Supporting the prescription of exercise in spinal cord injured populations

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Supporting the prescription of exercise in spinal cord injured populations: ratings of perceived exertion and inflammation-mediating plasma cytokines

By

Thomas Andrew William Paulson

A Doctoral Thesis

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To Mum & Dad, for all your support.

‘It always seems impossible until it is done’

(Nelson Mandela)
Abstract

Following a spinal cord injury (SCI), participation in regular exercise can enhance physical capacity and performance in activities of daily living. With this in mind, the use of subjective ratings of perceived exertion (RPE) may provide an easy-to-administer alternative to traditional methods of regulating exercise intensity (e.g. heart rate and power output (PO)). A physically active lifestyle is also associated with a reduced risk of cardiovascular disease, in part because exercise exerts ‘anti-inflammatory’ effects. Examining the plasma response of inflammation-mediating chemical messengers, known as cytokines, to traditional and novel exercise modalities may help maximise the anti-inflammatory potential of regular exercise.

Participants with a cervical level SCI successfully self-regulated a 20 min bout of moderate intensity wheelchair propulsion (Chapter three). No differences in physiological or PO responses were observed during the imposed-intensity and self-regulated wheelchair propulsion in the trained population group. In a non-SCI group of novice wheelchair-users, a differentiated RPE specific to the exercising muscle mass (RPE_p) was the dominant perceptual signal during submaximal wheelchair propulsion (Chapter four). The novice group successfully self-regulated a 12 min bout of moderate intensity wheelchair propulsion, comprising of a discontinuous 3 x 4 min protocol, using differentiated RPE_p. In contrast, a more accurate self-regulation of light intensity wheelchair propulsion was observed when employing traditional overall RPE compared to RPE_p.

Following strenuous wheelchair propulsion, plasma concentrations of the inflammation-mediating cytokine interleukin-6 (IL-6) were significantly elevated in non-SCI and thoracic level SCI participants (Chapter five). Impaired sympathetic nervous system (SNS) function was associated with a reduced IL-6 response in participants with a cervical level SCI. The plasma IL-6 response to 30 min moderate intensity (60% VO_2peak) arm-crank ergometry (ACE) was associated with an elevation in the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra) independent of SNS activation (Chapter six). Light intensity ACE resulted in a small, significant plasma IL-6 response but no IL-1ra response. The addition of functional electrical stimulation-evoked lower-limb cycling to concurrent hand cycling, termed hybrid exercise, resulted in a greater plasma IL-6 response compared to moderate intensity hand cycling alone in participants with a thoracic level SCI (Chapter seven).

Key words: Tetraplegia, paraplegia, wheelchair propulsion, hybrid exercise; arm-crank ergometry; immune system; cardiovascular disease.
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Preface

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Abbreviations

ACE = arm-crank ergometry
ADL = activities of daily living
ANOVA = analysis of variance
ASIA = American spinal injuries association
BF = breathing frequency
BLa^- = blood lactate concentration
BLa^-peak = peak blood lactate concentration
CI_diff = confidence intervals of the difference
Ca^{2+} = calcium ion
CSA = cross sectional area
CVD = cardiovascular disease
GE = gross mechanical efficiency
GXT = graded exercise test to exhaustion
H^+ = hydrogen ion
HDL = high density lipoprotein
HR = heart rate
HR_peak = peak heart rate
HRR = heart rate reserve
HPA = hypothalamic pituitary adrenal
IL = interleukin
IL-1ra = interleukin-1 receptor antagonist
non-SCI = non-spinal cord injured
PARA = paraplegic
PO = power output
PO_peak = peak power output
PF = push frequency
\dot{Q} = cardiac output
RER = respiratory exchange ratio
RER_peak = peak respiratory exchange ratio
RPE = ratings of perceived exertion
RPE_C = central ratings of perceived exertion
RPE_O = overall ratings of perceived exertion
RPE_p = peripheral ratings of perceived exertion
SD = standard deviation
SCI = spinal cord injury
TETRA = tetraplegic
TNF-\alpha = Tumour necrosis factor-alpha
VO_2 = oxygen uptake
\dot{VO}_2peak = peak oxygen uptake
VE = minute ventilation
VE/\dot{VO}_2 = ventilatory equivalent for oxygen
VER = verification test
WERG = wheelchair ergometry
1. General Introduction

Modern day developments in acute and long-term medical care mean life expectancies following a spinal cord injury (SCI) continue to rise. Subsequently, the focus of rehabilitation outcomes has seen a shift from the extension of life expectancy to the attainment of an optimal level of independent living. Today a large emphasis is placed on people’s health as well as their level of physical impairment. However, the physiological and psychosocial consequences of SCI can greatly challenge an individual’s ability to maintain a physically active lifestyle (Jacobs & Nash 2004). The metabolic sequelae of SCI, including the atrophy of paralysed muscle, reduced energy expenditure and gain in abdominal fat mass, combine with low levels of physical activity to elevate the risk of chronic disease (Cowan & Nash 2010). Cardiovascular disease (CVD) has become the leading cause of mortality and morbidity in chronic SCI populations (Myers et al. 2007). The prevalence of type 2 diabetes, a common risk factor for CVD, is three times higher than non-SCI populations of an equivalent age (LaVela et al. 2006).

A growing body of evidence supports the significant functional and health-related benefits of physical activity and exercise in people with a SCI. Despite this, physical activity levels are lower than those observed in non-SCI populations (Berg-Emons et al. 2008; Ginis et al. 2010). Physicians and rehabilitation professionals are in the best position to encourage and guide physical activity in persons with SCI, however, only 18-40% of patients report receiving exercise advice (Cowan et al. 2009). A number of barriers to exercise participation, including a lack of accessible facilities, lack of exercise knowledge and low self-esteem, challenge the ability to maintain a physically active lifestyle (Kehn & Kroll 2009). The impaired cardio-acceleration observed following a cervical level SCI and high cost of equipment can also mean traditional methods for regulating exercise intensity, including heart rate (HR) and power output (PO), are unavailable to many individuals. The subjective ratings of perceived exertion (RPE) may therefore provide a cost-effective, easy to administer method for prescribing exercise that can promote participation in structured exercise training following a SCI.
In both acute and chronic SCI populations, regular participation in exercise has been shown to significantly improve physical capacity and performance in activities of daily living (ADL) (Dallmeijer 1999a; Dallmeijer 1999b; de Groot et al. 2003; Valent et al. 2008). The positive association between cardiorespiratory fitness and many risk factors for CVD have led to calls for exercise to be prescribed as ‘medicine’ (Cowan & Nash 2010; Sallis 2009). In contrast, physical inactivity and obesity are causally linked to a ‘chronic low-grade inflammatory state’ (Osborn & Olefsky 2012). Subsequently, chronic inflammation has been shown to play a critical role in the aetiology of CVD and type 2 diabetes (Calder et al. 2011). It is proposed that physical activity and exercise may exert ‘anti-inflammatory’ effects that, combined with long-term reductions in adiposity and improved blood lipid-profiles, may provide a protective effect against CVD (Gleeson et al. 2011; Nash et al. 2011). However, the autonomic impairment associated with cervical level SCI and the small volume of muscle mass activated during traditional upper-limb exercise may limit the anti-inflammatory responses to acute exercise (Kouda et al. 2012; Yamanaka et al. 2010).

1.1 Anatomy of the spinal cord and nervous system

Enclosed within the vertebral column, the spinal cord is the major conduit through which motor and sensory information is relayed between the brain and the body (Kirschblum et al. 2011). With it’s one billion neurons, the spinal cord provides a key structure of the central nervous system (CNS), organising both conscious and unconscious motor and sensory function (Purves 2011). Observed in transverse, the spinal cord is roughly divided into two areas; with a centrally located H-shape of grey matter, surrounded by white matter (Figure 1.1). White matter contains longitudinally orientated spinal tracts containing ascending sensory ‘afferent’ and descending motor ‘efferent’ neurons (Purves 2011). The two anterior projections of grey matter, known as the ventral roots, contain cell bodies of lower motor neurons which synapse directly with skeletal muscle to initiate voluntary movement (Figure 1.1). The two posterior projections, known as dorsal roots, receive sensory afferent neurons carrying impulses from peripheral sensory receptors in the skin, muscles and internal organs (Purves 2011).
The dorsal and ventral roots fuse laterally to form the spinal nerves which emerge from the spinal vertebrae (Figure 1.1). In total, 31 pairs of spinal nerves (8 cervical, 12 thoracic, 5 lumbar, 5 sacral, 1 coccygeal) act as the bridge between the CNS and the motor and sensory components of the peripheral nervous system (Purves 2011). As shown in Figure 1.2, the innervation of target organs is organised in a segmental fashion. The cervical nerves supply the muscles of the upper-limbs (e.g. C5-C6 innervates biceps brachii (elbow flexor), C6-C8 innervates triceps brachii (elbow extensor)) and the lumbar and sacral nerves supply lower-limbs (e.g. L2-L4 innervates quadriceps (knee extensors)) (Marieb & Hoehn 2007).
While a large portion of motor function is under conscious control, the human body is highly sensitive to challenges against the stability of its internal environment. The autonomic nervous system, with its parasympathetic and sympathetic arms, exists as a division of the peripheral nervous system providing involuntary innervation of smooth muscle (blood vessels), cardiac muscle and secretory glands (Marieb and Hoehn 2007). Normal autonomic function provides a constant balancing act between ‘activity-supporting’ sympathetic tone and ‘rest-and-digest’ parasympathetic tone. With the exception of the sacral outflow to the bladder and genitals (Figure 1.2), neurons of the parasympathetic nervous system are located in the motor-cranial nerve of the brain stem and synapse directly to the target organ, predominantly via the vagus nerve (Marieb and Hoehn 2007). Among its many functions, parasympathetic tone decreases HR while promoting energy storage and digestion. In contrast, the grey matter of the thoracic and upper lumbar spinal cord between T1-L2 contains sympathetic nerve fibres within the lateral grey horns. Preganglionic sympathetic fibres enter the adjoining paravertebral sympathetic ganglion (Figure 1.2). Subsequent postganglionic fibres act directly via the neurotransmitter noradrenaline or indirectly via stimulating release of the catecholamines adrenaline and noradrenaline from the adrenal medulla (Marieb & Hoehn 2007). Sympathetic tone elevates HR and breathing rate, controls skeletal muscle blood flow via vasodilation and vasoconstriction of blood vessels in active and inactive muscles, and initiates the secretion of adrenaline and noradrenaline from the adrenal medulla (Figure 1.2).
Figure 1.2 A schematic representation of the somatic and autonomic nervous systems
1.2 Epidemiology of spinal cord injury

Traumatic spinal injuries occur when direct or indirect forces applied to the vertebral column lead to damage of the spinal cord, with motor vehicle collisions, falls, violence and sports activities among the leading causes (Lee et al. 2013). A bimodal distribution in injury prevalence is present with regard to age, with a first peak in young adults aged between 15-29 years and a smaller, but growing, second peak over the age of 60 (van den Berg et al. 2010a). Predicted global estimates suggest an incidence of 200,000 injuries per annum worldwide (Lee et al. 2013) with around 40,000 people living with a SCI in the UK (Webborn & Goosey-Tolfrey 2008). Incidence rates for non-traumatic injury are less clear, but an age-dependent relationship highlights the growing prevalence of tumour, degeneration and vascular related disorders in ageing populations (van den Berg et al. 2010a).

Whether a SCI is traumatic or non-traumatic, the resultant deficits in motor, sensory and autonomic functions are dependent on the level and the pattern of spinal lesion, mainly in the transverse plane. Remaining sensory (dermatome) and motor (myotome) function is examined according to international guidelines for neurological classification (American Spinal Injuries Association (ASIA)) to identify lesion level and completeness (Kirschblum et al. 2011). Injury level is described as the most caudal (‘away from the head’) spinal segment with remaining function. Tetraplegia refers to impairment or loss of motor and/or sensory function in the cervical segments of the spinal cord, and results in some impairment of the arms as well as typically the trunk, legs and pelvic organs (Kirschblum et al. 2011). Paraplegia refers to impairment or loss of motor function in the thoracic, lumbar and sacral segments of the spinal cord, with lesion level dependent losses in trunk, leg and pelvic function (Kirschblum et al. 2011). A complete SCI results in the complete loss of sensory and motor communication between the brain and tissue innervated below the lesion level (classified as ASIA impairment scale A). The neurology of incomplete injuries is complex and can differ greatly from one case to another (classified as ASIA score B-D) (Kirschblum et al. 2011). Damage to the neurons of anterior ventral roots (Figure 1.1) may lead to a loss of motor function but maintenance of sensory function, whereas the converse may apply with damage to neurons of posterior dorsal roots.
1.3 Thesis Aims and Outline

The current thesis aims to provide evidence to promote and support the prescription of exercise in SCI populations. The primary objectives were to:

1. Investigate the use of subjective ratings of perceived exertion (RPE) as a tool for the self-regulation of wheelchair exercise in novel participant groups, including individuals with tetraplegia and individuals inexperienced in wheelchair propulsion.

