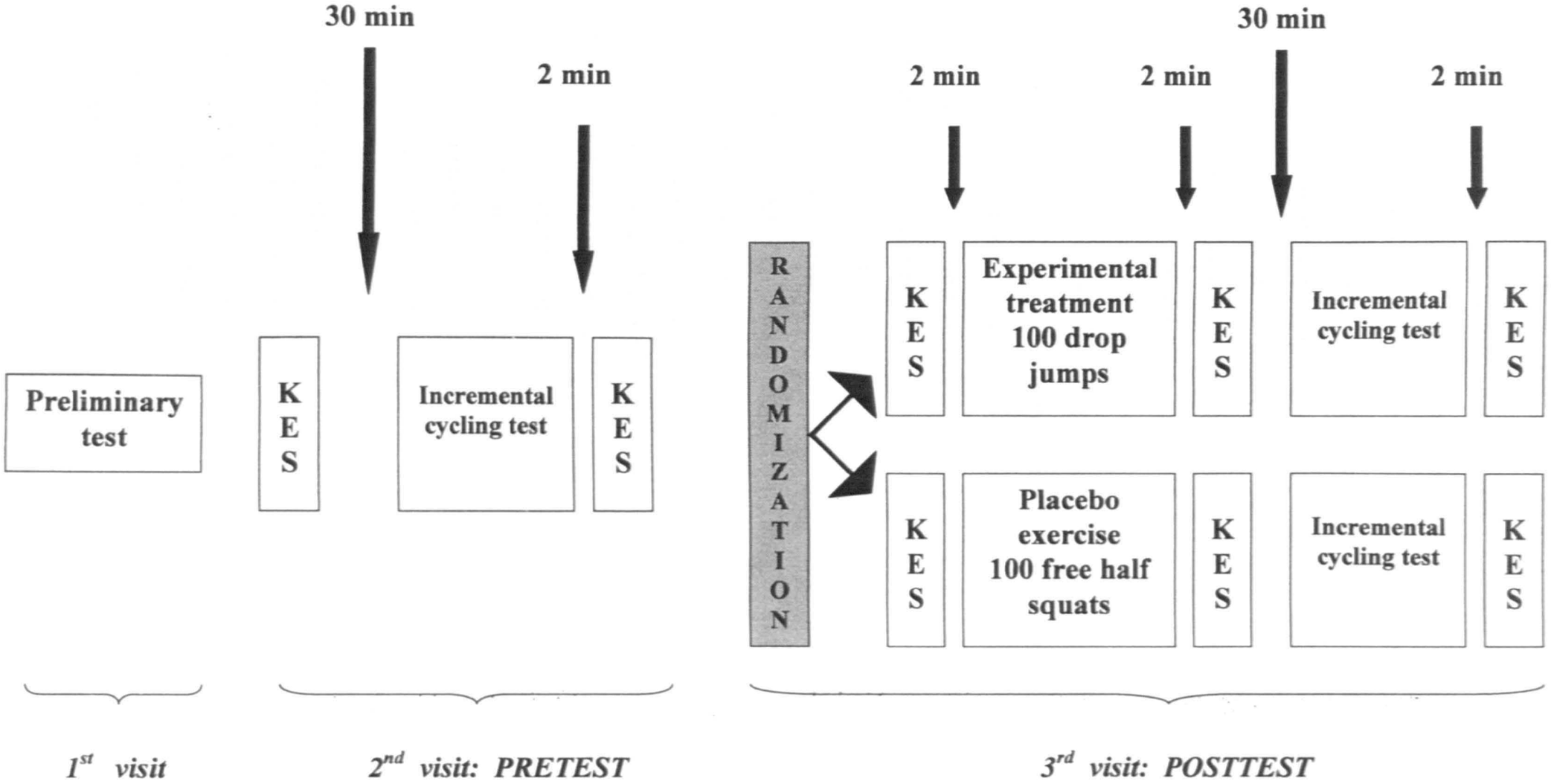

Figure 21

Study Design. KES, knee extensors strength.

During the first preliminary visit, body size and composition, and maximal oxygen consumption ($\dot{V}O_2max$) and peak power output (PPO) were measured (see *Preliminary testing* section for further details). After a minimum of 24 h, subjects came for the second visit (pretest). After general warm-up (consisting of 5 min cycling at 20% of PPO), knee extensor strength (KES) was assessed (pre-cycling KES). Thirty minutes after strength testing, exercise responses and performance were measured during an intermittent incremental cycling test until exhaustion (see *Intermittent incremental cycling test protocol* section for further details). Two minutes after exhaustion, KES was assessed again but without specific warm-up (post cycling KES). After a period of 48-72 hours, subjects returned to the Laboratory for the third and last visit (posttest). After general warm-up, KES was assessed (pre-treatment KES). Then subjects, which were matched for gender, were randomly assigned to either experimental or control treatment (see *Treatment* section for further details). After treatment, subjects repeated in the same order and timing the tests administered at pretest (pre-cycling KES, incremental cycling test, post cycling KES). Before each visit, subjects were asked to avoid vigorous exercise, smoking, alcohol, tea, coffee, and to drink 35 ml of water per kg of body mass in the 24 h preceding each visit. They were also instructed to have a light meal at least 3 h before reporting to the Laboratory, and to maintain their usual diet throughout the study. All visits were scheduled at the same time of the day, and environmental conditions in the Laboratory were always between 20 and 22 C° (temperature), and 40% and 50% (humidity).

Preliminary testing

Body size and composition were assessed by means of a wall-mounted stadiometer (Model 26SM, Seca, Hamburg, Germany) and a standing bioelectrical impedance analyzer (TBF-305, Tanita Corporation, Tokyo, Japan), using the proprietary sex-specific equations for athletes.

An incremental exercise test (2 min at 50 W + 50 W increments every 2 min) was performed until exhaustion [operationally defined as a pedal frequency of less than 60 revolutions per min (RPM) for more than 5 s despite strong verbal encouragement] on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) to measure maximal oxygen consumption ($\dot{V}O_2max$), and peak power output (PPO) which was calculated according to the equation of Kuipers et al. (Kuipers et al., 1985). The cycle ergometer was set in hyperbolic mode which allows the power output to be set independently of pedal frequency over the range of 30 to 120 RPM. Before the incremental exercise test the position on the cycle ergometer was adjusted for each subject, and settings were recorded so that they could be reproduced at each subsequent visit.

Tidal volume (VT), breathing frequency (BF), minute ventilation ($\dot{V}E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO2$) and respiratory exchange ratio (RER) were measured breath by breath using an automated metabolic gas analysis system (600Ergo Test, ZAN Messgerate, Oberthulba, Germany). This device was calibrated before each test using certified gases of known concentration (11.5% O2 and 5.1% CO2) and a 3.0 L calibration syringe (Series 5530, Hans Rudolph Inc., Kansas City, Missouri, USA). The highest $\dot{V}O_2$ value measured during the test was considered maximal ($\dot{V}O_2max$) when any two of the following criteria were met: a respiratory exchange ratio $\geq$ 1.15; a maximal heart rate higher than the 90% of the subject's age-predicted maximal heart rate; a plateau in the $\dot{V}O_2$ with an increase in work rate; a leg or chest RPE higher than 8 on the CR-10. The cycle ergometer was regularly checked for accuracy power output, and cadence.

During this test, subjects were also familiarised with the Borg category ratio 10 (CR-10) scale for leg effort, dyspnea, and leg pain (Borg, 1998a). Subjects were asked to rate exertional symptoms in the last 15 s of each stage and immediately after exhaustion.

Treatment

Subjects allocated to the experimental treatment group performed an eccentric exercise protocol aimed at reducing locomotor muscle force without inducing metabolic stress(Nielsen et al., 2005, Skurvydas et al., 2000). Subjects were asked to step on a 40 cm bench alternating the left and right leg. Once they were standing on the bench, subjects dropped on the floor with both feet and squatted down to about 90° knee angle before jumping in place as high as possible. This was repeated 100 times and a rest period of 20 seconds was observed between each jump (33 min in total). Throughout the entire protocol, subjects maintained their hands on the hips. To minimise the potential nocebo effect of fatiguing exercise, subjects allocated to the control group performed a protocol consisting of 100 free half squats separated by 20 seconds rest between each movement for a total of 33 min. During both the protocols subjects' heart rate was recorded on a heart rate monitor (Polar S 810, Kempere, Finland).

Knee extensor strength

Bilateral voluntary isometric strength of the knee extensors was measured with subjects seated in a rigid, straight-backed chair with a 90° knee and hip angle. After specific warm-up consisting of three submaximal trials (25%, 50%, 75% of maximal effort), subjects were asked three times to push maximally for 5 s against pads placed just proximal to their ankle joints and inextensibly attached to a load cell (Model No. 615, Tedea Huntleigh-Vishay, California, USA) connected to an A/D converter for data recording and analysis (Bridge Amp, Powerlab/16SP, Power Lab Chart V 4.2.3, Adi Instruments Pty Ltd., Bella Vista, Australia). Between all six trials 1 min rest was observed. During the maximal trials, strong verbal encouragement was given. Peak force produced during each of the three maximal trials was recorded and the best score noted for statistical analysis.

Intermittent incremental cycling test protocol

Subjects were required to cycle at four different intensities corresponding to the 20, 40, 60, 80% of their previously determined PPO. These stages lasted 4 minutes each, and were separated by a 1 min rest period. After that, the test continued with 1 min increments corresponding to 5% of PPO starting from 80% of PPO until exhaustion (see *Preliminary testing* section for further details). During the test a self selected cadence between 60 and 100 rpm was chosen by each subject. Feedback on revolution per minute was available, and subjects could choose the favourite cadence that was recorded every minute during the pretest and asked to be held during the posttest.

Physiological and perceptual responses to exercise

A bioimpedance device (Physioflow PF05L1, Manatec, Petit-Ebersviller, France) was used to measure heart rate (HR), stroke volume (SV) and cardiac output (CO). Two sets of two electrodes (Ambu Blue Sensor VL, Ambu A/S, Ballerup, Denmark), one transmitting and the other one receiving a low amperage alternating electrical current, were applied on the supraclavicular fossa at the left base of the neck and along the xiphoid. Another set of two electrodes was used to monitor a single ECG lead in the V1/V6 position. All electrode placement areas were shaved if necessary, cleaned with an alcohol pad and dried with a paper towel. Wires connected to the electrodes were fixed on the body using tape to reduce movement artifacts. Stroke volume (ml) is estimated by this computerized device from changes in transthoracic impedance during cardiac ejection according to the method described in detail by Charloux et al. (Charloux et al., 2000). Cardiac output (l min^{-1}) was calculated as CO = (HR x SVi x BSA) / 1000 where BSA is body surface area (m^2) calculated according to the Haycock formula [BSA = 0.02465 x body mass $(kg)^{0.5378}$ x stature $(cm)^{0.3964}$] and SVi (ml m^{-2}) = SV / BSA. Heart rate (min^{-1}) is based on the R-R interval determined from the first derivative of the ECG. All data were averaged over 1 min periods before statistical analysis. Accuracy of CO estimation has been

validated against direct Fick methods (Charloux et al., 2000) and in a group of 20 healthy men with characteristics similar to the subjects included in this investigation reproducibility during high-intensity cycling was high (coefficient of variation 3.4%) (Hsu et al., 2006). Before each test, the Physioflow was autocalibrated using a procedure based on 30 consecutive heartbeats recorded whilst the participant was resting in a seated position on the cycle ergometer, anthropometric data and resting systolic and diastolic blood pressure values (mmHg) (Charloux et al., 2000). These were the averages of two separate blood pressure recordings taken before and after the Physioflow autocalibration using an automated blood pressure monitor (Tango, SunTech Medical Ltd, Morrisville, North Carolina). The Tango device was interfaced to the Physioflow by an analog cable for the ECG trigger. The size of the cuff, which was placed on the left arm of the subject, was based on individual arm girth. Mean arterial pressure (MAP) (mmHg) was calculated as MAP = [(2 x diastolic pressure) + systolic pressure] / 3 Total peripheral resistance (TPR) (mmHg l^{-1}) was calculated as TPR = MAP / CO. Blood pressure was measured during the last minute of each stage.