2. Examine a) the acute response of inflammation-mediating plasma cytokines to traditional and novel exercise modalities available to individuals with a SCI, and b) how these responses may be affected by the autonomic impairment and the small muscle mass activated during upper-limb exercise.

A brief introduction to the chapters contained within this thesis is presented on the following page.
The following chapter (Chapter two) provides an overview of the physiological consequences of a SCI. Previous research investigating the acute plasma cytokine responses to upper-limb exercise is also discussed, followed by the proposed relationship between health and the ‘anti-inflammatory’ effects of regular exercise. Finally, a review of the current literature examining the self-regulation of exercise in both SCI and non-SCI populations using RPE is provided.

The first two experimental chapters of the thesis examine the utility of RPE as a method of self-regulating the intensity of wheelchair-based exercise training in novel participant groups. Specifically, Chapter three investigates the use of RPE to self-regulate exercise in individuals with tetraplegia. Subsequently Chapter four employs a non-SCI group with no prior experience of wheelchair propulsion to establish whether a mode-specific differentiated RPE, focusing on the localised upper-limb strain, may improve the self-regulation of exercise in individuals inexperienced in the demands of wheelchair exercise.

Chapter five examines the effect of SCI level on the acute response of inflammation-mediating plasma cytokines and stress hormones to acute wheelchair exercise. Chapter six then explores how the same responses are affected by the intensity of acute submaximal arm-crank exercise and the addition of lower-limb cycling to upper-limb exercise to form ‘hybrid’ whole body exercise in a non-SCI group. The differentiated RPE responses between different intensity arm and hybrid exercise are also compared. Chapter seven, the final experimental chapter, extends the previous chapters findings to compare the anti-inflammatory potential of voluntary upper-limb exercise with and without the addition of functional-electrical stimulation (FES)-evoked lower-limb cycling in participants with a thoracic level SCI. A general discussion of the key findings, implications and applications of this work is then presented in Chapter eight.
Figure 1.3 A needs analysis for the current thesis

Experimental Chapters:

Chapter three: Perceived exertion as a tool to self-regulate exercise in individuals with tetraplegia

Chapter four: Differentiated perceived exertion and self-regulated wheelchair exercise

Chapter five: Spinal cord injury level and the circulating cytokine response to strenuous exercise

Chapter six: Plasma cytokine and perceived exertional responses to submaximal arm-crank ergometry: The effect of exercise intensity and the addition of lower-limb cycling

Chapter seven: Exercise mode and the circulating cytokine response in persons with thoracic spinal injury: A feasibility study comparing hand cycling and hybrid exercise
2. Literature Review

2.1 Physiological consequences of spinal cord injury

Injury to the spinal cord presents a complex, lesion-level dependent challenge to respiratory, cardiovascular, autonomic, and skeletal muscle function. Due to the increasing loss of functional muscle mass and autonomic control, higher levels of injury result in impaired cardiovascular function and oxygen demand both at rest and during exercise (Haisma et al. 2006; Leicht et al. 2013a). The physiological consequences of SCI therefore have a large impact on an individual’s physical capacity and performance in ADL.

2.1.1 Respiration

Normal respiration and blood oxygenation requires both; 1) the co-ordinated motor control of both inspiratory and expiratory skeletal muscles, and 2) autonomic control of bronchial dilation (West et al. 2012). As shown previously in Figure 1.2, the complete paralysis of muscles of inspiration (diaphragm, scalenes and external intercostals) and expiration (internal intercostals and abdominals) with a SCI above C5 leaves individuals requiring ventilatory assistance to maintain breathing mechanics. Due to the maintenance of abdominal and intercostal control in low level paraplegia, respiratory muscle strength and function is comparable to non-SCI individuals (Haisma et al. 2006). Following a low cervical or high thoracic injury, voluntary inspiration via the diaphragm remains. However, the loss of the main muscles of expiration and the auxiliary muscles of inspiration impair lung volume parameters, including vital capacity and forced expiratory volume in 1 sec (Haisma et al. 2006; van Loan et al. 1987) (Figure 2.1). This compromised expiratory function can result in an ineffective cough and an inability to clear mucosal secretions (Schilero et al. 2009), leading to a high prevalence of morbidity resulting from pulmonary complications and infections (Brown et al. 2006). Highly trained sportsmen show superior pulmonary function compared to untrained individuals, including total lung capacity and vital capacity (West et al. 2012). Short-term inspiratory muscle training has also shown beneficial effects on respiratory muscle function (Mueller et al. 2008). Therefore some SCI related reductions in respiratory function may be partly reversed with training.
Figure 2.1 Cardiovascular and muscular consequences of a SCI and effect on peak aerobic capacity
2.1.2 Cardiovascular and autonomic control

At rest, and in response to exercise and orthostatic challenge, the redistribution of blood following a SCI is impaired due to the lack of sympathetic vasoconstriction in inactive tissue below the lesion level (Figoni 1993; Jacobs et al. 2002). The ‘pooling’ of blood in the lower-limbs and inactive venous muscle pump reduce ventricular re-filling and therefore stroke volume during exercise (Jacobs et al. 2002). Cardiac output (Q) is maintained by elevations in resting and submaximal HR in individuals with paraplegia (Hopman et al. 1993). Methods to increase ventricular filling, including exercising in a supine position, have been shown to increase stroke volume at submaximal exercise intensities (Hopman et al. 1998b). In individuals with tetraplegia, the redistribution of blood and ability to elevate Q is further limited due to the loss of autonomic control of vessels in the abdominal bed and cardiac tissue (Thijssen 2009). A spinal cord lesion above T5 results in the loss of sympathetic outflow to the heart and peak HR’s (HR
peak) of around 100-140 b·min⁻¹ (Valent et al. 2007a). Partial cardio-acceleration is maintained by the withdrawal of parasympathetic tone.

Depressed plasma concentrations of adrenaline and noradrenaline are observed at rest (Schmid et al. 1998a, Schmid et al. 1998b) and during exercise (Schmid et al. 1998a, Schmid et al. 1998b) following the loss of sympathetic outflow to adrenal glands at T6. This absent exercise induced systemic vasoconstriction and cardiac stimulation further limits the ability to augment Q during exercise (Hopman et al. 1993). This hypokinetic circulation results in a decreased left ventricular mass (~25% less than non-SCI) and smaller left ventricular volume in both untrained (de Groot et al. 2006) and trained (West et al. 2012) individuals with a cervical level SCI compared to non-SCI controls.

The loss of autonomic function following high thoracic and cervical level injury presents two distinct challenges to health and exercise performance; namely orthostatic hypotension and autonomic dysreflexia. Vasomotor centres in the medulla reflexively control the cardiovascular system by adjusting sympathetic and parasympathetic outflow to the heart and peripheral vasculature in order to maintain blood pressure (Krassioukov 2009). The loss of sympathetic nervous system outflow in tetraplegia results in bradychardia and chronic hypotension induced via a constant state of vasodilation (Somer 2010). Postural change to an upright position results in an un-
modulated drop in blood pressure called orthostatic hypotension. Extreme hypotension is transient following a SCI, resolving within a few weeks of injury due to a compensatory reduction in parasympathetic outflow and return of sympathetic reflexes (Somer 2010).

Autonomic dysreflexia is a potentially life-threatening bout of extreme, uncontrolled hypertension resulting from severe vasoconstriction and cardiac stimulation. Incidences occur when a noxious stimulus below the lesion level triggers an excessive sympathetic response due to the loss of inhibition descending from the medulla and/or hypersensitivity of sympathetic neurons (Krassioukov & Claydon 2006). Common causes include bladder or rectal distension and failure to remove the stimulus can lead to renal failure, cerebrovascular accidents and in extreme cases, even death (Krassioukov & Claydon 2006).

2.1.3 Skeletal muscle

A rapid decline in muscle fibre cross-sectional area (CSA) is observed in the lower-limbs following paralysis, with a direct relationship reported between the extent of muscle atrophy and injury duration (Castro et al. 1999). Just six months and one year post injury, quadriceps CSA in participants with motor complete injury were found to be 1/3 and 1/2 of age-matched non-SCI controls respectively (Castro et al. 1999; Spungen et al. 2000). Subsequent reductions in resting and daily energy expenditure are directly proportional to the volume of lean body mass lost (Bauman et al. 2008). This decline in muscle CSA is accompanied by a transformation of remaining muscle fibre from slow type-1 myosin heavy chain to fast type-2 myosin heavy chain isoforms (Castro et al. 1999). The changes in muscle fibre composition lead to concomitant reductions in oxidative metabolism (McCully et al. 2011) and increases in intramuscular fat (Elder et al. 2004). Following a SCI where the lower motor neuron remains intact, reflex contractions via the grey matter are evidenced by spasticity and the muscle remains responsive to electrically-evoked contractions passed through the corresponding peripheral nerve (Ragnarsson 2008). Where the lower-motor neuron is damaged, flaccid paralysis ensues, leading to an absence of muscle tone and spinal reflex activity (Martin et al. 2012).
2.1.4 Physical capacity

Physical capacity is the ability of the musculoskeletal, cardiovascular and respiratory systems to undertake a level of physical activity (Haisma et al. 2006). Peak anaerobic PO and muscular strength show a similar lesion-level dependent relationship to cardiovascular and respiratory function (Dallmeijer et al. 1996). This is an important factor as most daily activities during wheelchair propulsion, including negotiating curbs or a slope, require substantial anaerobic power (Hutzler 1998; Janssen et al. 2002). Subsequently individuals with tetraplegia exhibit higher physical strain during tasks of daily living, including transferring to bed and entering a car, than those with thoracic level injuries (Janssen et al. 1994). In turn, physical capacity shows an inverse relationship with physical strain during the same tasks (Dallmeijer et al. 1996; Janssen et al. 1994).

Gains in physical capacity are associated with reduced strain during ADL (Dallmeijer et al. 1999a; Janssen et al. 1994) and a greater time spent participating in leisure time physical activity (Hetz et al. 2009). However, physical strain during ADL is considered too low to improve or maintain cardiopulmonary fitness (Janssen et al. 1996). Dallmeijer et al. (1999b) found participation in sports activities to be the most important factors explaining relative changes in anaerobic power parameters following discharge from rehabilitation. Those involved in frequent sports competition frequently display higher aerobic and anaerobic capacity than untrained/recreational populations (Goosey-Tolfrey et al. 2006; Hutzler 1998; Lovell et al. 2012; Woude et al. 2002). Structured aerobic and resistance exercise training in addition to normal acute rehabilitative care (de Groot et al. 2003) and in persons with chronic SCI (Hicks et al. 2003; Jacobs 2009), has also been shown to improve both VO₂peak and PO_peak. Thus providing individuals with appropriate tools to actively participate in structured exercise training is an important requirement for maintaining or improving physical capacity.
2.1.5 Limitations to peak oxygen uptake: whole body versus upper limb exercise

As shown in Figure 2.1, injury to the spinal cord results in the impairment of mechanisms responsible for oxygen delivery to exercising limbs, including venous pooling and reduced stroke volume. Overall energy demand increases during exercise to sustain contractile activity in the active muscle, the respiratory muscles and myocardium. At maximal and supra-maximal intensities, \( \dot{V}O_2_{\text{peak}} \) is determined by; 1) systemic and limb oxygen delivery, and 2) muscle oxygen utilisation. Despite increasing metabolic demand, skeletal muscle perfusion, oxygen delivery and aerobic metabolism become progressively restricted during high intensity cycling exercise in non-SCI populations (Mortensen et al. 2008). Impaired limb \( O_2 \) delivery occurs secondary to a plateau in \( \dot{Q} \) and enhanced vasoconstrictor activity independent of increasing workload (Mortensen et al. 2008). Therefore central (\( \dot{Q} \), stroke volume) and peripheral (reduced blood flow, oxygen transport) factors determining limb oxygen delivery and utilisation primarily limit \( \dot{V}O_2_{\text{peak}} \) during whole body exercise (Mortensen et al. 2008; Saltin & Calbet 2006).

Persons with paraplegia and tetraplegia present lesion-level dependent impairments in oxygen delivery during exercise, including the loss of autonomic control of vessels in the lower-limb and abdominal vasculature, impaired cardio-acceleration and reduced respiratory muscle function (Figure 2.1). Despite these central limitations, Hopman et al. (1998a) observed no improvement in peak oxygen uptake (\( \dot{V}O_2_{\text{peak}} \)) or peak PO (\( PO_{\text{peak}} \)) in persons with paraplegia and tetraplegia when venous return was supported by supine exercise or concurrent functional electrical stimulation (FES)-evoked lower-limb contractions. It was therefore concluded that limitations to exercise in persons with a SCI may be located peripherally rather than centrally.