A 140 µl sample of whole fresh blood from the right earlobe was collected into a pre-heparinized capillary tube. To ensure arterialization of capillary blood, the earlobe was prepared with an ointment (Finalgon Extra Stark, Thomae, Biberach, Germany) to induce hyperemia. Samples were analyzed at 37 °C for pH, partial pressures of carbon dioxide (P_aCO_2) and oxygen (P_aO_2), [K^+] and Lactate (La) using an automated analyzer (GEM Premier 3000, Instrumentation Laboratory, Barcelona, Spain) which was calibrated before each test. Samples were directly analyzed or immediately capped, agitated gently and stored in an ice bath for later analysis (within 30 min). Samples were collected during the last 30 seconds of each stage

Tidal volume (VT), breathing frequency (BF), minute ventilation ($\dot{V}E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO2$) and respiratory exchange ratio (RER) were measured breath by breath using

the same automated metabolic gas analysis system during preliminary testing (600Ergo Test, ZAN Messgerate, Oberthulba, Germany). Borg's CR-10 scale was used to quantify leg effort, dyspnea, and leg pain following standard instructions and anchoring during the preliminary incremental exercise test (Borg, 1998a).

Rectal temperature was measured continuously with a disposable temperature probe (Henleys Medical Supplies Ltd, Herts, UK) inserted 10 cm beyond the anal sphincter and connected to a monitor (YSI 4000A, YSI Inc., Dayton, Ohio, USA). Temperature was recorded at the end of each stage.

For HR, CO, SV, Vt, Bf, $\dot{V}E$, $\dot{V}O_2$ $\dot{V}CO2$, the averages over the last minute of each stage and the last minute before exhaustion were considered for statistical analysis.

Statistical analysis

Data are presented as mean ± one standard deviation (SD). A three way (group x test x time) ANOVA with repeated measures on the test and time factors was used to assess the effect of the experimental treatment and high intensity constant cycling on KES. A significant second order interaction (group x test x time) was followed up by tests of simple interactions, i.e. two way (test x time) ANOVA with repeated measures on the test and time factors at the two time points (pre-cycling and post-cycling). If the second order interaction was not significant, only the first order (group x test) interaction was considered and followed up as described ahead in the text. Multiple three way (group x test x workload) ANOVAs with repeated measures on the test and workload factors, were used to assess the effect of the experimental treatment on the physiological and perceptual responses to high intensity constant cycling at the four different workloads (20, 40, 60, 80% of PPO). A significant second order interaction (group x test x workload) was followed up by tests of simple interactions, i.e. two way (group x test) ANOVA with repeated measure on the test factor at each of the four PPO percentage points. If the second order interaction was not

significant, only the first order (group x test) interaction was considered and followed up as described ahead in the text. Multiple two way (group x test) ANOVAs with repeated measure on the test factor was used to assess the effect of the experimental treatment on physiological and perceptual responses to high intensity constant cycling at exhaustion. A two way ANOVA with repeated measures was also used to assess the effect of experimental treatment on performance. Because both factors (group and test) have only two levels, a significant interaction was followed up by data plotting and visual exploration. In case of a non significant interaction, only the main effect of test was considered. Relevant assumptions were checked and appropriate correction employed if necessary. Significance was set at 0.05 for analyses.

RESULTS

Subjects and missing data

One participant withdrew from the study and was excluded from analysis. The characteristics of the remaining 23 subjects (9 males and 3 females in control group, and 8 males and 3 females in the treatment group) are shown in Table 5. Due to technical problems, 12 arterialised blood samples were missed over a total of 184 samples taken (6.5%). Ten O_2 saturation readings were also missed over a total of 230 samples taken (4%). All these missing data were replaced according to the "series mean" method.

Table 5

Subjects baseline characteristics.

Variable	Fatigue (n = 11)	Control (n = 12)
Sex (males/females)	8/3	9/3
Age (years)	24 ± 4	24 ± 4
Stature (cm)	175 ± 9	177 ± 6
Body mass (Kg)	68,8 ± 9.9	74,2 ± 6.9
Body fat (%)	13,0 ± 6.8	14,5 ± 7.1
$\dot{V}O_2$ max ($ml \cdot min^{-1} \cdot Kg^{-1}$)	52,1 ± 5.0	52,1 ± 9.5
Maximum heart rate (b.p.m.)	181 ± 9	189 ± 8
Peak Power Output (W)	298 ± 59	311 ± 57

Values are means ± standard deviation. $\dot{V}O_2$ max, maximal oxygen consumption.

Effects of experimental treatment and incremental exercise on locomotor muscle force

A significant, $F(1,21) = 6.254$, $p<.05$, $\eta^2 = .229$, ($P = 0.021$) second order (group x test x time) interaction showed an effect of the experimental treatment on KES. The follow-up tests for the Fatigue condition revealed the following significant test x time interaction, $F(1,10) = 29.543$, $p<.001$, $\eta^2 = .747$, ($P < 0.001$): 1) a reduction in pre-cycling KES at posttest compared to pretest (figure 22A); 2) a reduction in KES at pretest between pre-cycling and post cycling whilst no significant difference was detected between pre and post-cycling at posttest (figure 22A). The follow-up test for the Control condition did not show a significant test x time interaction, $F(1,11) = 1.902$, $p>.05$, $\eta^2 = .147$, ($P = 0.195$) but there was a significant main effect of time, $F(1,11) = 31.195$, $p<.001$, $\eta^2 = .739$, ($P < 0.001$). This effect demonstrates a significant reduction in KES between pre-cycling and post-cycling both at pretest and posttest (figure 22B).

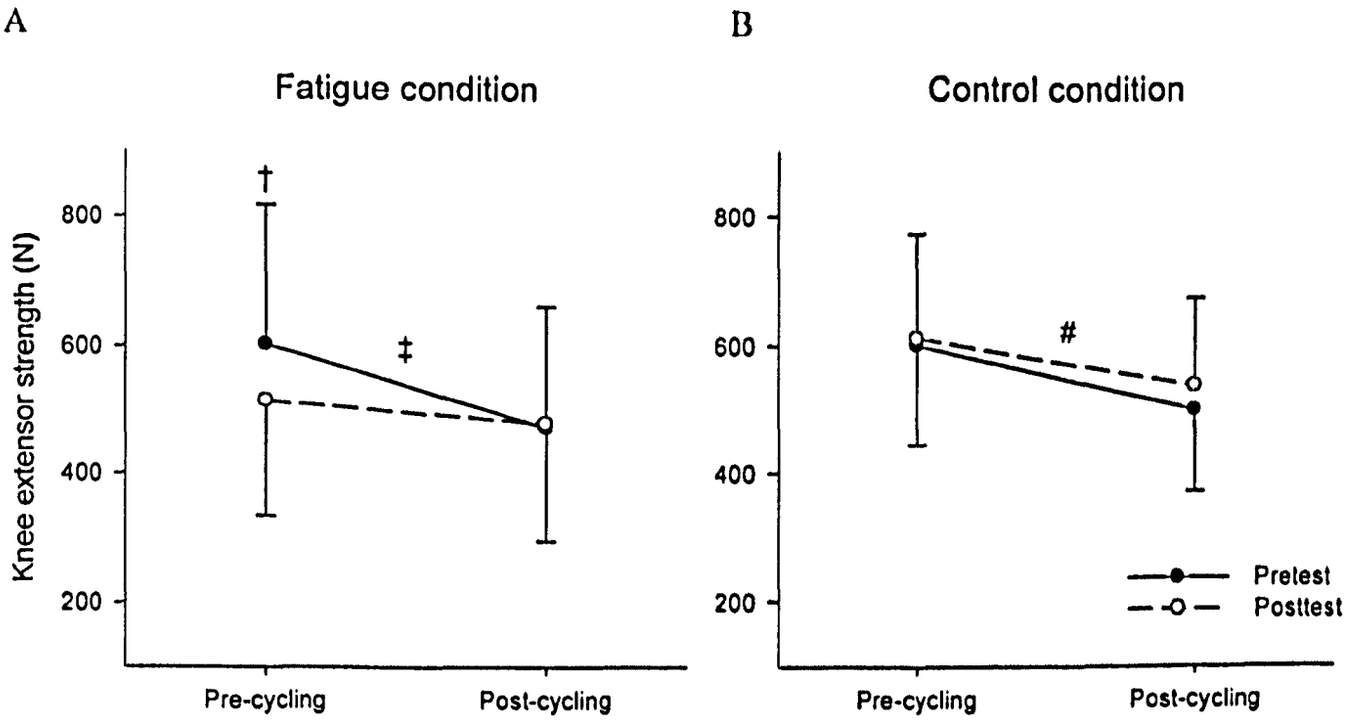

Figure 22

(Knee extensor strength)

Effects of experimental treatment and incremental cycling on knee extensor strength (KES). Values are means ± SD. Fatigue condition consisted of 100 drop jumps over 33 min at Posttest. Control condition consisted of 100 unloaded half squats over 33 min at Posttest. No treatment was administered to the subjects at Pretest in both Fatigue and Control condition. A significant second order group x test x time interaction by three way ANOVA was found (see "Result" section). #Significant main effect of time found by post hoc two way ANOVA for repeated measures ($p<0.001$). †Significant simple main effect of test according to Holm-Bonferroni method ($p<0.05$). ‡Significant simple main effect of time according to Holm-Bonferroni method ($p<0.05$).

Effect of reduced locomotor muscle force on exercise performance

The experimental treatment had a very small but significant group x test interaction, $F(1,21) = 5.830$, $p<.05$, $\eta^2 = .217$, ($P = 0.025$) effect on PPO (figure 23). Subjects in the Fatigue condition achieved a lower PPO (286 ±71W) at posttest than at pretest (290 ± 71 W). On the contrary, a higher PPO at posttest (307 ±72 W) was achieved by subjects in the Control condition compared to pretest (305 ±72 W).

Effects of reduced locomotor muscle force on the cardiorespiratory, metabolic, and perceptual response to exercise at submaximal workloads

Multiple three way ANOVAs to assess the effect of experimental treatment on the physiological and perceptual response to exercise at different workloads, did not reveal either second order (group x test x workload) or first order (group x test) significant interactions (Table 6). The only exception was significantly higher leg effort at posttest compared to pretest in the Fatigue condition (figure 24A) compared to no difference in the Control condition (figure 24B) (group x test interaction, $F(1,21) = 4.949$, $p<.05$, $\eta^2 = .191$, $P = 0.037$).

Effects of reduced locomotor muscle force on the cardiorespiratory and perceptual response to exercise at exhaustion

Subjects in the fatigue condition rated a higher leg effort at exhaustion at posttest compared to pretest whilst no change occurred in the control group (group x test interaction, $F(1,20) = 9.676$, $p<.01$, $\eta^2 = .198$, $P= 0.006$). In spite of a significant group by test interaction for the respiratory quotient, $F(1,21) = 5.171$, $p<.05$, $\eta^2 = .198$, ($P = 0.034$), follow-up tests did not show any significant difference when the Holm-Bonferroni correction was applied. None of the other physiological and perceptual variables at exhaustion were significantly affected by experimental treatment (Table 7).

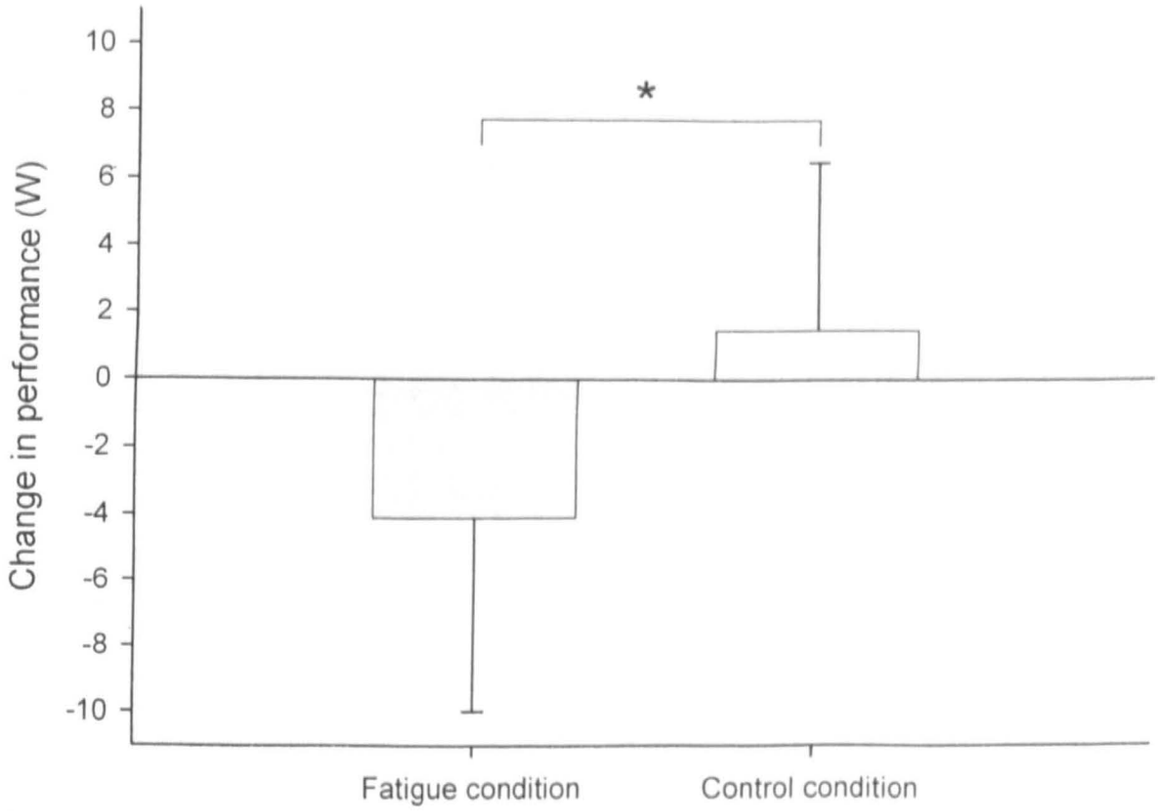

Figure 23

Effect of experimental treatment on performance. Values are means ± SD. Fatigue condition consisted of 100 drop jumps over 33 min at Posttest. Control condition consisted of 100 unloaded half squats over 33 min at Posttest. No treatment was administered to the subjects at Pretest in both Fatigue and Control condition. *Significant group x test interaction ($p<0.05$). The two bars represent the change score in each group calculated as the difference in Watts between the Posttest and Pretest Peak Power Output.

Variable	Group	20% PPO		40% PPO		60% PPO		80% PPO	
		Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
Ventilation ($l \cdot min^{-1}$)	Fatigue	30.8 ± 7.2	32.4 ± 5.6	45.4 ± 10.3	42.3 ± 8.7	63.5 ± 16.6	69.1 ± 11.9	95.5 ± 19.5	99.7 ± 18.3
	Control	30.2 ± 6.3	31.5 ± 6.3	48.0 ± 10.2	49.9 ± 10.1	68.8 ± 13.8	70.8 ± 13.7	102.9 ± 24.1	110.0 ± 26.9
Vt (l)	Fatigue	1.34 ± 0.26	1.41 ± 0.14	1.71 ± 0.26	1.76 ± 0.24	2.03 ± 0.33	2.12 ± 0.32	2.39 ± 0.41	2.42 ± 0.41
	Control	1.53 ± 0.25	1.49 ± 0.31	2.01± 0.30	1.96 ± 0.37	2.31 ± 0.38	2.34 ± 0.38	2.61 ± 0.45	2.75 ± 0.47
Bf (min^{-1})	Fatigue	23 ± 4	24 ± 4	27 ± 4	28 ± 4	32 ± 5	34 ± 7	41 ± 10	42 ± 8
	Control	21 ± 3	22 ± 2	25 ± 3	27 ± 4	30 ± 4	31 ± 5	40 ± 8	40 ± 6
$\dot{V}O_2$ ($l \cdot min^{-1}$)	Fatigue	1.37 ± 0.32	1.43 ± 0.22	1.96 ± 0.42	1.99 ± 0.39	2.55 ± 0.61	2.68 ± 0.41	3.25 ± 0.61	3.29 ± 0.49
	Control	1.41 ± 0.28	1.44 ± 0.28	2.12 ± 0.42	2.16 ± 0.43	2.74 ± 0.54	2.82 ± 0.53	3.32 ± 0.68	3.48 ±0.71
$\dot{V}CO_2$ ($l \cdot min^{-1}$)	Fatigue	1.25 ± 0.29	1.30 ± 0.29	1.93 ± 0.44	1.95 ± 0.39	2.64 ± 0.67	2.75 ± 0.46	3.60 ± 0.77	3.62 ± 0.67
	Control	1.23 ± 0.26	1.25 ± 0.29	2.02 ± 0.42	2.05 ± 0.45	2.77 ± 0.57	2.77 ± 0.57	3.66 ±0.82	3.81 ± 0.87
RER	Fatigue	0.91 ± 0.03	0.91 ± 0.04	0.98 ± 0.03	0.98 ± 0.04	1.04 ± 0.03	1.03 ± 0.04	1.11 ± 0.05	1.10 ± 0.07
	Control	0.87 ± 0.03	0.07 ± 0.07	0.96 ± 0.04	0.95 ± 0.05	1.02 ± 0.05	1.00 ± 0.05	1.10 ± 0.06	1.10 ± 0.06
Sat O_2 (%)	Fatigue	94 ± 4	95 ± 3	96 ± 2	97 ± 2	96 ± 2	97 ± 1	95 ± 3	94 ± 5
	Control	97 ± 1	97 ± 3	97 ± 1	97 ± 1	97 ± 1	97 ± 1	96 ± 2	96 ± 1
Heart rate (bpm)	Fatigue	105 ± 14	105 ± 15	128 ± 18	128 ± 16	152 ± 16	150 ± 16	171 ± 15	170 ± 14
	Control	97 ± 7	97 ± 8	122 ± 11	123 ± 12	150 ± 13	150 ± 14	176 ± 12	173 ± 13
SV (ml)	Fatigue	101 ± 18	102 ± 14	105 ± 19	107 ± 17	110 ± 23	112 ± 23	115 ± 24	114 ± 24
	Control	103 ± 14	103 ± 13	111 ± 18	109 ± 16	114 ± 20	113 ± 18	119 ± 22	115 ± 20
CO ($l \cdot min^{-1}$)	Fatigue	10.7 ± 2.6	10.7 ± 2.1	13.5 ± 3.4	13.6 ± 2.8	16.8 ± 4.2	16.8 ± 3.9	19.7 ± 5.0	19.4±4.5
	Control	10.0 ± 1.7	9.9 ± 1.5	13.5 ± 2.6	13.4 ± 2.3	16.9 ± 3.4	16.8 ± 3.1	20.9 ± 4 5	19.8±3.6
MAP (mmHg)	Fatigue	86 ± 10	84 ± 7	99 ± 8	95 ± 8	109 ± 10	104 ± 9	110 ± 14	109 ± 13
	Control	82 ± 9	86 ± 13	86 ± 8	88 ± 8	95 ± 7	93 ± 13	97 ± 8	98 ± 10
TPR ($mmHg \cdot l^{-1} \cdot min^{-1}$)	Fatigue	8.45 ± 2.29	8.35 ± 1.47	7.57 ± 1.96	6.62 ± 1.53	6.88 ± 1.96	5.87 ± 1.57	5.99±2 05	4.93 ± 1.63
	Control	8.21 ± 2.26	8.69 ± 1.21	7.24 ± 1.87	6.67±0.89	6.48 ± 1.79	5.60 ± 0.64	5 96±1.91	5.10 ± 1.17
pH ($-log[H^+]$)	Fatigue	7.41 ± 0.02	7.40 ± 0.01	7.38 ± 0.02	7.40 ± 0.02	7.36 ± 0.03	7.37 ± 0.03	7.31 ± 0.05	7.33 ± 0.04
	Control	7.41 ± 0.02	7.41 ± 0.02	7.40 ± 0.02	7.40 ± 0.02	7.39 ± 0.03	7 38 ± 0.03	7.34 ± 0.03	7.34 ± 0.03

(Table 6 continue)