In untrained populations with and without a SCI, a failure to attain age-predicted maximum HR or a plateau in \( \dot{V}O_2 \) during exhaustive upper-limb exercise is a commonplace, reflecting the peripheral limitation to peak aerobic capacity (Sawka et al. 1983; Lovell et al. 2012). The small diffusional surface area, shorter oxygen transit time and high intramuscular pressures experienced in the contracting upper-limb limit oxygen extraction compared to muscles of the lower-limb (Calbet et al. 2005; Volianitis et al. 2004). Improvements in \( \dot{V}O_2_{\text{peak}} \) associated with upper-limb exercise training are attributed to an increase in arm blood flow, a larger extraction of oxygen via a higher
capillary surface area and improved oxidative metabolism (Hooker et al. 1989; Volianitis et al. 2004). Persons with tetraplegia may face both central (blood redistribution) and peripheral (small active muscle mass) factors limiting peak aerobic capacity compared to paraplegic groups. Lesion-level dependent differences in peak aerobic capacity are shown in Table 2.1.

| Table 2.1 Characteristics of upper-limb exercise modalities and comparison of mode and injury-level specific peak aerobic responses with manual hand-rim propulsion |
| --- | --- | --- |
| Max GE (%) | Manual hand-rim propulsion | Arm-crank ergometry | Rec. hand cycling |
| Risk of upper-limb strain | <11 | 12-15 | ~15 |
| Form of outdoor mobility | High | Low | Low |
| Risk of upper-limb strain | Yes | No | Yes |
| Peak power output (W) | AB | 45-55 | ↑ (80-160) |
| | | 80-100 | ↑ (>100) |
| | | 50-70 | ↑ (80-100) |
| | TETRA | 20-50 | ↑ (~50-80) |


Note. GE = gross mechanical efficiency; AB = able-bodied; LP = low level paraplegia (below T6); HP = high level paraplegia (above T6); TETRA = tetraplegia; Rec = recreational.
2.2 Exercise modalities

2.2.1 Manual wheelchair propulsion

Manual wheelchair propulsion is a form of locomotion necessary for both daily ambulation and sports performance. The unconstrained, cyclic pattern of wheelchair propulsion involves a short push phase followed by a long recovery phase (Woude et al. 2001). Peak contact forces between the user and the hand-rim are observed in the middle of the push phase, with all contact forces applied during only 55% of the whole propulsion cycle (Arnet et al. 2012). In addition, braking forces, termed negative forces, applied at the beginning and end of each push cycle result from a non-optimal direction of force application (Woude et al. 2001). A high static component of force production stabilises the shoulder and wrist joints without contributing to external power production (Hintzy et al. 2002). Thus, gross mechanical efficiency (GE), defined as the ratio between external power production (PO) and internal energy liberation (oxygen cost under submaximal, steady-state conditions), is low during wheelchair propulsion, rarely exceeding 11% (Table 2.1) (Dallmeijer et al. 2004; Hintzy et al. 2002; Lenton et al. 2008).

Low intensity wheelchair propulsion training has a positive effect on GE via improvements in propulsion technique including; increased push time, reduced cycle frequency and reduced negative force (de Groot et al. 2002; de Groot et al. 2008). Experienced manual wheelchair users (8-11%) also show higher GE than novice user groups (4-8%), highlighting the role of familiarisation and habituation in improving coordination and force production (Brown et al. 1990; Dallmeijer et al. 2004; Lenton et al. 2008). However, high peak muscle forces in the rotator cuff muscles of the shoulder during low intensity propulsion are observed independent of wheelchair propulsion experience (Veeger et al. 2002). The high mechanical loads placed upon the wrist and shoulder joints by intermittent hand-rim coupling and the inefficient application of force result in a high prevalence of over-use injuries in both wheelchair athletes and everyday users (Boninger et al. 2002; Woude et al. 2001).

The employment of untrained, non-SCI participants as a model of novice wheelchair users in early rehabilitation (de Groot et al. 2008; Lenton et al. 2008) and for comparison between exercise modes (Dallmeijer et al. 2004; Hintzy et al. 2002) is
common in the wheelchair propulsion literature. Findings cannot be immediately generalised to wheelchair users with a SCI, particularly those with cervical level injury. Yet the application of novice, non-SCI participants reduces confounding factors of upper-limb training, wheelchair propulsion experience and upper-extremity pain within research design. If appropriate, experimental findings from these non-SCI populations can be extended to novice and experienced wheelchair users for verification.

2.2.2 Arm-crank ergometry

Asynchronous, stationary ACE provides a more mechanically efficient (~15%) mode of exercise than wheelchair propulsion (Hintzy et al. 2002; Sawka et al. 1980). The greater GE reflects the continuous application of force throughout 360° of the cyclic movement and a simultaneous push and pull with contralateral arms (Sawka et al. 1980). Lower submaximal \( \dot{V}O_2 \), HR and physical strain indicate ACE is inherently less strenuous than wheelchair propulsion at the same submaximal workload (Hintzy et al. 2002). When energy expenditure from unloaded exercise (0 W) is excluded from total energy expenditure, wheelchair propulsion and ACE present similar levels of work efficiency at submaximal intensities (Hintzy et al. 2002). Therefore, biomechanically induced energy losses inherent with intermittent hand-rim coupling and joint stabilisation during wheelchair propulsion are the cause of the mode specific GE. The greater GE observed during ACE results in higher levels of \( P_O^{\text{peak}} \) during ACE (~30%) than wheelchair propulsion, with little difference seen in \( \dot{V}O_2^{\text{peak}} \) (Table 2.1).

2.2.3 Hand cycling

Hand cycling provides a daily and recreational form of ambulation that can be performed over longer distances and at higher speeds than wheelchair propulsion (Hettinga et al. 2010). Add-on crank units fitted to conventional daily hand-rim wheelchairs provide an effective form of aerobic training during active rehabilitation (Valent et al. 2008). The growing interest in sports performance has also led to the development of light-weight, rigid frame, two and three-wheeled specialised competition hand cycles (Abel et al. 2006; Hettinga et al. 2010). The majority of hand cycles employ synchronous propulsion, which present higher GE, \( P_O^{\text{peak}} \) and lower submaximal physical strain than asynchronous modes (Woude et al. 2008). It is proposed that increased co-contraction of the upper-extremities and, if active, the trunk,
to combine power production with stable steering may contribute to reduced efficiency during asynchronous propulsion (Faupin et al. 2011; Woude et al. 2008).

As with ACE, the constant application of force over the whole propulsion cycle and absence of coupling-uncoupling actions makes hand cycling more mechanically efficient than wheelchair propulsion (Dallmeijer et al. 2004). At a fixed PO of 35 W, Dallmeijer et al. (2004) reported significantly lower VO₂, HR, RPE and a higher GE during hand cycling than wheelchair propulsion. Further, Arnet et al. (2012) reported a significantly lower shoulder joint loading and muscle force production during hand cycling than wheelchair propulsion at POs ranging from 25-55 W. A greater distribution of force production around the contributing muscles was evident during hand cycling, with relative muscle stress less than 10% of individual muscle predicted maximums (Arnet et al. 2012). Due to the relatively low physical strain and range of accessible equipment, hand cycling has become a popular exercise mode among both rehabilitation practitioners and sports competitors (Janssen et al. 2001; Valent et al. 2008; Valent et al. 2009).

2.2.4 Functional electrical stimulation (FES)-evoked lower-limb exercise

The application of electrical currents via intact lower-motor neurons to restore control over absent bodily functions is known as functional electrical stimulation (FES) (Ragnarsson 2008). FES-evoked lower-limb cycling and resistance training are popular therapeutic tools employed to offset muscle atrophy and elevate energy expenditure via involuntary muscle contractions (Baldi et al. 1998; Perret et al. 2010). Baldi et al. (1998) concluded that FES-evoked cycling in the acute phase of injury (4-15 weeks post injury) reduced SCI related atrophy of lower-limb lean tissue. A significant increase in gluteal and quadriceps lean mass was reported following 6 months cycle training (Baldi et al. 1998). Despite the large muscle mass activated by FES-evoked cycling, mechanical efficiency is very low (<2%) due to the external control of contraction timing and involuntary recruitment of large, highly fatigable anaerobic muscle fibres (Hunt et al. 2007). Subsequently achievable POs are very low (<20 W) compared to those observed with voluntary cycling (Berry et al. 2008; Hunt et al. 2007).

Conversely, the low GE observed during FES-evoked exercise induces a large metabolic stress within paralysed skeletal muscle that may prove beneficial to health.
Following 12 weeks resistance training, improvements in the CSA of whole thigh (28%), knee extensor (35%) and knee flexor (16%) coincided with a reduction in intramuscular fat, plasma triglyceride levels and increased plasma high-density lipoprotein (HDL) levels (Gorgey et al. 2012). Intensive FES-evoked cycling programmes improve insulin-mediated glucose transport and metabolism via increased GLUT-4 (Mohr et al. 2001) and glycogen synthase activity respectively (Hjeltnes et al. 1998). Thijssen et al. (2005) also reported an increased femoral artery diameter and acute blood flow following just four weeks FES-evoked cycle training, however, the effects are lost following just 2 weeks of de-training (Thijssen et al. 2006). However the low aerobic capacity of deconditioned limbs and low GE result in lower cardiorespiratory stress, including VO₂ and HR, during FES-evoked exercise than voluntary arm exercise at submaximal and maximal intensities (Hasnan et al. 2013).

2.2.5 Hybrid exercise

The addition of FES-evoked contractions to concurrent upper-limb exercise, termed hybrid exercise, has been shown to augment submaximal and maximal cardiorespiratory demands compared to upper-limb or FES-evoked exercise alone (Hasnan et al. 2013). Hybrid exercise can be performed with an adapted stationary arm-crank ergometer mounted over an FES-evoked leg cycling system, FES-rowing ergometers, or roadworthy integrated hybrid recumbent bikes. As well as increased oxygen demand, the activation of the lower-limb muscle pump during hybrid exercise is proposed to prevent venous pooling and augment via an improved ventricular preload and stroke volume (Davis et al. 1990; Hooker et al. 1992). In 12 participants with paraplegia, Davis et al. (1990) reported a ~30% increase in \( \dot{Q} \) during steady-state ACE with the addition of isometric lower-limb contractions. Likewise, Hooker et al. (1992) reported a significantly higher (50%) stroke volume during hybrid versus arms only exercise in individuals with tetraplegia. However, reports on venous muscle pump activity during hybrid exercise are conflicting. Beekvelt et al. (2000) reported a significantly lower muscle pump activity in a SCI group in response to electrical stimulation versus non-SCI controls. The extensive leg muscle atrophy and diminished vascular bed in the SCI group were proposed as mechanisms for the ineffective muscle pump activity (Beekvelt et al. 2000). Conflicting findings may also be explained by the
large effect of stimulation intensity on the skeletal muscle pump response (Beekvelt et al. 2000).

2.3 Inflammation, chronic disease and exercise

2.3.1 Physical activity levels and chronic disease risk

Physical activity levels are low in SCI populations. An estimated 50% of people are not engaged in regular physical activity, compared with around 35% of the able-bodied population (Anneken et al. 2010; Buchholz et al. 2003; Ginis et al. 2010). Berg-Emons et al. (2008) reported a small increase in activity levels during rehabilitation, with a significant decline in dynamic physical activity immediately upon discharge. Whilst activity levels were restored 1 year post-discharge, total physical activity was still significantly lower than reported in non-SCI controls (Berg-Emons et al. 2008).

Physical activity status is influenced by a number of barriers to participation and physical function (Saebu & Sørensen 2011). ‘Internal’ barriers include neurological pain, lack of basic fitness, low self-esteem, a failure to come to terms with injury, fears about incontinence and a lack of confidence (Rimmer et al. 2004). In contrast, ‘external’ barriers include cost or economic constraints and a lack of accessible facilities (Rimmer et al. 2004). Anneken et al. (2010) found wheelchair dependent individuals with paraplegia are significantly more likely to engage in physical exercise than those with tetraplegia. Therefore, the greater impairments in motor and cardiovascular function following cervical level SCI may act as an additional barrier to a physically active lifestyle. Individuals with paraplegia and incomplete injuries display a greater improvement in the duration of dynamic exercises in the year post-discharge than those with complete injuries and tetraplegia (Berg-Emons et al. 2008).

Prolonged physical inactivity and the reduction in energy expenditure associated with SCI predispose individuals to a debilitating cycle of greater losses of physical capacity and increased risk of chronic disease. Cardiovascular mortality rates are higher than those for non-SCI groups, with mortality and morbidity occurring earlier in life (Myers et al. 2007). The gain in relative adiposity, particularly visceral or abdominal adipose tissue, provides one of the biggest risk factors for disease in individuals with a SCI (Myers et al. 2007). Persons with tetraplegia are at a 16% greater risk of CVD than persons with paraplegia, with complete injuries 44% more at risk than incomplete
Through its association with insulin resistance, obesity is a major driver behind numerous cardiovascular disease risk factors including chronic low-grade inflammation, elevated resting blood glucose following impaired pancreatic β-cell insulin production, known as type 2 diabetes, hypertension and adverse lipid profiles.