P_aO_2 (mmHg) §	Fatigue	84 ± 4	88 ± 4	83 ± 4	86 ± 4	82 ± 5	86 ± 6	83 ± 7	81 ± 5
	Control	85 ± 3	84 ± 4	85 ± 4	86 ± 4	82 ± 6	84 ± 4	82 ± 6	83 ± 5
P_aCO_2 (mmHg)	Fatigue	42 ± 2	41±3	43 ± 2	41 ± 3	40 ± 2	39 ± 3	37 ± 3	36 ± 2
	Control	42 ± 3	41±3	42 ± 2	41 ± 3	40 ± 3	39 ± 3	36 ± 4	35 ± 4
$[K^+]$ (mmol·l^{-1}) *	Fatigue	5.59 ± 0.76	5.15 ± 0.37	5.61 ± 0.54	5.38 ± 0.25	5.86 ± 0.56	5.65 ± 0.54	6.32±0.45	6.22 ± 0.36
	Control	5.28 ± 0.50	5.23 ± 0.33	5.37 ± 0.30	5.40 ± 0.32	5.57 ± 0.31	5.52 ± 0.27	6 28±0.59	6.38 ± 0.44
Blood Lactate (mmol·l^{-1})	Fatigue	1.4 ± 0.4	0.5±0.9	3.0 ± 1.4	2.5 ± 1.0	5.0 ± 1.9	5.0 ± 1.9	9.8 ± 3.0	9.2 ± 2.8
	Control	1.0 ± 0.2	1.4± 0.6	2.0 ± 0.7	1.8 ± 1.0	3.9 ± 1.2	4.0 ± 1.5	8.8 ± 2.4	8.7 ± 2.5
Core temperature (C°)	Fatigue	37.5 ± 0.3	37.6 ± 0.2	37.6 ± 0.3	37.6 ± 0 2	37.7 ± 0.3	37.6 ± 0.2	37.8 ± 0.3	37.8 ± 0.2
	Control	37.3 ± 0.2	37.3 ± 0.2	37.3 ± 0.2	37.3 ± 0.2	37.4 ± 0.2	37.4 ± 0.2	37.6 ± 0.3	37.6 ± 0.2
Dyspnea (0 – 10)	Fatigue	0.3 ± 0.3	0.3 ± 0.4	1.0 ± 0.5	0.9 ± 0.7	2.5 ± 0.7	2.4 ± 1.1	4.6 ± 1.2	4.5 ± 1.7
	Control	0.8 ± 0.5	0.5 ± 0.4	1.5 ± 0.8	1.1 ± 0.6	2.5 ± 1.1	2.4 ± 0.7	3.9 ± 1.3	3.8 ± 1.6
Leg pain (0 – 10)	Fatigue	0.4 ± 0.6	0.7 ± 0.9	0.8±0.9	1.2±1.1	2.1 ± 1.8	3.0 ± 2.2	4.1 ± 2.6	4.7 ± 2.8
	Control	0.5 ± 0.5	0.4 ± 0.3	1.1±0.9	1.3±0.9	2.0 ± 1.4	2.3 ± 1.1	4.1 ± 2.2	4.1 ± 2.1

Table 6

Effects of experimental treatment on respiratory, cardio-circulatory, metabolic, thermal and perceptual responses to constant cycling at four different workloads corresponding to 20, 40, 60, and 80% of the Peak Power Output (PPO).

Fatigue and Control groups include 11 and 12 subjects respectively. Values are means ± SD. All main effects of workloads $p < 0.001$. No significant second-order group x test x workloads interaction nor group x test interaction by three way ANOVA with repeated measures on the test and workloads factors were found for each variable (all values of $p > 0.05$). The only exceptions are represented by a significant second-order interaction for P_aO_2 ($p = 0.010$) and a significant group x test interaction for $[K^+]$ ($p = 0.52$), for follow up tests see "Results" section. Vt, tidal volume; Bf, breathing frequency; VO_2 , oxygen uptake; VCO_2 , carbon dioxide production; RER, respiratory exchange ratio; Sat O_2 , oxygen saturation; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; TPR, total peripheral resistance; P_aO_2, partial pressure of oxygen in mixed venous blood P_aCO_2, partial pressure of carbon dioxide in mixed venous blood $[K^+]$, blood potassium concentration; RPE, ratings of perceived exertion. §Significant second order group x test x workloads interaction. *Significant group x test interaction.

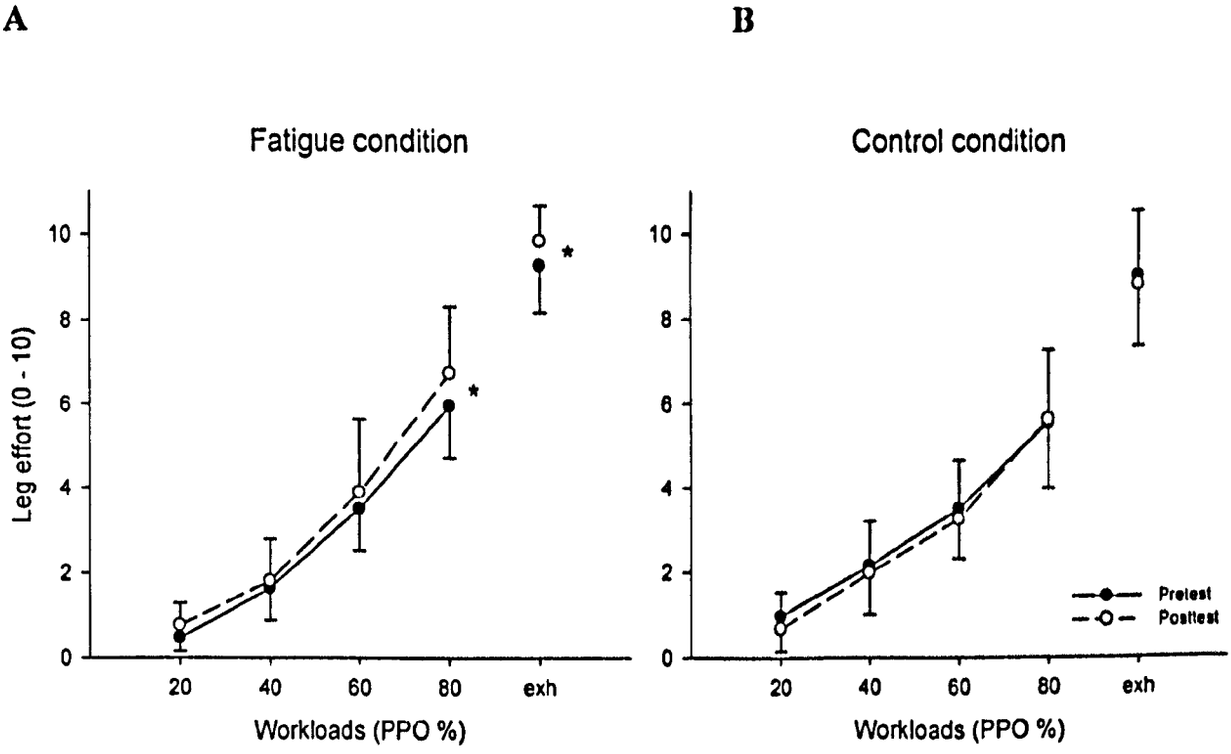

Figure 24

Effects of experimental treatment on ratings of perceived exertion of the lower limbs (Leg effort). Values are means ± SD. Fatigue condition consisted of 100 drop jumps over 33 min at Posttest. Control condition consisted of 100 unloaded half squats over 33 min at Posttest. No treatment was administered to the subjects at Pretest in both Fatigue and Control condition. No significant second order group x test x workloads interaction by three way ANOVA was found. *Significant group x test interaction ($p<0.05$) (see "Result" section).

Table 7

Effects of experimental treatment on respiratory, cardio-circulatory, thermal and perceptual responses to constant cycling at exhaustion.

Variable	Condition	Pretest	Posttest
Ventilation ($l \cdot min^{-1}$)	Fatigue	139 ± 34	138 ± 34
	Control	144 ± 30	155 ± 35
Vt (l)	Fatigue	2.46 ± 0.54	2.45 ± 0.49
	Control	2.50 ± 0.45	2.67 ± 0.67
Bf (min^{-1})	Fatigue	57 ± 9	57 ± 9
	Control	58 ± 8	59 ± 10
$\dot{V}O_2$ ($l \cdot min^{-1}$)	Fatigue	3.58 ± 0.73	3.62 ± 0.68
	Control	3.57 ± 0.67	3.81 ± 0.93
$\dot{V}CO_2$ ($l \cdot min^{-1}$)	Fatigue	4.17 ± 1.04	4.14 ± 0.97
	Control	4.16 ± 0.96	4.45 ± 1.07
RER *	Fatigue	1.16 ± 0.1	1.14 ± 0.1
	Control	1.16 ± 0.1	1.17 ± 0.1
Sat O_2	Fatigue	88 ± 11	91 ± 7
	Control	93 ± 5	94 ± 4
Heart rate (bpm)	Fatigue	186 ± 7	185 ± 5
	Control	191 ± 7	190 ± 8
SV (ml)	Fatigue	120 ± 26	122 ± 26
	Control	125 ± 26	125 ± 24
CO ($l \cdot min^{-1}$)	Fatigue	22.2 ± 5.2	22.3 ± 5.1
	Control	23.8 ± 5.3	23.8 ± 4.5
Core temperature (C°)	Fatigue	38.1 ± 0.3	38.0 ± 0.1
	Control	37.9 ± 0.4	37.9 ± 0.3
Dyspnea (0 – 10)	Fatigue	8.1 ± 1.2	8.3 ± 1.5
	Control	7.9 ± 2.6	7.5 ± 1.9
Leg pain (0 – 10)	Fatigue	7.3 ± 3.2	7.2 ± 3.3
	Control	7.9 ± 2.1	8.4 ± 2.1

Table 7. Fatigue and Control groups include 11 and 12 subjects respectively. Values are means ± SD. No significant group x test interaction by two way ANOVA with repeated measures on the test factor was found for each variable (all values of $p > 0.05$). The only exceptions is represented by a significant group x test interaction for RER ($p = 0.034$), for follow up tests see "Results" section. *Significant second order group x test interaction. Vt, tidal volume; Bf, breathing frequency; VO_2 , oxygen uptake; VCO_2 , carbon dioxide production; RER, respiratory exchange ratio; Sat O_2 , oxygen saturation; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; TPR, total peripheral resistance; P_aO_2, partial pressure of oxygen in mixed venous blood P_aCO_2, partial pressure of carbon dioxide in mixed venous blood $[K^+]$, blood potassium concentration; RPE, ratings of perceived exertion.

DISCUSSION

Effects of experimental treatment and incremental cycling exercise on locomotor muscle force

As expected, experimental treatment significantly reduced KES by 13% (pre treatment versus pre-cycling at posttest in the Fatigue condition). This strength loss is slightly lower than previous reports in which the same eccentric exercise protocol was used to induce locomotor muscle fatigue (approximately 20%) (Nielsen et al., 2005, Skurvydas et al., 2002, Skurvydas et al., 2000). Nevertheless, this 13% reduction in locomotor muscle force is clinically relevant because it is similar to the 15% reduction in KES found in patients with cardiorespiratory diseases when compared to healthy controls (Hamilton et al., 1995). Interestingly, the average reduction in locomotor muscle force measured 2 min after exhaustion (pre-cycling vs. post cycling in Figure 22) found in both groups at pretest and in the control group at posttest is 14%. A similar effect has been reported by Sandiford (Sandiford et al., 2005, Man et al., 2003) and Man (Man et al., 2003). From a physiological point of view, the similarity between strength loss induced by the eccentric exercise protocol and incremental cycling exercise suggests that the same pool of muscle fibres are affected although by different mechanisms: sarcomere damage and/or impaired calcium release from the sarcoplasmic reticulum during eccentric exercise (Nielsen et al., 2005, Warren et al., 2001, Allen, 2001, Proske and Morgan, 2001); metabolic fatigue during the incremental cycling test (Fitts, 1994, Fitts, 2008, Allen et al., 2008). In fact, both eccentric exercise and metabolic fatigue seems to affect mostly fast-glycolitic fibres(Sargeant, 2007, Rijkelijkhuizen et al., 2003, Proske and Morgan, 2001). Once the function of these fatigue-sensitive fibres has been impaired, no further strength loss can occur because the remaining fibre types (fast-oxidative and slow-oxidative) are fatigue-resistant (Sargeant, 2007). This hypothesis is supported by the finding that locomotor muscle force is not further reduced by the incremental exercise test when cycling is preceded by eccentric exercise (see pre-cycling vs. post cycling at post test in the experimental group, Figure

22). Similar results have been obtained in our previous locomotor muscle fatigue study (Chapter 3) and by Amann and Dempsey (Amann and Dempsey, 2008b) who found no further strength loss after a 5km time trial preceded by an exhaustive bout of intense constant-power cycling exercise. In our present and previous studies, however, locomotor muscle fatigue was induced by an eccentric exercise protocol that does not increase metabolic stress and sensitivity of type III and IV afferents to metabolic stimuli (Nielsen et al., 2005). Therefore, the inhibitory afferent feedback system proposed by Amann and Dempsey to prevent locomotor muscle fatigue trespassing a certain threshold is not necessary to explain why similar levels of muscle fatigue are found after maximal exercise tests in different experimental conditions (Marcora, 2008a). Because exercise performance was measured 30 min after eccentric exercise, afferent feedback related to overt muscle tissue damage and inflammation, and delayed-onset muscle soreness was controlled for as these phenomena occur several hours-days after eccentric exercise (Peake et al., 2005). Indeed, the early strength loss observed after eccentric exercise is caused by altered calcium release from the sarcoplasmic reticulum and damage at molecular level, i.e. the sarcomere (Allen, 2001, Proske and Morgan, 2001, Warren et al., 2001). Indeed, this phenomenon has been called the "insensitive fatigue".

Finally, it is important to emphasise that, from a functional point of view, it is irrelevant whether muscle weakness is caused by muscle wasting, eccentric exercise or metabolic stress(Fitts, 2006). Therefore, our experimental model should be relevant to the reduction in locomotor muscle force observed in cachectic patients with cardiorespiratory diseases (Hamilton et al., 1995, Jones and Killian, 2000) and after incremental exercise tests (Hamilton et al., 1995, Jones and Killian, 2000). However, the lack of further muscle fatigue after incremental exercise in the experimental treatment group means that our human model does not simulate the overall muscle dysfunction affecting patients with cardiorespiratory disorders where muscle weakness and increased fatiguability are combined (Gosker et al., 2000).

Effects of reduced locomotor muscle force on physiological responses to incremental cycling exercise

All physiological variables showed patterns typical of incremental exercise (Vollestad et al., 1994, Dempsey, 2006a). As expected, the eccentric exercise protocol used to induce locomotor muscle fatigue did not affect metabolic responses and rectal temperature. Albeit statistically significant, the small effects of experimental treatment on PaO2 and K+ in arterialised blood are not physiologically important (Dempsey and Wagner, 1999, Vollestad et al., 1994), and may be type I errors due to the numerous dependent variables included in the present study (Huck, 2004).

We hypothesised that even with similar metabolic stress, the higher central motor command necessary to cycle at the same power outputs with weaker locomotor muscles would exacerbate the cardiorespiratory responses to incremental exercise, particularly in the final stages of the test when the fatigued fast-glycolitic fibres are recruited (Coyle, 2000, Altenburg et al., 2007). However, despite significantly higher perception of leg effort (a marker of central motor command)(Gallagher et al., 2001, Norton et al., 1999, Smith et al., 2003), we failed to measure any effect on the various cardiorespiratory parameters included in this study. The discrepancy between our present and previous studies using the same eccentric exercise protocol may be explained by the smaller reduction in locomotor muscle force: 13% vs an average of 18% (Chapter 3). In fact, previous studies using curare (Galbo et al., 1987) suggest that a dose-response relationship may exist between reduced locomotor muscle force and increased cardiorespiratory strain during exercise (Galbo et al., 1987). There is also evidence that the effect of eccentric muscle actions on physiological responses differ between constant-power and incremental exercise tests. For example, DOMS-inducing exercise has been found to determine a higher response of the main cardiorespiratory parameters to submaximal cycling compared to a control condition(Gleeson et al., 1995) but not during incremental exercise(Gleeson et al., 1998). Whatever the reason, our study

demonstrates that a physiologically and clinically relevant 13% loss of locomotor muscle force has no significant effect on cardiorespiratory responses during incremental cycling exercise. This finding suggests that the reason for exaggerated cardiorespiratory responses in patients with CHF and COPD is either a) the very high central command required to overcome the combined effect of muscle weakness and increased fatiguability, and/or b) an exagerated exercise pressor reflex (Piepoli et al., 1996, Ponikowski et al., 2001, Schmidt et al., 2005).

Effects of reduced locomotor muscle force on exertional symptom intensity and exercise performance

All perceptual variables showed a typical increase during the incremental exercise tests (Hamilton et al., 1995). Importantly, experimental treatment did not significantly affect leg muscle pain thus confirming that afferent feedback related to muscle damage, inflammation and delayed-onset muscle soreness was well controlled by our fatiguing protocol. Given no significant effect on respiratory responses, it is no surprise that dyspnea was also unaffected by experimental treatment. However, the reduced locomotor muscle force induced by eccentric exercise forced subjects in the experimental treatment group to increase their central motor command which is perceived, via corollary discharge, as the sensation of leg effort (Marcora, 2008b). This effect is clearly demonstrated by the significant increase in ratings of leg effort during submaximal workloads (Figure 3 At 20-40-60-80% of PPO).

This finding suggests that potential motivation (i.e., the maximum effort an individual is willing to exert to satisfy a motive) (Wright, 2008) was increased by experimental treatment. Despite this positive psychological effect of our eccentric exercise protocol, exercise performance (measured as PPO during the incremental cycling tests) was significantly reduced by locomotor muscle fatigue. The effect, however, is relatively small if we consider that PPO in patients with cardiorespiratory disorders is reduced by 24% (Hamilton et al., 1995). Therefore, other factors are necessary to explain reduced exercise

performance in these patients. Obviously, the combined effect of muscle weakness and increased fatiguability may play an important role (Gosker et al., 2000). Cardiorespiratory symptoms (i.e., angina and dyspnea during exercise) and the leg discomfort associated with increased metabolic stress may also affect exercise performance in these patients (Hamilton et al., 1995, Jones and Killian, 2000). What is clear from our investigation, is that reduced locomotor muscle force can impair performance during an incremental exercise test independently of afferent feedback from metabolically stressed locomotor muscles. This is an important finding from a physiological point of view. Indeed, it has been recently proposed that subjects stop exercising at VO2max because limited convective oxygen delivery causes severe metabolic stress in the locomotor muscles which activates type III and IV afferents leading to cessation of central motor drive and voluntary effort (Levine, 2008). In our investigation, however, VO2max and exercise performance have been experimentally dissociated. In fact, experimental treatment did not affect convective oxygen delivery, metabolic stress, and VO2max despite a significant reduction in PPO. According to our alternative psychobiological model based on motivational intensity theory (Chapter 3), this negative effect of reduced locomotor muscle force on exercise performance is mediated by the significant increase in the sensation of leg effort, a major contributor to overall perceived exertion during cycling exercise (Robertson et al., 1979).

Conclusions

From a clinical point of view, the findings of our study suggest that muscle weakness or increased fatiguability in isolation can not explain the exaggerated cardiorespiratory responses and greatly reduced performance observed during incremental exercise tests in patients with cardiorespiratory disorders. The combination of muscle weakness and increased fatiguability and/or the influence of other factors (e.g., an exaggerated exercise pressor reflex) is necessary to explain these

important clinical phenomena. From a physiological point of view, however, our study provides evidence that reduced locomotor muscle force *per se* can impair performance during incremental cycling exercise independently of metabolic stress. This finding argues against the inhibitory afferent feedback model proposed by Levine to explain why people stop exercising at VO2max (Levine, 2008), and provides support to the psychobiological model of exercise performance based on centrally-generated perception of effort and potential motivation (Chapter 3).

CHAPTER 5

GENERAL DISCUSSION

Overview

The aim of this general discussion is to summarize and compare the main findings across all four studies on the effects of muscle fatigue and damage. First, endurance exercise performance is discussed, and then the physiological and perceptual responses to exercise. In the conclusion these two aspects are integrated to discuss the physiological and perceptual mechanisms mediating the effects of locomotor muscle fatigue and damage on endurance exercise performance.

Effects of muscle fatigue and damage on endurance exercise performance

We demonstrated for the first time that an isolated decrease of locomotor muscle force and exercise-induced muscle damage can impair endurance exercise performance. This finding is consistent across three different studies in which performance has been assessed using various protocols. In order to compare the results of the three studies, a standardized effect size measure (eta squared) has been used (Table 8).

Table 8

Relative (%) and standardized measures of the effects of muscle fatigue and damage on endurance exercise performance

	Decrease in muscle force compared to baseline	Relative decrease in performance	Partial η^2
Study 1 (Muscle damage)	- 12%	- 4%	0.207
Study 2 (Muscle fatigue)	- 15%	- 15%	0.637
Study 4 (Muscle fatigue)	- 13%	- 2%	0.217

The greatest effect on endurance performance was found in the second study in which, interestingly, the experimental treatment caused the

largest loss of force. This evidence might suggest the existence of a dose-response relationship between the drop in MVC and the decreased performance. It seems that higher drops in MVC, such as that obtained in study 2 compared to study 1 and 4, determines a bigger detrimental effects on performance. The dose-response relationship seems to be supported by a neuroblockade study (Galbo et al., 1987) in which the waning effect of curare during an incremental cycling exercise test elicited simultaneously an increase in muscular force and in peak power output. However, we need to take into consideration the much larger changes in force induced by curare administration compared to eccentric exercise.

The different decreases in MVC across the studies might be a consequence of the different eccentric protocols used to cause muscle damage and muscle fatigue, and the period at which locomotor force was assessed(Clarkson and Hubal, 2002). However, a comparison of the decrease in force in study 2, 3 and 4, where the protocol and time of force assessment are the same, shows a difference in the loss of force caused by the same eccentric exercise. The fibre type composition of the participants recruited in the different experiments is the most likely explanation for this discrepancy. According to the literature eccentric exercise affects mainly type II fibres (Proske and Morgan, 2001, Rijkelijkhuizen et al., 2003). Therefore, muscle fibre composition might determine, at least in part, the extent of force loss. Furthermore, we can not exclude different levels of accustomization to eccentric exercise even if one of the inclusion criteria for our studies was not to be engaged in any regular activity involving eccentric muscle contractions.