Sedentary SCI populations frequently exhibit resting concentrations of inflammatory biomarkers, including interleukin-6 (IL-6) and C-reactive protein (CRP), consistent with high CVD risk (resting IL-6 > 2 pg·ml⁻¹; CRP > 3 pg·ml⁻¹) (Gibson et al. 2008; Lee et al. 2005; Nash et al. 2011; Wang et al. 2007). Independent of SCI level and injury duration, a significantly higher plasma IL-6 and CRP concentration was observed in individuals with a chronic SCI (>1 year) (20 tetraplegics; 42 paraplegics) compared to age-matched, non-SCI controls (Wang et al. 2007). In a cross-sectional analysis of 93 persons with SCI, Lee et al. (2005) observed a significant association between plasma CRP concentrations and the prevalence of insulin resistance and dyslipidaemia. Manns et al. (2005) reported a significant association between physical activity levels and resting CRP concentrations, as well as a lower HDL, higher fasting glucose, and a larger abdominal diameter (Manns et al. 2005). Elsewhere, individuals reporting >25 min per day of leisure time physical activity presented lower CRP concentrations, lower BMI and a higher fat free mass compared to physically inactive counterparts (Buchholz et al. 2009). Following 10 weeks FES-evoked cycling training, Griffin et al. (2009) reported a significant reduction in circulating concentrations of IL-6, tumour necrosis factor-alpha (TNF-α) and CRP. The improved inflammatory profile was associated with an improved glucose and insulin response to an oral glucose tolerance test and increased lean muscle mass but no change in adipose tissue mass (Griffin et al. 2009). However, despite the exercise induced reduction, post-training plasma CRP concentrations (12.94 ± 0.78 pg·ml⁻¹) and IL-6 (3.79 ± 0.52 pg·ml⁻¹) were still consistent with a high-risk of CVD.

Type 2 diabetes is three times more prevalent in individuals with a SCI than able-bodied persons of an equivalent age (LaVela et al. 2006). In a sample of 100 people with a SCI and 50 non-SCI controls, 82% of controls presented normal glucose tolerance, compared to only 38% and 50% for persons with tetraplegia and paraplegia respectively (Bauman & Spungen 1994). Raymond et al. (2010) performed oral glucose tolerance tests in 25 community-based individuals with paraplegia and tetraplegia greater than six months post-injury. A significantly greater glucose tolerance and
greater non-exercise related mobility was present in those reporting participation in moderate-vigorous strength exercise independent of level of SCI (Raymond et al. 2010). Serum HDL levels are significantly lower than non-SCI controls, with an inverse association observed between plasma HDL and triglyceride concentrations (Bauman et al. 1999). Engaging in a physically active lifestyle post-rehabilitation, predominantly via sports performance, has also been shown to maintain serum HDL concentrations in persons with long-term tetraplegia (Dallmeijer et al. 1997). In a large cohort of 206 subjects with a chronic SCI (78 with tetraplegia), greater physical capacity (PO\textsubscript{peak}; VO\textsubscript{2peak}; muscle strength) was associated with higher HDL and lower triglyceride concentrations (de Groot et al. 2007).

2.3.2 Aetiology of type 2 diabetes and CVD

Inflammation is a vital defence mechanism that initiates pathogen killing and tissue repair. Pro-inflammatory chemical signals, including the cytokines TNF-\alpha, IL-6 and IL-1 activate cells of the innate and acquired immune systems and recruit them to the site of cellular infection or damage. The secretion of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1ra) provides a negative feedback mechanism within the immune system that down-regulates inflammation and protects the host against excessive tissue damage. The dysregulation of this inflammatory response, as evidenced by chronic, low-grade elevations in circulating concentrations of the pro-inflammatory biomarkers TNF-\alpha, IL-6 and hepatic derived CRP, is an influential process in the development of type 2 diabetes and CVD (Osborn & Olefsky 2012) (Figure 2.2).

Adipose tissue is an endocrine organ that influences metabolic regulation via the secretion of a range of pro-inflammatory cytokines (TNF-\alpha, IL-6, IL-1\beta). During periods of over-nutrition and physical inactivity, pro-inflammatory signalling pathways are initiated by a growth in adipocyte size (Cancello et al. 2005) (Figure 2.2). This local inflammation activates immune cells residing within adipose tissue, leading to a further infiltration of inflammatory cells and a conversion of activated cells to inflammatory phenotypes (Cancello et al. 2005; Lumeng et al. 2007). Systemic, low-grade elevations in adipose-derived inflammatory cytokines, including TNF-\alpha and IL-6, are driven predominantly by activated macrophage and T cells within adipose tissue. The exposure of adipose, hepatic, skeletal muscle and endothelial cells to systemic inflammatory
mediators interferes with the down-stream signalling of insulin receptors (Halse et al. 2001; Li et al. 2011). The reduction in tissue insulin sensitivity, termed insulin resistance, leads to metabolic dysregulation, evidenced by elevations in plasma glucose and triglyceride concentrations, and subsequent oxidative stress and inflammation-induced endothelial dysfunction (Figure 2.2). Thus, available evidence suggests systemic, chronic low-grade inflammation causally links obesity to the development of insulin resistance, type 2 diabetes, atherogenic lipid profiles and endothelial dysfunction, all of which increase the risk of developing CVD.

Figure 2.2 Aetiology of CVD risk and proposed anti-inflammatory action of exercise
2.3.3 The anti-inflammatory potential of regular exercise

Evidence from large scale, epidemiological studies demonstrates an inverse, dose-response relationship between physical activity and fitness and the risk of CVD-related mortality (Blair & Morris 2009; Lee et al. 2005; Mora et al. 2007; Tanasescu et al. 2003). With respect to chronic disease risk, the greatest reductions are observed in those who improve physical activity from sedentary to moderate levels (Blair & Morris 2009). Previously, the incidence of type 2 diabetes was more than halved in high-risk population groups following lifestyle intervention including bodyweight control, physical activity and dietary modification (Knowler et al. 2009; Lindstrom et al. 2006). Independent of weight loss, achieving over four hours per week of physical activity resulted in an 80% lower risk of type 2 diabetes than remaining sedentary in overweight men and women with impaired glucose tolerance (Lindstrom et al. 2006). In the same cohort, decreases in CRP and IL-6 were predicted by moderate to vigorous leisure-time physical activity independent of BMI (Herder et al. 2009). In a prospective study of 27,055 apparently healthy women, CVD risk decreased linearly with increased physical activity, with the most active quartile at a 41% lower risk of experiencing a CVD event than the least active quartile (Mora et al. 2007). Inflammatory biomarkers made the biggest contribution (32.6%) to the reduction in CVD risk, with the highest quartile of fitness presenting a 37% lower CRP concentration than the lowest quartile of fitness (Mora et al. 2007).

As reviewed by Gleeson et al. (2011), a growing body of literature suggests regular exercise may exert anti-inflammatory effects that protect against chronic inflammation. Significant inverse relationships are present between physical activity levels and fitness and the circulating concentration of inflammatory biomarkers (Libby & Ridker 2004; Shanley et al. 2013). In 1000 community-based individuals, these inverse relationships remained evident even after adjustment for age, body mass index (BMI) and smoking habits (Shanley et al. 2013). A number of mechanisms have been proposed whereby regular activity and exercise may exert anti-inflammatory effects. However, the mechanism that has received the greatest attention over the last two decades is the transient elevations in skeletal muscle derived IL-6 following acute exercise (Pederson 2012). Elevations in plasma IL-6 concentrations are then associated with an anti-inflammatory cascade in the post-exercise period, increasing circulating
concentrations of the anti-inflammatory cytokines IL-10 and IL-1ra (Ostrowski et al. 1999; Steensberg et al. 2003).

Contracting skeletal muscle is a major source of circulating IL-6 (Starkie et al. 2001; Steensberg et al. 2000) with up to 100-fold elevations in plasma concentrations observed following prolonged, high intensity exercise (Ostrowski et al. 1999). In contrast to chronic low-grade elevations in adipose derived IL-6, contraction induced elevations in IL-6 are transient, returning to resting levels 2 to 3 h post-exercise (Scott et al. 2011). IL-6 is proposed as a paracrine and endocrine mediator of substrate availability, elevating fat oxidation in skeletal muscle during exercise (Wolsk et al. 2010). Importantly, IL-10 inhibits pro-inflammatory cytokine expression, prevents the differentiation of cells to inflammatory phenotypes, and down-regulates immune cell activation (Moore et al. 2001). As a naturally occurring antagonist to pro-inflammatory signalling of IL-1α and IL-1β, plasma IL-1ra is an important modulator of systemic inflammation and innate immune responses. IL-1ra inhibits the inflammatory actions of IL-1β via competitive binding of the IL-1 receptor (Freeman & Buchman 2001). Repeated elevations in circulating anti-inflammatory cytokines with chronic exercise training may therefore initiate a longer-lasting anti-inflammatory environment that could have the potential to down-regulate chronic low-grade inflammation (Figure 2.2).

The regulation of IL-6 release from skeletal muscle involves a complex cross-talk between signalling pathways responsive to intracellular calcium concentrations, mechanical stimuli, glycogen depletion and sympathetic nervous system activation (Chan et al. 2004; Frost et al. 2004; Keller et al. 2006; Welc & Clanton 2013; Whitham et al. 2012) (Figure 2.2). Therefore the magnitude of the plasma IL-6 response is dependent on the intensity and duration of exercise (Fischer et al. 2006). Current evidence suggests a ‘threshold’ of exercise intensity must be overcome to initiate an anti-inflammatory cytokine response to acute exercise (Peake et al. 2004; Peake et al. 2005; Scott et al. 2011). Recently, Scott et al. (2011) reported 5-fold, 6-fold and 12-fold elevations in plasma IL-6 in response to 60 min treadmill running at 55% \( \dot{V}O_{2\text{peak}} \), 65% \( \dot{V}O_{2\text{peak}} \) and 75% \( \dot{V}O_{2\text{peak}} \), respectively (Scott et al. 2011). In contrast, plasma IL-1ra concentrations remained unchanged in response to exercise at 55% \( \dot{V}O_{2\text{peak}} \), with a 0.5-fold elevation observed at 65% \( \dot{V}O_{2\text{peak}} \) and a 2-fold elevation at 75% \( \dot{V}O_{2\text{peak}} \) (Scott et al. 2011). Plasma IL-1ra concentrations remained elevated 2 and 3 h post the 75%
VO_{peak} trial, where they were significantly higher than both 55% VO_{peak} and 65% VO_{peak} trials (Scott et al. 2011).

The findings of Peake et al. (2004; 2005) support the effect of exercise intensity on the circulating IL-6 and IL-1ra response. Significant elevations in plasma concentrations of IL-6 were observed following 60 min moderate intensity (60% VO_{peak}) and high intensity (85% VO_{peak}) treadmill running, with concentrations significantly greater for the high intensity versus the moderate intensity trial (Peake et al. 2004). A 1.3-fold elevation in plasma IL-1ra concentration was observed following moderate intensity exercise compared with a 5-fold elevation following the high intensity trial (Peake et al. 2005). Further, a 6-fold increase in plasma IL-10 was seen in the high intensity trial, with no response in the moderate intensity trial (Peake et al. 2005). With respect to exercise duration, plasma IL-6 responses express a non-linear relationship with increasing exercise duration, with the magnitude of response increasing as exercise continues (Fischer et al. 2006). No effect of exercise intensity was observed in the plasma IL-6 response reported by Scott et al. (2011) after 20 min of exercise.

During submaximal whole-body exercise, IL-6 release is higher from the contracting upper-limb muscles compared with the lower-limb, potentially as a result of a higher rate of carbohydrate metabolism or greater force production per unit area of muscle (Helge et al. 2011). Elevations in plasma IL-6 following wheelchair basketball performance occurred independently of elevations in TNF-α or CRP (Kinoshita et al. 2013). Elsewhere, Umemoto et al. (2011) reported similar post-exercise elevations in plasma IL-6 (10-fold) following 2 h moderate intensity (60% VO_{peak}) ACE compared to those reported following one h of treadmill running at 75% VO_{peak} by Scott et al. (2011). The smaller (5-fold) elevation in plasma IL-6 concentrations observed in the thoracic SCI group working at the same relative intensity may be explained by a lower absolute PO, as evidenced by lower adrenaline concentrations (Umemoto et al. 2011). In contrast, participants with a cervical level SCI displayed no increase in circulating IL-6 concentrations in response to 20 min ACE at 60% VO_{peak}, despite having higher concentrations at rest than a non-SCI control group (Kouda et al. 2012). The relatively small (1-fold) increase in the non-SCI group (Kouda et al. 2012) reflects the short duration of exercise performed compared to the work of Umemoto et al. (2011).
Therefore, it is questionable whether the attenuated IL-6 response observed in the tetraplegic group was a result of impaired SNS action on contracting muscle or simply the low exercise intensity and short duration (Kouda et al. 2012). No study to date has identified whether exercise intensities and durations appropriate for general exercise prescription in SCI populations can initiate an IL-6 response of a magnitude sufficient to initiate the anti-inflammatory cascade post-exercise.

An intensity dependent elevation in adrenal gland derived, plasma stress hormone concentrations are also observed during exercise via activation of the SNS and hypothalamic pituitary adrenal (HPA) axis. HPA axis derived plasma cortisol directly up-regulates IL-10 production in T-lymphocytes of the acquired immune system, while down-regulating the secretion of pro-inflammatory cytokines by monocytes and macrophages belonging to the innate immune system (Elenkov 2004). In addition, adrenaline and noradrenaline down-regulate the action of the innate immune system via the stimulation of IL-10 secretion and the down-regulation of TNF-α secretion (Platzer et al. 2010; van der Poll et al. 1996). The stress hormone response to exercise shows a strong positive relationship with the intensity dependent anti-inflammatory cytokine response previously described (Peake et al. 2004; Scott et al. 2011). At exercise intensities sufficient to stimulate the SNS and HPA axis, plasma stress hormones may therefore contribute to the anti-inflammatory action of acute exercise.