Another factor which may explain the different effects of isolated locomotor muscle fatigue on endurance performance in study 2 and 4 is the different test modality: time to exhaustion and peak power output during incremental exercise respectively. Indeed, this is not the first case in the literature where the same experimental treatment gave different responses on performance assessed by a time to exhaustion

(Harms et al., 2000) or an incremental exercise test (Romer et al., 2007b).

The negative effect of muscle damage on endurance performance demonstrated for the first time in Study 1, has been recently confirmed by other authors (Davies et al., 2008). In this study, performance during severe intensity cycling exercise was significantly reduced by 16% compared to control condition in presence of muscle damage which was induced by repeated jumps as in our study. These results extend to humans previous animal studies also showing reduced time to exhaustion after muscle damage (Carmichael et al., 2005, Carmichael et al., 2006). Overall, it is evident that endurance exercises performance at different modalities (cycling and running) and duration/intensity (from 6-7 min to 30 min) can be negatively affected by muscle damage.

To the best of our knowledge, there are no other studies on the effect of isolated locomotor muscle fatigue on endurance performance. Indeed, in previous studies a significant decrease of locomotor muscle force was induced by: i) fatiguing whole-body exercise (Amann and Dempsey, 2008b), or ii) neuromuscular blockade (Galbo et al., 1987) which some have proposed as a model of fatigue (Dettmers et al., 1996). Although significant reductions in 5 km time trial (Amann and Dempsey, 2008b) and incremental exercise performance (Galbo et al., 1987) were demonstrated, these experimental interventions have several limitations which may confound the actual effect of muscle fatigue *per se*. First of all, prior whole-body fatiguing exercise causes metabolic perturbations which augment sensory afferent feedback (i.e., leg discomfort). According to the model proposed by Amann and colleagues (Amann, 2007, Amann et al., 2006), such feedback may impose a "sensory tolerance limit" on exercise performance. In addition, whole-body fatiguing exercise causes significant fatigue of the respiratory muscles (Babcock et al., 2002, Johnson et al., 1993). This may be an important confounding factor as both inspiratory and expiratory muscle fatigue can significantly reduce endurance exercise

performance (Romer and Polkey, 2008). Similarly, the weakening effect of neuromuscular blockades are not limited to locomotor muscles but also reduce force of the respiratory muscles(Johansen et al., 1964).
Considering all these concerns, it is clear that the advantage of our model consists in the isolation of reduced locomotor muscle force, i.e. fatigue, per se.

Effects of muscle fatigue and damage on physiological and perceptual responses to exercise

With the exception of the final stage of the incremental test in study 4, high intensity exercise performed in a condition of muscle fatigue or damage was associated with an augmented cardiorespiratory response compared to the control condition. In fact, in studies 2 and 3, we were able to demonstrate significant changes in heart rate, cardiac output, breathing frequency, and ventilation. Similarly, during the high-intensity 30-min time trial in study 1, average heart rate was 91% of maximum despite lower average running speed after EIMD.
On the contrary, locomotor muscle fatigue/damage did not affect physiological responses during low-to-moderate intensity exercise. Indeed, during steady state running at 70% VO2max in study 1 and during the first part of the incremental exercise (20, 40, and 60% of peak power output) in study 4, we could not measure any significant difference between the experimental and control condition. It is important to highlight that at these low-to-moderate exercise intensities the fatigued and damaged type II muscle fibres are not recruited to a large extent (Altenburg et al., 2007, Coyle, 2000). Therefore, the task can be performed almost entirely by recruiting the fatigue- and damage-resistant type I muscle fibres. For this reason, it is not necessary to significantly increase central motor command and, consequently, the input to the cardiorespiratory centres remains largely unaltered (Waldrop, 1996).

Altogether, it seems that the augmented physiological responses to exercise in presence of muscle fatigue and damage is dependent upon the intensity at which endurance exercise is performed. The lack of effect during the final stage of the incremental test in study 4 may be explained by the fatiguing effect of the previous three stages. Indeed, subject in the control group might had been performing the final stage of the incremental test in study 4 with a degree of muscle fatigue not greatly different from the one induced by the 100 drop jumps protocol. Such effect could potentially cancel or greatly reduce the effect of experimental treatment on the physiological responses to exercise. Furthermore, as mentioned earlier in the performance section, some studies present in the literature suggest that the same experimental treatment may have different physiological effects during constant workload exercise (Gleeson et al., 1995, Harms et al., 2000) compared to incremental exercise (Gleeson et al., 1998, Romer et al., 2007b). The higher ventilatory responses caused by muscle fatigue in study 2 and 3 may also be due to the unusual respiratory pattern observed during high intensity time to exhaustion tests. Indeed, these tests elicit an extraordinary tachypneic response compared to incremental exercise (Dempsey, 2006b).

The cardiorespiratory and metabolic responses to running exercise has been found to be augmented at both low and moderate-to-high intensities after downhill running (Braun and Dutto, 2003, Chen et al., 2007). Other damaging exercise protocols did not cause the same physiological alterations (Hamill J, 1991, Paschalis et al., 2005, Scott et al., 2003). Furthermore, results from a recent study suggests that a higher discharge of the group III and IV afferent fibres due to the distension of the vessels located in proximity to damaged fibres may augment the cardiorespiratory responses to exercise (Davies et al., 2008). Unlike the increased central motor command due to reduced contractility of type II fibres, such afferent mechanism should affect physiological responses at all exercise intensities. However, in our muscle damage study, we found no significant differences in cardiorespiratory responses during the 10 min steady state running at

70% of VO2 max. On the contrary, the significant effect of experimental treatment on average running speed during the 30-min time trial (about 90 – 93% of VO_2max) without corresponding changes in heart rate and RPE suggest that central motor command may be more influential than the mechanism proposed by Davies and colleagues (Davies et al., 2008). Therefore, more research is necessary to better understand the interaction between muscle force losses and exercise intensity/fibre type recruitment in determining the effects of muscle damage on cardiorespiratory responses.
Unfortunately, we can compare our findings relative to isolated muscle fatigue only to those using neuromuscular blockades in which reduced locomotor force was accompanied by respiratory muscle weakness and/or metabolic stress which are important confounding factors. Nevertheless, significant muscle weakness (less than 50% of baseline values) caused by neuromuscular blockade significantly affected the cardiorespiratory responses to exercise (Asmussen et al., 1965, Galbo et al., 1987, Gallagher et al., 2001). Such changes were detected both at very low intensity (around 1.2-1.6 l/min VO2 and 20% of VO2max) (Asmussen et al., 1965, Gallagher et al., 2001) and medium-to-high intensity during incremental exercise (Galbo et al., 1987). This does not contradict our previous argument about the interaction between muscle force losses and exercise intensity because curarization greatly affects type I fibres (Gallagher et al., 2001, Galbo et al., 1987).

One of the most consistent finding is the significant effect of experimental treatment on perception of effort. We found a trend ($P = 0.076$) during submaximal constant-speed running in Study 1 and statistically significant increases in perception of effort during the 30-min time trial (when related to running speed) in Study 1 and in Study 3 and 4. Lack of statistical power is the most likely explanation for the non significant effect of experimental treatment in Study 2 because the increase in RPE is similar to that observed in the other studies. The fact that the increase in RPE observed after muscle damage is

similar to that measured after locomotor muscle fatigue without significant metabolic stress or DOMS suggests that the increase in central motor commands necessary to run/cycle with weaker locomotor muscles and to hyperventilate are the main sensory inputs for perceived exertion. Indeed, evidence (including the curarization studies cited previously) has been recently reviewed to suggest that perceived exertion during whole-body exercise is not generated by afferent feedback from skeletal muscles, heart and lungs (Marcora, 2008b). The suggestion is that perception of effort is generated by corollary discharges from pre-motor and/or motor areas to the somato-sensory sensory cortex. Our findings are certainly consistent with this hypothesis although we can not totally exclude a significant effect of afferent feedback from damaged and sore leg muscles on RPE in Study 1. The increase in inflammatory cytokines in the brain measured after muscle damage in animal studies (Carmichael et al., 2005, Carmichael et al., 2006) may also affect perception of effort during exercise by influencing processing of both centrally- and peripherally-generated sensory signals. More studies are necessary to assess the individual contribution of all these possible mechanisms to the increase in RPE observed after muscle damaging exercise.

Theoretical implications and conclusions

In the literature several theoretical models have been proposed to explain the limits to exercise performance. In this section we review these models to see whether they can explain the impairment in endurance exercise performance caused by locomotor muscle fatigue and damage. A novel psychobiological model will also be proposed as the most likely explanation for the performance effects of experimental treatment demonstrated in three of our studies.

The traditional peripheral fatigue model asserts that performance during high-intensity exercise protracted until exhaustion is directly limited by the development of muscle fatigue. This is because the involvement of the anaerobic energy system is required to sustain the

elevated energy demands with consequent accumulation of fatiguing metabolites (Fitts, 2006). The important role of calcium and potassium changes in causing muscle fatigue has also been demonstrated (Fitts, 2008, Fitts, 2006, Allen et al., 2008). The assumption of this model is that these peripheral changes would result in a progressive reduction in muscle contractility until the required force or power can not be longer produced. At this point the exercise is terminated (Allen et al., 2008, Sejersted and Sjogaard, 2000, Jones et al., 2008). Can we use this model to explain the reduced endurance performance found in our studies? According to the traditional peripheral fatigue model, subjects with fatigued muscles should have attained more rapidly the point at which they were no longer able to produce the workload required by the exercise task. Indeed, time to exhaustion was reduced in both Study 1 and 4. However, two other findings are in contrast with the fundamental assumptions underpinning the traditional model. First of all, it is known that about 20% of the MVC is required to cycle at VO2max (Lollgen et al., 1980). Therefore, despite significant muscle fatigue, the locomotor muscle force (80% of MVC) we found after exhaustion both in the fatigue and control condition is more than sufficient to cycle at the workload at which exhaustion occurred. This evidence does not support the direct role of muscle fatigue in determining the limit to endurance performance. It can be argued that we measured MVC 2 min after exhaustion, and this may represents one of the limits of our research program. However, it is unlikely that profound losses of strength, such those hypothetically necessary to prove the validity of the peripheral fatigue model, might recover so quickly after exhaustion. Moreover, experimental data show that the MVC measured immediately after an isometric submaximal exercise to exhaustion is much higher than that required by the task (Fulco et al., 1996, Gondin et al., 2006). Furthermore, unpublished data from our lab show that, immediately after a high intensity cycling test to exhaustion, a power output 2-3 times higher than the one required during the task can be generated by the fatigued muscles.