Additional anti-inflammatory mechanisms relate to the phenotypic switching of circulating and adipose residual immune cells. In an animal model, regular exercise reduced macrophage infiltration and induced phenotypic changes in adipose-derived macrophages cells during high fat feeding (Kawanishi et al. 2010). Macrophages switched from inflammatory cytokine secreting M1 cells to anti-inflammatory cytokine secreting M2 cells following 16 weeks exercise training (Kawanishi et al. 2010). Little is currently known about the effects of exercise in humans. Physically active humans also display a lower percentage of circulating activated monocytes (CD14<sup>low</sup>CD16<sup>+</sup>) and exercise training in elderly males and females results in a reduction in monocyte pro-inflammatory cytokine (TNF-α) secretion following bacterial stimulation (Timmerman et al. 2008). Along with the reduction in visceral fat mass and adipocyte size, both factors could contribute to a reduction in chronic low-grade inflammation following prolonged improvements in physical activity levels.
2.4 Current tools for prescribing and regulating exercise

2.4.1 Exercise guidelines

The dose-response relationship between cardiorespiratory fitness and CVD risk was described in section 2.2.3. Despite this relationship, current understanding of the optimal dose (intensity x duration x frequency) of activity required to improve fitness and reduce cardiovascular and metabolic disease risk factors remains unclear. It has been suggested that training above 45% $\text{VO}_2\text{peak}$ is required to improve aerobic capacity in untrained population groups, with higher intensities required as fitness levels increase (Swain & Franklin 2002). This is supported by the significant enhancement in physical capacity, including sprint power output, following 7 weeks low intensity wheelchair training (van den Berg et al. 2010b). Based on the available evidence, exercise prescription guidelines for the improvement in cardiorespiratory fitness and reduction in chronic disease risk in persons with a SCI remain similar to those for healthy able-bodied adults (Garber et al. 2011; Figoni 2009; Ginis et al. 2011). Table 2.2 describes aerobic training zones as outlined by the American College of Sports Medicine (Garber et al. 2011). Recommendations suggest participation in either 1) moderate intensity aerobic activity for $\geq$30 min per day, $\geq$5 days per week in order to achieve a total of 150 min wk$^{-1}$; 2) vigorous intensity aerobic activity $\geq$20 min per day, 3 days per week totalling at least 75 min wk$^{-1}$ or 3) a combination of the two to achieve energy expenditures of 1000-2000 kcal·wk$^{-1}$ (Figoni 2009). Cardiorespiratory training should be supported by resistance exercise two to three days per week involving each of the remaining muscle groups to promote strength for functional independence and efficiency during activity (Figoni 2009).
Table 2.2 Typical exercise intensities employed for upper and lower–limb cardiorespiratory training (reproduced from Garber et al. 2011)

<table>
<thead>
<tr>
<th></th>
<th>%HRR or % VO₂R</th>
<th>% HR̴̂_peak</th>
<th>% VO₂̴̂_peak</th>
<th>RPE (Borg 6-20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very-light</td>
<td>&lt;30</td>
<td>&lt;57</td>
<td>&lt;37</td>
<td>&lt;9 very light</td>
</tr>
<tr>
<td>Light</td>
<td>30-39</td>
<td>57-63</td>
<td>37-45</td>
<td>9-11 very light to fairly light</td>
</tr>
<tr>
<td>Moderate</td>
<td>40-59</td>
<td>64-76</td>
<td>46-63</td>
<td>12-13 fairly light to somewhat hard</td>
</tr>
<tr>
<td>Vigorous</td>
<td>60-89</td>
<td>77-95</td>
<td>64-90</td>
<td>14-17 hard to very hard</td>
</tr>
<tr>
<td>Near-maximal</td>
<td>&gt;90</td>
<td>&gt;96</td>
<td>&gt;91</td>
<td>&gt;18 very very hard</td>
</tr>
</tbody>
</table>

2.4.2 Heart rate and oxygen uptake

Traditionally HR and VO₂ are employed as indicators of training intensity due to their strong linear relationship in both non-SCI populations (Garber et al. 2011) and individuals with paraplegia (Goosey-Tolfrey & Tolfrey 2004; Hooker et al. 1993; Tolfrey et al. 2001). Exercise intensity is then prescribed relative to an individual’s VO₂̴̂_peak, HR̴̂_peak or PÔ̴_peak (Garber et al. 2011). As shown in Table 2.2, %HR̴̂_peak underestimates exercise intensity with respect to %VO₂̴̂_peak. Despite this apparent limitation, the use of HR for controlling training intensity has great value due to its ease of measurement compared to VO₂ and PO. Therefore, an alternative strategy for exercise prescription is proposed based upon the heart rate reserve (HRR) and VO₂ reserve (VO₂R) methods first developed by Karvonen and colleagues (1957). The HRR and VO₂R represent a measure of difference between the resting and maximal values for HR and VO₂ respectively (da Cunha et al. 2011). During exercise prescription, the reserve method may be applied to remove any error introduced to individual VO₂-HR relationships by inter-individual variation in resting and maximal responses (da Cunha et al. 2011). Subsequently, it is suggested that the VO₂R and HRR methods to provide a more direct (1:1) interpretation of exercise intensity between HR and VO₂ (Lounana et al. 2007; Swain & Leutholz et al. 1997).

The attenuated sympathetic innervation and low levels of physical capacity resulting from a cervical level SCI mean the use of HR for exercise prescription may be unsuitable for individuals with tetraplegia (Coutts et al. 1983; McLean et al. 1995; Valent et al. 2007a). Valent et al. (2007a) reported an adequate linear relationship
between $\dot{V}O_2$-HR in only 8 out of 18 subjects during a discontinuous graded hand cycling exercise. This is supported by McLean et al. (1995) who reported low correlation co-efficient during both sitting and supine ACE in cervical (above C7) SCI. The decreased HR$_{\text{peak}}$ and subsequent reduction in HRR may result in large fluctuations in %$\dot{V}O_2$$_{\text{peak}}$ when prescribing exercise based on HR in this population (Leicht et al. 2012; McLean et al. 1995). Therefore, alternative methods for the regulation of exercise include PO and subjective RPE (McLean et al. 1995).

2.4.3 Subjective ratings of perceived exertion

The use of effort perception as a ‘tool’ for monitoring strain during physical work was first proposed by Gunnar Borg. The strong correlation between RPE and physiological variables including HR, $\dot{V}O_2$ and blood lactate concentration ($BLa^-$) has led to the employment of the Borg 6-20 category scale as a subjective measure of physical exertion across a range of exercise modes (Borg 1982; Borg et al. 1987; Eston & Brodie 1986; Noble et al. 1973). Overall RPE (RPE$_O$) provides a summation of exertional cues from the exercising muscles and joints, cardiovascular and respiratory systems, and the environment (Borg et al. 1982). Rather than directly attending to changes in physiological processes, subjective RPE represents the externalisation of these processes which can be perceived via sensory afferent systems (Noble et al. 1973). For example, an increase in metabolic rate with exercise elevates minute ventilation (VE), respiratory rate and skin temperature. In addition, the accumulation of hydrogen ions ($H^+$) in contracting muscle is associated with lactate production during exercise, which in turn reduce muscle pH and induce metabolic acidosis (Robertson et al. 1986). Afferent signals from these physiological cues are then combined with psychological (mood, motivation, anxiety) and performance (pacing strategy, exercise duration) factors to describe the overall perception of effort (Robertson & Noble 1997).

The importance of differentiated perceived exertion was first highlighted by Ekblom & Golobarg (1971). Perceived exertion was found to be higher for a given intensity of arm work than leg work (Ekblom & Golobarg 1971). In turn higher RPE were reported during lower-limb cycling compared to running (Ekblom & Golobarg 1971). Therefore it is suggested that exercise mode may differentially affect peripheral (muscle strain and joint) and central (cardiorespiratory factors including ventilation) physiological factors driving RPE$_O$. Subsequently the differentiated RPE model
describes the exertional responses to specific peripheral (RPE_p) and central (RPE_c) signals of exertion (Robertson & Noble 1997).

The term *perceptual signal dominance* describes the dominance of a differentiated RPE (either peripheral or central) when forming undifferentiated RPE_o (Robertson & Noble 1997). RPE_p is frequently reported to be higher than RPE_c during upper-limb exercise tasks, including manual wheelchair propulsion (Lenton et al. 2008) or ACE (Al-Rahamneh et al. 2011; Pandolf et al. 1984). The elevation in push frequency (PF) from 40 to 70 p/min\(^{-1}\) at a fixed workload (32 W) during synchronous wheelchair propulsion resulted in an increase in RPE_p that mirrored increases in HR and \(\dot{V}O_2\), while no change was reported in RPE_c or RPE_o (Goosey-Tolfrey & Kirk 2003). Higher RPE_p were also reported in non-wheelchair trained versus trained participants during wheelchair ergometry at the same workload, reflecting the more efficient technique and push cycle parameters of experienced users (Lenton et. al 2008). At two submaximal exercise intensities (25 W and 32 W), Dallmeijer et al. (2004) reported lower overall physical strain in hand cycling versus wheelchair ergometry exercise, reflecting the more efficient and less mechanically straining nature of manually propelling a hand cycle. Differentiated RPE may therefore provide an exercise mode specific tool that is sensitive to variations in GE and mechanical strain driven by both physiological and biomechanical parameters during different upper-limb exercise modalities.

*Application in SCI populations*

Strong linear relationships have been reported between all differentiated RPE and physiological indices of exercise intensity during graded ACE in individuals with paraplegia (Al-Rahamneh & Eston 2011) and poliomyelitis (Al-Rahamneh et al. 2010). RPE has also been shown to follow physiological trends during wheelchair propulsion (Goosey-Tolfrey & Kirk 2003; Lenton et al. 2008) and hand cycling exercise (Goosey-Tolfrey et al. 2010) in trained individuals with paraplegia. Elsewhere the utility of RPE as a marker of submaximal exercise intensity in populations with a SCI has been questioned (Cowan et al. 2012; Lewis et al. 2007). Cowan et al. (2012) reported a weak relationship between submaximal \(\dot{V}O_2\) and RPE in 12 community and rehabilitation based participants with paraplegia during ACE. However, the authors drew their conclusions from a linear regression analysis using \(\dot{V}O_2\) and RPE data collected
between 60 and 90 sec of each 2 min stage during a ramped-incremental test to exhaustion (Cowan et al. 2012). RPE and VO$_2$ cannot be considered to display a steady-state relationship under these conditions and therefore cannot be used to confirm the utility of RPE at submaximal intensities. Similarly, Lewis et al. (2007) challenged the relationship between RPE and both HR and VO$_2$ in a group of paraplegic and tetraplegic participants during graded ACE. Both groups performed exercise at the same fixed workloads, displaying linear increases in all physiological variables (Lewis et al. 2007). However, the RPE-physiological relationships were assessed on a group basis within each workload rather than individually across exercise intensities (Lewis et al. 2007). Neither of the aforementioned studies accounted for differentiated RPE during exercise, which may have further influenced their findings from relatively untrained populations. Further work is required to provide a greater understanding of the effect of cardiovascular and functional impairments associated with a SCI on the use of RPE at submaximal exercise intensities.

Estimation-production for the regulation of exercise

The application of RPE for the prescription and self-regulation of exercise is growing in popularity (Parfitt et al. 2012). In the ‘estimation-production’ paradigm, exercise intensity is prescribed at an RPE elicited during an exercise task at a prescribed workload (Goosey-Tolfrey et al. 2010). Alternatively a fixed RPE (e.g. ‘hard’) can be applied across a whole cohort (Parfitt et al. 2012). Individuals then self-regulate their exercise intensity via adjustments in speed, gradient or resistance in accordance with their perceived exertion and the RPE scale. The successful self-regulation of exercise has been reported for a range of exercise modalities including hand cycling (Goosey-Tolfrey et al. 2010) and wheelchair racing (Muller et al. 2004) in SCI populations, and lower-limb cycling and treadmill running in non-SCI cohorts (Buckley et al. 2000; Eston & Williams 1987; Kang et al. 2003; Kang et al. 2009). Frequent reports of the improved precision of self-regulated exercise at higher exercise intensities (Eston & Williams 1987; Muller et al. 2004) and an increased reliability with repeated exercise sessions suggest familiarisation with RPE and the specific exercise mode are important (Eston & Williams 1987). However, no work to date has regulated exercise solely on the dominant, mode specific differentiated RPE. Accounting for the dominance in perceptual signals may further improve the precision of self-regulated exercise. Currently the use of RPE for exercise prescription is acknowledged, but evidence is
insufficient to support these methods as primary methods for regulating exercise (Garber et al. 2011). As RPE provides a cost-effective and easy to administer method for regulating exercise intensity, further work is required to validate its utility as a tool for the regulation of exercise intensity.

2.5 Summary

Injury to the spinal cord provides a unique challenge to motor, sensory and autonomic function. The increased prevalence of chronic disease risk factors and low cardiorespiratory strain during ADL suggest exercise prescription has an important role for maintaining or improving cardiovascular health and fitness. Exercise prescription must manage the risk of upper-limb over-use injuries, whilst employing intensities sufficient to achieve the specific goals of the training programme. RPE provides a tool for the self-regulation of exercise that, in its differentiated form, can monitor subjective strain on the peripheral exercising muscles and central cardiorespiratory system. Regular exercise may protect against the development of chronic diseases, including type 2 diabetes and cardiovascular disease, via anti-inflammatory mechanisms associated with contracting skeletal muscle and circulating inflammation-mediating cytokines. Little is currently known about the effects of SCI-related impairments in autonomic function (reduced HR response; impaired adrenaline secretion) and the primary reliance on upper-limb exercise modalities (wheelchair propulsion, ACE or hand cycling) on 1) the use of RPE for the self-regulation of exercise, and 2) the proposed anti-inflammatory benefits of acute exercise.

The proceeding experimental chapters aim to answer the following questions:

- Does the impaired autonomic and motor function observed in persons with a cervical level SCI affect the RPE-workload relationship and the ability to utilise RPE for the self-regulation of exercise?
- Does the elevated peripheral fatigue and low GE experienced by novice wheelchair users influence the use of RPE to self-regulate wheelchair propulsion?
- Does the impaired autonomic function observed in persons with a cervical level SCI affect the transient inflammation-mediating cytokine response to acute exercise?
• Does the small volume of muscle mass activated during traditional upper-limb exercise modalities affect the same response at intensities in accordance with current exercise prescription guidelines?

• Can FES-evoked contractions augment the anti-inflammatory cytokine response when combined with voluntary upper-limb exercise during novel hybrid exercise?
3. Perceived exertion as a tool to self-regulate wheelchair exercise in individuals with tetraplegia

This chapter has been published in a slightly modified form in the *European Journal of Applied Physiology*:

3.1 Abstract

**Purpose:** To investigate the use of subjective RPE as a tool to self-regulate the intensity of wheelchair propulsive exercise in individuals with tetraplegia.

**Methods:** Eight motor complete tetraplegic (C5/6 and below; ASIA impairment scale = A) participants completed a submaximal incremental exercise test followed by a graded exercise test to exhaustion to determine $\dot{V}O_2^{\text{peak}}$ on a wheelchair ergometer. On a separate day, a 20-min exercise bout was completed at an individualised imposed PO equating to 70% $\dot{V}O_2^{\text{peak}}$. On a third occasion, participants were instructed to maintain a workload equivalent to the average RPE for the 20-min imposed condition. $\dot{V}O_2$, HR and PO were measured at 1-min intervals and BLa⁻ was measured at 0, 10 and 20 min.

**Results:** No differences ($p>0.05$) were found between mean $\dot{V}O_2$, %$\dot{V}O_2^{\text{peak}}$, HR, %HR$_{\text{peak}}$, BLa⁻, velocity or PO between the imposed and RPE-regulated trials. No significant ($p>0.05$) time-by-trial interaction was present for $\dot{V}O_2$ data. A significant interaction ($p<0.001$) for the PO data represented a trend for an increase in PO from 10 min to the end of exercise during the RPE-regulated condition. However, post-hoc analysis revealed none of the differences in PO across time were significant ($p>0.05$).

**Conclusion:** In conclusion, these findings suggest that RPE can be an effective tool for self-regulating 20 min of wheelchair propulsion in a group of trained participants with tetraplegia who are experienced in wheelchair propulsion.
3.2 Introduction

Ratings of perceived exertion scales provide a quantifiable measure of an individual’s subjective feeling of fatigue and strain during a physical task (Borg 1982). Since its conception, the Borg 6-20 scale has been one of the most widely applied means of monitoring and evaluating exercise tolerance in both healthy adult and clinical populations (American College of Sports Medicine 2010; Noble 1982). The strong linear relationship between RPE and $\dot{V}O_2$ and HR during exercise in both active and sedentary non-spinal cord injured adults (Eston & Brodie 1986; Faulkner & Eston 2007; Lambrick et al. 2009; Pandolf et al. 1984) has led to the application of RPE as a tool in the prescription and self-regulation of exercise. The intensity of an acute bout of exercise can be prescribed according to an RPE that reflects a given HR, $\dot{V}O_2$ or blood lactate value (Faulkner & Eston 2008). Individuals can then use this RPE to self-regulate the intensity of exercise according to the RPE scale and associated verbal anchors (Buckley et al. 2000; Dunbar et al. 1992; Eston et al. 1987; Eston & Williams 1988; Goosey-Tolfrey et al. 2010; Kang et al. 2003; Kang et al. 2009).

In individuals with a SCI, regular exercise has been shown to have beneficial effects on physical capacity (Valent et al. 2007b) and performance during ADL (Dallmeijer et al. 1999a; Janssen et al. 1994). As the equipment required to assess PO and $\dot{V}O_2$ during exercise training may be cost-prohibitive for many rehabilitation centres and recreationally active individuals, the use of RPE to prescribe and regulate exercise intensity may provide an effective alternative. In addition, the utilisation of HR as a traditional tool for prescribing exercise intensity may not be appropriate in all individuals with tetraplegia (Leicht et al. 2012; Valent et al. 2007a). The term tetraplegia refers to the loss of motor and/or sensory function in the cervical segments of the spinal cord (Kirshblum et al. 2011). The level of injury will determine the extent of the motor loss, which can vary from impaired hand function to complete upper-limb paralysis. A complete injury typically results in the loss of all function, motor and sensory, in the lowest sacral segments of the spinal cord (S4/S5) whereas some neurological function may be maintained in this region with an incomplete injury (Kirshblum et al. 2011).

High thoracic and cervical level lesions result in attenuated sympathetic innervation of the heart, with the increase in HR during exercise mostly due to the
withdrawal of vagal parasympathetic tone (Valent. et al. 2007a). This leads to a reduced maximum HR response (120-140 b·min⁻¹) and a potential disturbance in the $\dot{V}O_2$-HR-relationship (Valent et al. 2007a). Disturbed sympathetic nervous system control also results in the lack of vasoconstriction of inactive tissues below the lesion and an inhibited re-distribution of blood flow to active muscles (Thijssen et al. 2009). This vascular insufficiency reduces cardiac pre-load via limiting venous return, and in turn impairs exercise stroke volume and $Q$ (Figoni 1993). The vasodilation of exercising muscles without compensatory vasoconstriction can lead to orthostatic hypotension, further limiting exercise capacity in persons with tetraplegia (Figoni 1993).

A strong linear relationship has been reported between RPE and $\dot{V}O_2$ in persons with paraplegia (Al-Rahamneh & Eston 2011) and poliomyelitis (Al-Rahamneh et al. 2010) during ACE to volitional exhaustion. Further, Goosey-Tolfrey et al. (2010) reported a group of paraplegic participants were successfully able to self-regulate the intensity of hand cycling exercise using the Borg 6-20 scale. No differences were observed in $\dot{V}O_2$, HR and PO between exercise bouts performed at imposed exercise intensities and subsequent bouts where the same participants self-regulated exercise intensity using RPE (Goosey-Tolfrey et al. 2010). Elsewhere, perceived exertion scales have been employed to self-regulate training in wheelchair racing athletes (Muller et al. 2004). Participants successfully self-regulated propulsion speeds at a range of intensities from ‘1= warm-up’, ‘3= aerobic training’ and ‘5= race intensity’ (Muller et al. 2004). On the other hand, the utility of RPE to monitor the intensity of exercise in a group of participants with paraplegia and tetraplegia has previously been questioned (Lewis et al. (2007). This discrepancy with the aforementioned work is likely to be caused by the employment of absolute rather than relative workloads and the analysis of RPE-$\dot{V}O_2$ relationships on a group rather than an individual basis. Consequently, translations of the work conducted by Lewis and co-workers (2007) with those studies utilising relative exercise intensities for exercise prescription cannot be made.

Subjective RPE represents the psychological interpretation of cardiorespiratory, musculoskeletal and metabolic signals of exertion (Faulkner & Eston 2008). Self-regulating exercise using RPE therefore requires consideration in individuals with tetraplegia, as the loss of sympathetic innervation and sensorimotor function may affect this interpretation, and consequently, RPE responses. The purpose of this investigation
was to examine the use of subjective RPE as a tool to self-regulate the intensity of wheelchair propulsive exercise in participants with tetraplegia. Specifically, the primary aim was to compare exercise responses (HR, $\dot{V}O_2$ and PO) during a 20-min bout of imposed intensity wheelchair propulsion against a 20-min bout of propulsion where participants self-regulated the intensity of exercise according to their RPE recorded during the corresponding imposed intensity trial. It was hypothesised that exercise responses between the imposed intensity trial and the RPE-regulated trial would not differ. In addition, Goosey-Tolfrey et al. (2010) have previously reported an increase in exercise intensity during a 20-min bout of self-regulated hand cycling at a fixed RPE. This so-called ‘end-spurt’ in intensity was reported as the termination of the exercise task approached (Goosey-Tolfrey et al. 2010). A secondary aim of this study was, therefore, to identify whether sustained changes in exercise response (PO and $\dot{V}O_2$) occurred during the 20-min bout when using RPE to self-regulate exercise intensity.

3.3 Methods

3.3.1 Participants

Eight male wheelchair dependent participants with a cervical SCI at C5/6 and below voluntarily consented to participate in the study. All participants provided written informed consent. All procedures were approved by the Loughborough University Ethical Advisory Committee and performed in accordance with the Declaration of Helsinki. Participants’ characteristics and disability classifications are presented in Table 3.1. All subjects had motor complete lesions classified on the ASIA impairment scale (Kirshblum et al. 2011). Inclusion criteria for the study required participants to be involved in regular national or regional wheelchair rugby training and to have a minimum of three years of manual daily wheelchair propulsion experience. A separate health screen questionnaire was completed by all participants to ensure all participants were free from any illness or injury that may have prevented the safe completion of exercise testing.
### Table 3.1 Participant characteristics

<table>
<thead>
<tr>
<th>Part. No.</th>
<th>Body Mass (kg)</th>
<th>Time since SCI (yr)</th>
<th>Lesion Level</th>
<th>ASIA Impairment Scale</th>
<th>Exercise Training (h·week&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64.0</td>
<td>13</td>
<td>C5/6</td>
<td>A</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>72.0</td>
<td>3</td>
<td>C5/6</td>
<td>A</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>56.3</td>
<td>10</td>
<td>C6</td>
<td>A</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>93.9</td>
<td>14</td>
<td>C6/7</td>
<td>A</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>63.4</td>
<td>14</td>
<td>C6/7</td>
<td>A</td>
<td>15</td>
</tr>
<tr>
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<td>67.7</td>
<td>3</td>
<td>C7</td>
<td>A</td>
<td>16</td>
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<td>7</td>
<td>69.1</td>
<td>20</td>
<td>C7</td>
<td>A</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>73.1</td>
<td>7</td>
<td>C7/T2</td>
<td>A</td>
<td>8</td>
</tr>
<tr>
<td>Mean</td>
<td>69.9</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>SD</td>
<td>11.1</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

#### 3.3.2 Experimental design

The study utilised a repeated measures design with participants visiting the laboratory on three separate occasions. During the first visit, participants completed a submaximal incremental test and a graded exercise test to exhaustion to determine \( \dot{V}O_2\text{peak} \). The following day, participants performed a 20-min exercise bout at a velocity equating to 70% \( \dot{V}O_2\text{peak} \) and were asked to recall RPE<sub>O</sub> at five-min intervals. On a separate occasion, participants were instructed to produce and maintain a workload equivalent to the RPE recorded during the imposed intensity bout. Visits one and two were always performed on consecutive days. Visits two and three were separated by at least six days but no longer than seven days.

#### 3.3.3 Instrumentation

All tests were performed in the participants’ own rugby chair mounted on a wheelchair ergometer (Appendix I) interfaced with a computer (Compaq Armada 1520, Series 2920A, Compaq Computer Corporation, Taiwan). The wheelchair ergometer consisted of a single roller (length, 1.14 m; circumference, 0.48 m). A flywheel sensor connected to the roller and interfaced to a computer calculated wheelchair velocity and displayed it visually on a computer monitor. Upon each visit participants performed two
deceleration tests to allow resistance to be calculated according to the principles described by Theisen et al. (1996). Briefly, for each deceleration trial the participant was asked to accelerate the roller to maximum velocity and to then stop pushing and sit stationary as if in a position to perform the next push. The velocity was recorded as the chair slowed to a standstill and the average deceleration calculated from the slope of this velocity-time data. PO was calculated from the torque applied to the wheels and their angular velocity. The torque applied is a function of one total internal torque of 1) the wheelchair ergometer-wheelchair system, 2) the rotational moment of inertia of the rear wheels, 3) the one of the roller, and 4) its angular acceleration (Lenton et al. 2008). Tyre pressure was self-selected by the participants on the first visit according to their individual preference for sports performance and standardised for each subsequent visit using a manual tyre pump with psi gauge (Topeak sport bike pump, Halfords, UK). Before all sessions, participants were given standardised instructions detailing the use of the Borg 6-20 scale and the verbal anchors associated with the scale (Appendix II) (Borg 1998).