Secondly, we found that high-intensity cycling exercise does not further reduce force of pre-fatigued locomotor muscles. This interesting finding has been recently confirmed by (Amann and Dempsey, 2008b), and suggests that once the fatigue-sensitive type II fibres are impaired, no further strength losses can occur because the remaining fibre types (fast-oxidative and slow-oxidative) are fatigue-resistant (Sargeant, 2007). Therefore, it is unlikely that fatigue of human muscles with mixed fibre type composition can reduce force to the low level necessary to cause exhaustion during even very high-intensity cycling exercise tests. Overall it seems that, paradoxically, the traditional peripheral fatigue model can not even explain the negative effect of locomotor muscle fatigue on exercise performance.

Two different research groups have proposed interesting alternative models to explain the limits to exercise performance. Both these models are based on afferent feedback regarding the physiological conditions of the body. The model proposed by Amann and Dempsey (Amann and Dempsey, 2008b, Amann and Dempsey, 2008a) explain exercise termination during constant-power tests and the regulation of exercise intensity during time trials by an inhibitory afferent feedback system aimed at preventing peripheral muscle fatigue to develop beyond a critical threshold. According to its proponents, after this point potential damage to muscle tissue may occur (Amann, 2007, Amann and Dempsey, 2008b) and the reduction in central motor command serves as a protective mechanism. Physiologically, the inhibition of the central motor output would be mediated by inhibitory afferent feedback from type III and IV afferent nerve fibres situated in the working muscles and stimulated by the accumulation of fatiguing metabolites (e.g. H^+, inorganic phosphate) (Amann et al., 2006). Noakes and colleagues (St Clair Gibson and Noakes, 2004, Noakes et al., 2004) have proposed a more complex central governor model in which afferent sensory feedback coming from many different organs (e.g., skeletal muscles, heart, lungs, skin, and the brain itself) is processed at subconscious level by an intelligent system located in a

not yet identified part of the brain. Central to this model is the hypothesis that exercise duration or intensity (depending upon the type of performance test) is set in anticipation by the CNS in order to avoid a catastrophic failure of homeostasis.

Can these CNS models of exercise performance regulation explain our findings? Like Noakes and Marino (Noakes and Marino, 2007) and Marcora (Marcora, 2008a) have done previously, we argue that the significant increase in running velocity measured at the end of the 30-min time trial (the so-called "end-spurt") is not compatible with the inhibitory afferent feedback model proposed by Amann and Dempsey. Indeed, metabolic stress (and related afferent feedback) should be higher at the end rather than at the start or middle part of an intense time trial. Thus, it should not be possible to significantly increase central motor drive when the "finishing line" is in sight.

At first glance, the results of our muscle damage study could be explained by the central governor model. Indeed, afferent feedback related to inflammation may inform this intelligent system to reduce central motor drive to the locomotor muscles and, thus, running speed during the 30-min time trial. It is not clear, however, how this could be done subconsciously given that running speed on our treadmill had to be consciously regulated by the subject by operating buttons on one of the sidebars.

Furthermore, neither the inhibitory afferent feedback model nor the central governor model can explain the significant reduction in performance measured during intense constant-power cycling in Study 2 and incremental cycling test in Study 4. This suggestion is based on two main facts. First of all, the body does not have receptors capable of detecting changes in MVC. Secondly, afferent feedback related to fatiguing metabolites and muscle injury was controlled by using a non metabolically stressful 100 drop jumps protocol, and by testing subjects 30 min after eccentric exercise when exaggerated afferent sensory feedback (as indicated by DOMS) has yet to develop. Finally, it is known that eccentric exercise does not affect the function of muscle spindles and Golgi tendon organs (Gregory et al., 2002,

Gregory et al., 2004). Therefore, afferent sensory inputs directly inhibiting supraspinal centres or feeding information to the central governor were similar in the fatigue and control condition. So why was exercise performance reduced by experimental treatment in both studies?

We believe that exercise performance should not be viewed only as the product of physico-chemical machine (physiological approach) but as a motivated behaviour that can be explained, like other motivated behaviours, by psychological theory. Specifically, we have proposed the application of Brehm's motivational intensity theory to exercise performance, the so-called psychobiological model (Chapter 3). According to this theory (Wright, 2008), people engage in a task until the effort required reaches the maximum level of effort they are willing to invest for succeeding in the task (the so-called potential motivation, Figure 25 A and B) or when the task is perceived as impossible despite very high potential motivation (Figure 25 C).

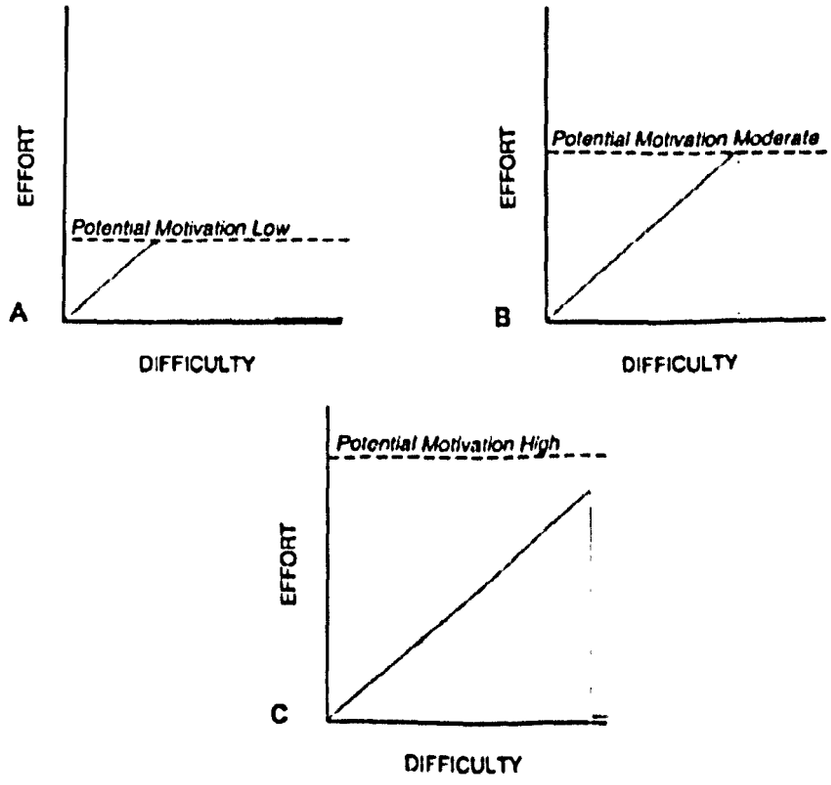

Figure 25

According to this model, well-motivated subjects voluntary terminate any form of submaximal endurance exercise when perception of effort reaches high levels which is a common observation during exhaustive exercise tests. Because potential motivation (as measured by maximal RPE) was either unchanged or even increased by experimental treatment, the effect of locomotor muscle fatigue on exercise performance in Study 2 and 4 can be explained by its effect on perception of effort described and explained in the previous section of the General Discussion. Interestingly, (Wright, 2008) found similar effects when testing Brehm's motivational intensity theory during cognitive tasks as illustrated in Figure 26. Such generalization speaks in favour of such theory.

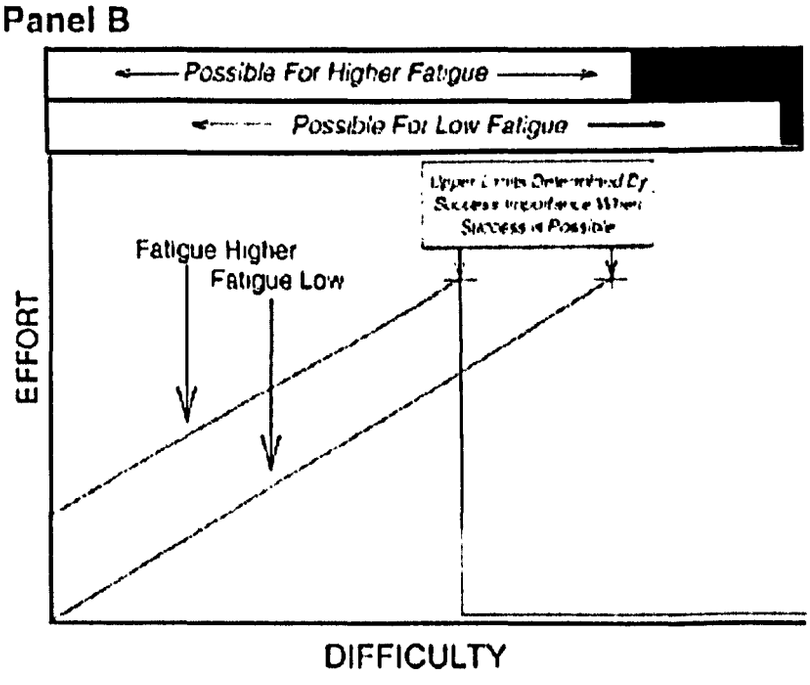

Figure 26

Indeed, this model is also applicable to time trial performance in Study 1. However, in this more complex task, the conscious decision-making process is informed not only by i) perception of effort and ii) potential motivation, but also by iii) memory of perception of effort during previous exercise bouts of different intensities and durations, iv) knowledge of total time trial time, and v) knowledge of elapsed time/time remaining. In other words, we propose that subjects regulate

their pacing in order to run at the highest possible speed whilst, at the same time, avoid reaching maximal RPE before the end of the time trial. This is why they reduced their running speed in the muscle damage condition compared to control. This effort-based decision-making model is also compatible with the "end-spurt" phenomenon. Indeed, precise conscious anticipation of perceived exertion and running speed at the end of the time trial is not possible. Because finishing the race is paramount, athletes usually choose a slightly conservative pace for most of the time trial. Near the end, when the information provided by the conscious sensation of effort at a certain running speed is more reliable, subjects realise that they can significantly increase running speed without reaching exhaustion before the finishing line, and decide to go for an end-spurt.

Future research

The scarceness of studies about the effects of muscle damage and fatigue on endurance exercise performance represents an open field for future research. To date our studies and the small number of other studies present in the literature have investigated only high intensity endurance performance between 5 and 30 min duration (Davies et al., 2008, Twist and Eston, 2009). At these intensities, both type IIa and IIb muscle fibres are known to be recruited (Coyle, 2000, Altenburg et al., 2007). Whether fatigue/damage of these muscle fibres affects lower intensity exercise performance of > 30 min duration is an issue that deserves to be addressed in the future. In fact, it is possible that performance at an exercise intensity that requires only the recruitment of slow twitch fibres may not be affected by muscle fatigue and damage. The hypothesis of the dose-response relationship between the degree of muscle force loss and endurance performance also needs to be tested in the future. Because of the variability in muscle fatigue induced by eccentric exercise, it should be possible to divide subjects who respond with higher and lower force losses to the 100 drop jumps

protocol into two different groups and investigate the effect on endurance performance.

Moreover, to understand whether the detrimental effect of muscle damage on endurance performance is mediated by the reduction in force or soreness, it would be appropriate to investigate the time course of endurance performance after eccentric exercise. Indeed, the assessment of endurance performance immediately after and then again at 24/48 hours would discern the relative importance of these two factors.