3.3.4 Session 1: Preliminary measures

For the submaximal incremental test, participants performed on average five 4-min constant load exercise stages at ascending velocities to elicit physiological responses covering a range from 40% to 80% \( \text{VO}_{2\text{peak}} \) according to Goosey-Tolfrey (2008). Initial velocities were selected from previous testing experience with this group. Subsequently, velocity increments of 0.2 or 0.3 m.s\(^{-1}\) were used. HR was monitored continuously using radio telemetry (Polar PE 4000, Kempele, Finland). On-line respiratory gas analysis was carried out throughout each 4-min stage via a breath-by-breath system (Cortex metalyser 3B, Cortex, Leipzig, Germany) to provide \( \text{VO}_2 \) data. A small capillary blood sample was obtained from the earlobe at the start of the test and during a 1-min break between stages to determine \( \text{BLa}^- \) using a YSI 1500 SPORT Lactate Analyser (Appendix III) (YSI Inc, Yellow Springs, OH). The lactate analyser was calibrated with a lactate standard of 5mmol·l\(^{-1}\). The Borg 6-20 scale was used to attain participants differentiated perceived exertion at the end of each stage (Borg 1998). Participants were asked to rate their perceived exertion relating to the active muscle mass and joints (RPE\(_P\)), the cardiorespiratory system (RPE\(_C\)) and an overall RPE (RPE\(_O\)) combining both local and central ratings.
After a 15-min rest period, a continuous incremental test was performed to determine \( \dot{V}O_2 \text{peak} \). The test involved 0.1 m.s\(^{-1}\) increments every minute from an initial velocity of 1.5±0.6 m.s\(^{-1}\). Initial velocities were determined on an individual basis equivalent to blood lactate threshold to achieve a test duration of 6 to 10 min. Participants performed the test at a freely chosen PF until volitional exhaustion. HR and expired gas were measured continuously throughout the test, and the final RPE was recorded as previously described. Breath-by-breath data allowed the highest 30 s rolling average \( \dot{V}O_2 \) value recorded during the exercise test to be taken as the \( \dot{V}O_2 \text{peak} \). For each participant, a simple linear regression analysis was performed using the linear workload-\( \dot{V}O_2 \) relationship. The regression line created from the paired submaximal velocity and \( \dot{V}O_2 \) data was employed to interpolate individual velocities corresponding to an exercise intensity of 70% \( \dot{V}O_2 \text{peak} \).

3.3.5 Session 2: Imposed power

A 20-min warm up at an RPE of no greater than 11 (‘light’ exertion) was performed prior to performing the imposed intensity and RPE-regulated bouts. Participants were then informed of the imposed velocity required and were asked to maintain it for 20 min. Participants had full vision of their velocity on the computer monitor throughout the whole session. During the exercise bout \( \dot{V}O_2 \) and HR were measured constantly and averaged over 1-min periods. RPE\(_o\) was recalled every 5 min and BLA\(^-\) was measured at 10 min and the end of exercise. A Borg 6-20 RPE scale was visible to participants at all times during testing. The average recorded RPE during the 20-min bout was taken as the anchor for the intensity of the RPE-regulated bout.

3.3.6 Session 3: RPE-regulated

Participants were informed of the average RPE recorded during the imposed intensity trial and were instructed to reproduce a workload equating to these RPE for 20 min. Participants were blinded to their velocity but were informed of time elapsed. \( \dot{V}O_2 \), HR and BLA\(^-\) were measured in accordance with the imposed intensity trials. PO was recorded and averaged over 1-min intervals. GE (%) was then calculated according to Woude et al. (1986). Participants were reminded of their target RPE at 5-min intervals.
3.3.7 Statistical analysis

All data was analysed using the statistical package SPSS for windows version 18 (SPSS inc, Chicago, IL). Normal distribution of the outcome variables was confirmed by Shapiro-Wilk test \(W(8) = 0.89 - 0.99, P = 0.07 - 0.99\). All descriptives are provided as mean ± standard deviation (SD) apart from ordinal RPE data which are reported as median and quartile ranges. Paired values for \(\dot{VO}_2\), \%\(\dot{VO}_2\)peak, PO, velocity, HR, \%HRpeak and BLaˉ averaged during the 20-min exercise bout between the imposed and RPE-regulated trials were examined using Student’s dependent t-tests. 95% confidence intervals of the differences (95% CI\(_{diff}\)) are also provided. Minute-by-minute data were available for \(\dot{VO}_2\) and PO, so separate 2 x 20 (trial x time) within measures analysis of variance (ANOVA) were performed to examine a potential interaction between the trial (imposed and RPE-regulated) and time. Post-hoc analysis consisted of a one-way repeated measures ANOVA for \(\dot{VO}_2\) and PO data in the RPE-regulated trial across time. GE data from the RPE-regulated trial was also analysed using a one-way repeated measures ANOVA. Tukey post-hoc tests were used to perform multiple time comparisons. Non-parametric Friedman tests and Wilcoxon signed-rank tests were used to analyse differences in differentiated RPE data at termination of \(\dot{VO}_2\)peak test. Pearson’s product moment correlation analysis was performed to analyse the individual HR-\(\dot{VO}_2\) and RPE-\(\dot{VO}_2\) relationships during the submaximal exercise test. Significance was set a priori at \(p \leq 0.05\). A Bonferroni adjustment was performed on the alpha value when performing multiple comparisons.

3.4 Results

The peak physiological responses and PO\(_{peak}\) are shown in Table 3.2. No difference \((p > 0.05)\) was seen between the differentiated RPE at termination of the \(\dot{VO}_2\)peak test (Table 3.2). Table 3.3 shows the individual HR-\(\dot{VO}_2\) relationship during the submaximal exercise stages. Strong linear relationships \((p < 0.05)\) were found between \(\dot{VO}_2\) and \(\text{RPE}_p\) \((r = 0.91)\), \(\text{RPE}_C\) \((r = 0.88)\) and \(\text{RPE}_O\) \((r = 0.90)\).

A comparison of the physiological responses during the imposed and RPE-regulated trials is shown in Table 3.4. None of the between trial differences in \(\dot{VO}_2\), \%\(\dot{VO}_2\)peak, HR, \%HRpeak, BLaˉ, velocity or PO were statistically significant \((p > 0.05)\) (Table 3.4).
<table>
<thead>
<tr>
<th>Participant No.</th>
<th>VO₂ (L·min⁻¹)</th>
<th>VO₂ (ml·kg·min⁻¹)</th>
<th>PO (W)</th>
<th>HR (b·min⁻¹)</th>
<th>BLa⁻ (mmol·L⁻¹)</th>
<th>RER</th>
<th>Local</th>
<th>Central</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.19</td>
<td>18.6</td>
<td>38.2</td>
<td>132</td>
<td>2.93</td>
<td>1.13</td>
<td>19</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>1.11</td>
<td>15.4</td>
<td>25.0</td>
<td>124</td>
<td>2.47</td>
<td>1.32</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>1.18</td>
<td>21.0</td>
<td>38.8</td>
<td>126</td>
<td>3.51</td>
<td>1.18</td>
<td>18</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>2.01</td>
<td>21.4</td>
<td>53.0</td>
<td>137</td>
<td>3.20</td>
<td>1.20</td>
<td>17</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>1.97</td>
<td>31.1</td>
<td>45.2</td>
<td>143</td>
<td>4.35</td>
<td>1.14</td>
<td>18</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>1.48</td>
<td>21.9</td>
<td>41.1</td>
<td>121</td>
<td>2.96</td>
<td>1.26</td>
<td>20</td>
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<tr>
<td>7</td>
<td>1.84</td>
<td>26.6</td>
<td>51.6</td>
<td>120</td>
<td>2.74</td>
<td>1.22</td>
<td>17</td>
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<td>8</td>
<td>1.92</td>
<td>26.3</td>
<td>47.6</td>
<td>160</td>
<td>3.52</td>
<td>1.20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean/Median</td>
<td>1.59</td>
<td>22.8</td>
<td>42.6</td>
<td>133</td>
<td>3.21</td>
<td>1.21</td>
<td>19</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>SD/Quartiles</td>
<td>0.39</td>
<td>5.0</td>
<td>9.0</td>
<td>14</td>
<td>0.58</td>
<td>0.06</td>
<td>17,20</td>
<td>16,20</td>
<td>17,20</td>
</tr>
</tbody>
</table>

Note. RPE data is median and quartiles; all other data are presented as mean and SD.
Table 3.3  Individual HR-$\dot{V}O_2$ relationships during incremental submaximal exercise

<table>
<thead>
<tr>
<th>Participant No.</th>
<th>HR-$\dot{V}O_2$ relationship (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.95</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.96</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.81</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>0.93</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>0.61</td>
<td>0.58</td>
</tr>
<tr>
<td>6</td>
<td>0.93</td>
<td>0.03</td>
</tr>
<tr>
<td>7</td>
<td>0.60</td>
<td>0.40</td>
</tr>
<tr>
<td>8</td>
<td>0.93</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Note. $r$ = Pearson’s correlation co-efficient

Table 3.4  Physiological responses during 20 min imposed intensity and RPE-regulated wheelchair propulsion

<table>
<thead>
<tr>
<th></th>
<th>Imposed intensity</th>
<th>RPE-regulated</th>
<th>95% CI diff</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE</td>
<td>12 (11,14)</td>
<td>12 (11,14)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (L·min$^{-1}$)</td>
<td>1.09 ± 0.30</td>
<td>1.12 ± 0.31</td>
<td>-0.15 to 0.93</td>
<td>0.60</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (ml·kg·min$^{-1}$)</td>
<td>15.6 ± 3.6</td>
<td>16.0 ± 4.0</td>
<td>-2.34 to 1.36</td>
<td>0.55</td>
</tr>
<tr>
<td>% $\dot{V}O_2$ peak</td>
<td>68 ± 4</td>
<td>71 ± 9</td>
<td>-11 to 6</td>
<td>0.47</td>
</tr>
<tr>
<td>HR (b·min$^{-1}$)</td>
<td>100 ± 15</td>
<td>106 ± 19</td>
<td>-16 to 4</td>
<td>0.18</td>
</tr>
<tr>
<td>% HR peak</td>
<td>75 ± 7</td>
<td>79 ± 10</td>
<td>-12 to 3</td>
<td>0.20</td>
</tr>
<tr>
<td>BLa$^-$ (mmol·L$^{-1}$)</td>
<td>0.98 ± 0.24</td>
<td>1.14 ± 0.57</td>
<td>-0.52 to 0.20</td>
<td>0.33</td>
</tr>
<tr>
<td>Velocity (m·s$^{-1}$)</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.6</td>
<td>-0.5 to 0.2</td>
<td>0.31</td>
</tr>
<tr>
<td>PO (W)</td>
<td>27.0 ± 7.1</td>
<td>30.6 ± 9.3</td>
<td>-9.1 to 1.9</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Note. RPE data are median (Quartiles); all other data is mean ± SD.
For the minute-by-minute \( \dot{V}O_2 \) data, no time-by-trial interaction was present during the exercise (\( p=0.43 \); Figure 3.1) showing that the exercise responses during the 20-min bout were consistent across trials. Post-hoc analysis revealed no differences (\( p>0.05 \)) in \( \dot{V}O_2 \) across time during either the imposed or RPE-regulate bout. Analysis of PO across time revealed a significant time-by-trial interaction (\( p=0.001 \)). As shown in Figure 3.1, PO during the RPE-regulated trial was seen to increase from around 10 min onwards to the end of the bout compared to the imposed intensity bout. However, post-hoc analysis revealed no significant differences (\( p>0.05 \)) in PO during the exercise bout. GE showed a significant increase (\( p<0.05 \)) across time during the RPE-regulated trial (Min 1 = 8.9±3.1 % vs. Min 20 = 9.5±3.7%).

![Figure 3.1](image_url)  
**Figure 3.1** Oxygen uptake and power output during 20 min wheelchair propulsion at 70% \( VO_{2peak} \) during imposed and RPE-regulated trials. Data are mean ± SD.
3.5 Discussion

The employment of RPE as a tool for the self-regulation of exercise intensity has been validated in both able-bodied and paraplegic populations (Eston & Williams 1988; Kang et al. 2003; Goosey-Tolfrey et al. 2010). However, the efficacy of implementing such procedures in persons with tetraplegia has received little attention. The results from this study involving a group of well-trained participants with tetraplegia demonstrate encouraging support for the use of RPE scales to self-regulate exercise in this population. In support of the initial hypothesis, the present study found that RPE elicited from fixed load exercise bouts at 70% \( \dot{\text{VO}}_{2\text{peak}} \) could be used to self-regulate wheelchair propulsive exercise. These findings support the work of Goosey-Tolfrey et al. (2010) who reported the successful self-regulation of light and moderate intensity hand cycling exercise in a group of participants with SCI at T4 and below.