In conclusion, this research programme has provided the first experimental evidence that locomotor muscle fatigue and damage significantly reduce high-intensity exercise performance. Our results also suggest that the increase in central motor command associated with reduced locomotor muscle force has a significant influence on cardiorespiratory responses during high-intensity constant-power and time trial tests, but not during an incremental exercise test. Finally, the effects of locomotor muscle fatigue and damage on exercise performance seem to be mediated by their effects on perception of effort as predicted by the psychobiological model based on motivational intensity theory. Further research is necessary to better understand the neurocognitive and physiological factors determining perceived exertion in conditions of muscle fatigue and damage, and to explore their effects on endurance performance of longer duration.

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APPENDIX

Example of letter from *School Ethics Committee* (Study 4).

ETHICS REVIEW AND APPROVAL FORM

1. Title of project: *Effect of decreased maximal strength of lower limbs on aerobic capacity and performance during an incremental exercise test.*

2. Name of researcher(s): Andrea Bosio

3. Name of supervisor: Samuele Marcora

4. Proposed starting date: November 2006 Proposed duration: 5 months

5. Briefly describe the sample of persons to be used in this study (include ages, gender, and special status, e.g. learning disabled).

Twenty physically active male and female subjects between the age of 18 and 45 familiar with maximum exercise testing on a cycle ergometer and ratings of perceived exertion (RPE) will be recruited among student and staff of the School of Sport, Health and Exercise Sciences (SSHES) of the University of Wales-Bangor and among the local cycling, triathlon and running clubs.

6. Methods of recruiting participants (describe):

Participants will be recruited through public advertisements, mailing list, letter to local sport clubs. Direct contacts to athletes who took part in previous experiments.

7. Where will the study take place, e.g. university, school, hospital?

The study will take place at the School of Sport, Health and Exercise Sciences (SSHES) of the University of Wales-Bangor

8. Give an estimate of the amount of time you will require of each participant in the study/project.

The estimated amount of time for each participants will be 5 hours split into three different occasions

9. Do you intend to pay participants for their participation? NO
(If yes, what form will the payment take).

10. Will you be using any form of deception? NO

11. Will this study involve any of the following manipulations?:

a Physiological NO

b. Psychological NO

c. Other controversial or potentially risky manipulations NO

d. In the case of questionnaire formats, will the study involve any questions which may be upsetting? NO

e. Do the hypotheses of your study involve the induction of negative effects upon the participants (e.g. learned helplessness) NO

12. If your study has the potential for "upsetting" participants (e.g. affective manipulation) and/or for identifying distressed or disturbed individuals you must make "a priori" arrangements to mitigate such effects (e.g. debriefing). Please specify the nature of such arrangements, if required.

The study doesn't have the potential for "upsetting" participants

13. Is there any risk to participants (physical and/or psychological)?: NO

14. How do you plan to handle the requirement of confidentiality?

15. During your data collection will supervision or assistance be required? YES
(e.g., for experiments in the physiology laboratory)

If yes, how will supervision be arranged?

The assistance will be arranged with the help of an undergraduate student and a member of staff.

16. Will informed consent be obtained? YES

(Unless there are very good reasons for not obtaining informed consent, the School guidelines state that you must use informed consent procedures). If no, explain why you will not be using an informed consent procedure.

17. Will a medical questionnaire need to be administered? YES

18. Will a pre-study questionnaire need to be administered? YES

19. Does your project involve using children under the age of 18 as you're participant population? NO

20. Does your project use special participants NO

21. Thus, is parental/guardian consent required for your project? NO

22. If your project requires you to have any **unsupervised** access to children under the age of 16, police screening needs to be carried out. This requires a Criminal Records Bureau Disclosure Form to be completed (see Appendix 5 for details).

23. Does your project require you to have any **substantial unsupervised** access to children between the age of 16 and 18? NO
If yes; Please provide further details

With reference to items 22 or 23 above, does police screening need to be carried out? **NO**

Taking into account the questions on this form and after discussion with the supervisor of the project, what ethics rating is proposed?

Category A: Project involving participants in which there is no element of psychological or physiological distress or harm other than that experienced in normal life.

Category B1: Project involving participants in which there is some risk of psychological or physiological distress or harm above that experienced in normal life. However steps have been taken to inform participants of the risk, minimise this risk, and debrief participants and the researcher demonstrates the necessary skills to do this.

Category B2: Project involving participants in which there is some risk of psychological or physical distress or harm above that experienced in normal life but steps have not been taken to inform participants of the risk, minimise the risk and debrief participants and/or the researcher does not show the necessary skills to do this.

Category C: Project involves participants in which there is risk of psychological or physical distress or harm above that experienced in normal life.

PROPOSED ETHICS RATING **B1**

ETHICS APPROVAL ACTION
To be completed by two staff members

IF RATING IS A OR B1

1. Staff members will sign below.
2. Submit signed copy of this form to Katharine

_____ _____
Staff signature Date

_____ _____
Staff signature Date

IF RATING IS B2 or C

1. Submit this form, the information sheet, the customised consent form (Form 2 or 3 as appropriate) and the Protocol to the SSHES Ethics Committee.
2. SSHES Ethics Committee considers application (see Flow Chart on Page 6 for details).
3. If approved, Ethics Committee Chairperson will sign below.
4. Submit signed copy of this form to Katharine.

_____ _____
Ethics Committee Chairperson signature Date

Example of *Information sheet* (Study 2).

Effects of Locomotor Muscle Fatigue on the Physiological and Perceptual Responses to Exercise

Subject Information Sheet

Testing Procedures

This study involves subjects fatiguing their leg muscles, primarily the quadriceps, prior to performing an exercise test on a cycle ergometer. Fatigue will be induced to the leg muscles by the subjects following a drop jump protocol, which consists of 100 jumps from a 40cm high aerobics step performed over 33 minutes. The subjects will be tested for the isometric maximal voluntary contraction force of their quadriceps both before and after the drop jump protocol has been performed.

The subjects will then perform the cycling exercise test, in which they will cycle at 80% of their peak power output until exhaustion.

Time period Involved

Subjects will be required to perform 3 separate sessions, each of 2-hours duration. However only 1 of these sessions will involve the muscle fatigue protocol. The first session will be a VO_2 max, and peak power output test, and the third session will be a control trial. The initial max test has to be at least 48 hours apart from the first trial, then there will be 10-14 days between each trial.

Cash Prizes

There will be a prize of £50 for the people who go for the longest time on the time trial. A £30 and £10 prize for the second and third performance respectively.

Contact Details

If you are interested in participating in this study then please contact Andrea Bosio:

Tel: 07717583304 Mike
Tel: 07854034925 Andrea

Andrea Bosio
Tel: 01248 38 3495
Mobile 07854034925
E-mail: pex001@bangor.ac.uk

Example of *Consent form* (Study 1).

Effects of muscle damage on aerobic metabolism and endurance performance

Please read the following carefully

Person will be considered unfit to do the experimental exercise task if they:

- Have a fever, suffer from fainting spells or dizziness;
- Have suspended training due to a joint or muscle injury;
- Have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- Have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- Have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- Have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella,..)
- Have history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum;

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiment during the period of .....

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I can withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature of Participant: .....

Date: ...../...../.....

Signature of experimenter: .....

Date: ...../...../.....

Example of *Data collection form* (Study

PRE-STUDY QUESTIONNAIRE

Name: .....

Date: .....

Researcher: .....

YES/NO

1. Have you had any kind of illness or infection in the last two weeks? .....

   If YES what? .....

2. Are you taking any form of medication? .....

3. Do you have any form of injury? .....

4. Have you eaten in the last hour? .....

5. Have you consumed any alcohol in The last 24 hours? .....

6. Have you performed exhaustive Exercise within the last 48 hours? .....

If the answer to any of the above questions is YES, then you must consult a member of staff before undergoing any exercise test.

Signature of participant: .....

VO2 max Test

Astrand

Bike: ***Lode Excalibur*** Date of test: ...../...../..... Time: .....

Temperature C°: ..... Humidity %: ..... Barometric Pressure kPa: .....

Saddle: Vertical ..... Handlebars: Vertical .....
Horizontal ..... Horizontal .....

Time (min)	*Load (W)*	*Cadence (rpm)*	*HR (bpm)*	*RPE (6 – 20)*	*VO_2 ($l \cdot min^{-1}$)*
1	50			L	
2	50			C	
3	100			L	
4	100			C	
5	150			L	
6	150			C	
7	200			L	
8	200			C	
9	250			L	
10	250			C	
11	300			L	
12	300			C	
13	350			L	
14	350			C	
15	400			L	
16	400			C	
17	450			L	
18	450			C	

VO_2max: ..... $l \cdot min^{-1}$ RER: .....

BF: ..... $1 \cdot min^{-1}$ VE/VO_2: .....

Effects of leg muscle weakness on exercise capacity

Name: .....

Date of test ...../...../..... Time.....

Temperature: ..... Humidity: ..... Barometric Pressure: .....

DOMS

Please tick the sentence below that best describes your level of muscle soreness over the past 12 hours

- ☐ 0 A complete absence of soreness
- ☐ 1 A light pain felt only when touched / a vague ache
- ☐ 2 A moderate pain felt only when touched / a slight persistent pain
- ☐ 3 A light pain when walking up or down stairs
- ☐ 4 A light pain when walking on a flat surface / painful
- ☐ 5 A moderate pain, stiffness or weakness when walking / very painful
- ☐ 6 A severe pain that limit my ability to move

Blood Creatine Kinase

CK values at rest before warm-up: ..... UI/l

Lactate

Before starting time to exhaustion: ..... mmol/l

After time to exhaustion: ..... mmol/l

Haemoglobin

Before starting time to exhaustion: ..... g/dl

After time to exhaustion: ..... g/dl

Average HR during 100 drop jumps: ***..... (bpm)***

Strength measurement

Warm-up: 5 min cycling 10% Peak power output Workload: (W).....

25% 1 min 50% 1 min 75% (of the maximal force)

Chair set-up: Lever ..... Back of the seat .....

Quadriceps Maximal Voluntary Contraction ***before treatment***

Trial	1	2	3
Force (N)			

Quadriceps Maximal Voluntary Contraction ***immediately after treatment*** O *A* O *B*

Trial	1	2	3
Force (N)			

Physioflow calibration: blood pressure

1st measurement: ...../..... (syst/diast)

2nd measurement: ...../..... (syst/diast)

3rd measurement: ...../..... (syst/diast)

Average: ...../..... (syst/diast)

CO: ..... l/min ***SV: ..... ml***

Time to exhaustion

Mask size:.....

Lode bike Excalibur Set-up

Saddle vertical ..... Handlebars vertical .....

Saddle horizontal ..... Handlebars horizontal .....

3 min Warm-up 10% Peak power output **Workload (W): .....**

Time to exhaustion 80% Peak power output **Workload (W): .....**

Time (min)	*HR (bpm)*	*Cadence (RPM)*	*RPE (legs)*	*RPE (chest)*	*Blood pressure*
At rest					
End warm-up					
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
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