A mean \( \dot{\text{VO}}_{2\text{peak}} \) of 1.58 L·min\(^{-1}\) suggests the group were well-trained for their disability level (Haisma et al. 2006; Leicht et al. 2013\(a\); 2013\(b\)). Moreover, the mean \( \text{HR}_{\text{peak}} \) of 133±14 b·min\(^{-1}\) suggested that all participants had an attenuated HR response as a result of their disability. Interestingly, the RPE reported during a 20-min bout of steady-state exercise in this group (12 (quartiles: 11-14)) were lower than those reported by Goosey-Tolfrey et al. (2010) during exercise at a similar intensity (16 (quartiles: 15-16)). This RPE was found to be at the lower end of the average RPE range recommended by ACSM guidelines to cause physical adaptation (12 to 16) (American College Sports Medicine 2010). The comparatively low RPE recorded during the 20-min steady-state exercise bouts are indicative of the low velocities associated with a fixed exercise intensity of 70% \( \dot{\text{VO}}_{2\text{peak}} \) when employing this protocol in persons with a reduced maximal aerobic capacity. The present study found \( \text{BLa}^- \) of 0.98 ± 0.24 mmol·L\(^{-1}\) in response to 20 min imposed intensity exercise at 70% \( \dot{\text{VO}}_{2\text{peak}} \), whereas Goosey-Tolfrey et al. (2010) reported \( \text{BLa}^- \) of 3.01 ± 1.00 mmol·L\(^{-1}\) in response to exercise of the same relative intensity and duration. The reduction in muscle pH associated with lactic acid metabolism has previously been suggested as an influential factor in the increase in RPE with exercise intensity during upper-limb exercise (Pandolf et al. 1984). Therefore, the lower RPE reported in this study may be a consequence of the lower \( \text{BLa}^- \).
When analysing the HR data, it was clear that a large inter-individual variability was present during the imposed intensity trial (80-115 b·min⁻¹). This was shown by the large SD for both HR and %HR_{peak} (Table 3.4) and represents the individual variation in HR seen within this population when exercising at fixed exercise intensities. One of the purposes of this study was, therefore, to determine whether this variable HR response, combined with higher levels of sensorimotor loss, affects the ability of persons with tetraplegia to utilise RPE scales. Previous work by Valent et al. (2007a) has noted a linear HR-\(\dot{V}O_2\) relationship may be present in some, but not all individuals with tetraplegia and that the relationship should be determined on an individual basis. The findings from this study are consistent with those previously reported by Valent et al. (2007a), however, the small participant number in this study limits the ability to determine the effect of lesion level on this relationship. Despite the differences in the linearity of the HR-\(\dot{V}O_2\) relationship within the participants, the lack of linear relationship did not affect the differences between the imposed and RPE-regulated trials. Thus, these findings suggest that HR may not be a dominant factor in all participants when determining undifferentiated perceived exertion during exercise.

Previously it has been suggested that local factors (exercising muscle) are more dominant than central factors (ventilation and HR) in determining perceived exertion when exercise involves only a small muscle mass (Al-Rahamneh & Eston 2011; Pandolf et al. 1984), as exercise capacity is limited by peripheral rather than central factors (Sawka et al. 1983). Al-Rahamneh et al. (2010) have reported higher local RPE than RPE_{O} at the termination of a graded exercise test in persons with paraplegia and polio. In addition, Lenton et al. (2008) have also reported that RPE_{P} were consistently higher than RPE_{C} when performing wheelchair propulsion at a range of push frequencies. Findings from this study, however, show no difference in differentiated RPE upon termination of the \(\dot{V}O_{2peak}\) test. The results suggest that central factors, such as ventilation rate, may still play a role in determining perceived exertion when exercise is of a maximal intensity, despite the attenuated HR response. Similarly there was a strong linear \(\dot{V}O_2\)-RPE relationship for individual RPE_{P} and RPE_{C} as well as RPE_{O} during the submaximal exercise stages. A limitation of the protocol employed in this study is the lack of differentiated RPE data during the imposed intensity steady-state trial. This data may have provided the authors with more information with which to explain the factors determining RPE_{O} and the self-regulation of exercise in this group.
Further investigation is therefore required into the individual contributions that local and central feelings of perceived exertion make to the formation of an RPEO to self-regulate wheelchair exercise. The effect that level of SCI and the associated differences in active muscle mass and cardiorespiratory responses to exercise have on these differentiated responses warrants further investigation.

It should be noted that all participants were experienced in wheelchair propulsion and, in addition to being given standardised instructions for the use of the Borg scales before each session, they had used these methods previously as part of their wheelchair rugby training. Previous work has suggested less active or sedentary participants may require practice to familiarise themselves with self-regulating regulating exercise intensity using RPE scales (Faulkner et al. 2007). Al-Rahamneh & Eston (2011) also reported that predictions of VO2peak from perceptually rated exercise tests in persons with paraplegia were more accurate following a familiarisation trial with the RPE scale. Further, Muller et al. (2004) recommend that a learning trial is necessary to ensure high levels of reproducibility when employing perceived exertion as a method of training control in wheelchair racing athletes. Therefore, familiarity with the RPE scale should be ensured prior to the prescription of exercise requiring self-regulation using perceived exertion.

A secondary aim of this work was to examine the participants’ exercise responses across time during the self-regulated intensity bout. The results show a variation across time for PO when exercise intensity is set at a fixed RPE (Figure 3.1). From the halfway point of the self-regulated trial, PO displays a gradual increase across time towards the end of exercise, although post-hoc analysis revealed the increases in PO were non-significant. Analysis of the VO2 data during the imposed and self-regulated trials showed no difference in the response during the two trials (Figure 3.1). The authors propose that the increase in PO without the concomitant rise in VO2 can be explained by the increase in GE after 10 min pushing during the self-regulated bout. Previous work has reported that during exercise, afferent feedback detailing the physiological response to exercise workload is collected by the brain and used in a ‘feedforward’ manner to regulate exercise intensity in order to prevent premature fatigue (Tucker & Noakes 2009). In closed loop tasks where knowledge of the end-point of exercise is explicit, this feedback allows the conscious adjustment of workload to be made during exercise and permits the increase in workload when the end-point of exercise is anticipated. This
increase in workload towards the end of an exercise bout has been a common finding when exercise is performed at a fixed RPE over a known period of time and is widely referred to as the ‘end-spurt’ (Goosey-Tolfrey et al. 2010; Tucker 2009). However, the increase in PO data found in this study is supported by the increase in GE across time rather than the role of the anticipatory calculations of work rate during self-regulated exercise.

3.6 Conclusion

In conclusion, the results from the present study provide encouraging support for the use of RPE as a tool to self-regulate the intensity of wheelchair propulsive exercise in persons with high level cervical and thoracic SCI. These findings show a strong relationship between differentiated RPE and \( \text{VO}_2 \) during submaximal exercise and that the Borg 6-20 scale can provide a cost-effective and valid alternative to HR when setting and regulating target exercise intensities in this population. The conclusions derived from this study must, however, be interpreted with caution due to the training status and experience of the participants. This group showed a high level of fitness compared to sedentary or untrained persons with similar disability. All participants had experience with manual wheelchair propulsion and the majority were familiar with the Borg 6-20 scale. Further, the submaximal incremental exercise test, \( \text{VO}_2\text{peak} \) test and the imposed intensity exercise trial all preceded the RPE-regulated trials in the study protocol. It may therefore be that the ability of the participant to self-regulate intensity from the RPE scales was facilitated by the performance of these previous sessions and the experience gained using RPE scales. This remains a limitation of the present study. Future work should look to validate these findings in untrained persons with tetraplegia. The ability of participants inexperienced in both wheelchair propulsion and the use of the RPE scale to self-regulate wheelchair exercise are also both important areas for investigation, as is the role that differentiated RPE can play in self-regulating wheelchair exercise.
4. Differentiated perceived exertion and self-regulated wheelchair exercise in novice users

This chapter has been published in a slightly modified form in the Archives of Physical Medicine & Rehabilitation:


In Chapter three, individuals with tetraplegia experienced in wheelchair propulsion successfully self-regulated exercise utilising subjective perceived exertion.

The following chapter investigates whether the same tool can be employed in a novice (non-SCI) user group and if a differentiated RPE specific to peripheral strain in the exercising muscle can improve the self-regulation process.
4.1 Abstract

**Purpose:** To investigate the utility of the differentiated RPE as a tool for the self-regulation of submaximal wheelchair propulsion in novice users.

**Methods:** Eighteen, healthy able-bodied participants with no prior experience of wheelchair propulsion completed a submaximal incremental test and a graded test to exhaustion to determine $\dot{V}O_{2peak}$ on a wheelchair ergometer. On a separate day, two 12-min intermittent bouts consisting of three 4-min stages were completed at individualised imposed PO equating to ‘light’ (40% $\dot{V}O_{2peak}$) and ‘moderate’ (60% $\dot{V}O_{2peak}$) intensity exercise. On a third occasion, participants were assigned to either the overall group or peripheral group and were required to self-regulate 12 min intermittent exercise according to either RPE$_O$ or RPE$_P$ reported during the corresponding imposed intensity trial. Differences in physiological variables, including $\dot{V}O_2$, HR and BLa, PO were then examined between the imposed and self-regulated exercise trials.

**Results:** No difference was found in physiological responses between the moderate intensity imposed and RPE-regulated trials in the peripheral group, whereas a significant ($p<0.05$) under-production in $\dot{V}O_2$ (1.76±0.31 vs. 1.59±0.25 L·min$^{-1}$) and BLa$^-$ (2.60±0.90 vs. 2.21±0.83 mmol·L$^{-1}$) was seen in the overall group. In contrast a significant ($p<0.05$) over-production was seen in the peripheral group at a light exercise intensity, whereas no difference was observed between physiological variables during the light intensity imposed and RPE-regulated trials in the overall group.

**Conclusion:** RPE$_P$ provided the dominant perceptual signal during submaximal wheelchair exercise. When self-regulating exercise based on perceptual exertional signals, RPE$_P$ enabled a more precise self-regulation of moderate intensity wheelchair exercise in a novice user group than RPE$_O$. In contrast, RPE$_P$ resulted in a significant over-estimation of light intensity exercise. Therefore it is recommended that RPE$_O$ should be employed for the self-regulation of light intensity exercise prior to further familiarisation with differentiated RPE.
4.2 Introduction

The majority of wheelchairs employed for daily ambulation and sports performance are hand-rim propelled, which is reported to be one of the least efficient forms of locomotion (Woude et al. 1999). However, wheelchair propulsion training and experience of manual wheelchair use show favourable effects on mechanical efficiency and physiological strain (Dallmeijer et al. 2004; de Groot et al. 2008; Lenton et al. 2008). Therefore, wheelchair practice is encouraged to enable participants to refine their propulsion technique, reduce feelings of physical strain and to ultimately encourage the confidence necessary to increase exercise adherence (de Groot et al. 2008; MacPhee et al. 2004). Short term wheelchair skills training can also improve factors determining quality of life, including self-esteem (MacPhee et al. 2004), and regular manual wheelchair exercise has been shown to improve cardiorespiratory fitness and endurance capacity (Grange et al. 2002; Woude et al. 1999). Low intensity wheelchair training offers a method of improving physical capacity (van den Berg et al. 2010b) and wheelchair propulsion technique (de Groot et al. 2008) in untrained wheelchair users, whilst minimising local discomfort and strain in the upper-limb.

Assessments of exercise intensity can be made during wheelchair propulsion training using standard open circuit spirometry procedures, in which oxygen uptake (VO₂) and PO are measured. However, the rehabilitation practitioner may not have access to the equipment required for these assessments on a day-to-day basis. Regulating exercise intensity solely on HR may also be unsuitable for some individuals with a high thoracic (paraplegia) or cervical (tetraplegia) SCI due to an attenuated sympathetic innervation of the heart in response to exercise (Valent et al. 2007a). It is therefore proposed that RPE may provide a convenient and inexpensive alternative to the aforementioned methods for regulating exercise intensity (Borg 1998; Parfitt et al. 2012).

RPE have previously been employed for the prescription and self-regulation of exercise intensity across a range of exercise modalities, including treadmill exercise, cycling, ACE, hand cycling and wheelchair propulsion (Eston & Williams 1988; Goosey-Tolfrey et al. 2010; Muller et al. 2004; Chapter three). Muller et al. (2004) reported small coefficients of variation (2.6–7.8%) when self-regulating high intensity wheelchair racing training according to a modified perceived exertion scale. In Chapter
three, a group of trained athletes with tetraplegia successfully employed RPE to self-regulate 20 min of moderate intensity, manual wheelchair exercise. However, it is recognised that the strength of perceptual signals from the peripheral exercising limbs and joints (RPE<sub>P</sub>) are greater than central signals from the cardiorespiratory system, such as HR and ventilation (RPE<sub>C</sub>), during submaximal wheelchair propulsion (Lenton et al. 2008). Lenton et al. (2008) also observed that individuals inexperienced in wheelchair propulsion reported higher RPE<sub>P</sub> compared to experienced users at the same relative exercise intensity. It is therefore important to consider the role of differentiated RPE in forming overall perceived exertion during manual wheelchair propulsion. However, to date no study has examined the ability of novice wheelchair users to self-regulate exercise or the potential role of RPE<sub>P</sub> in improving the accuracy of self-regulated upper-limb exercise.

The differentiated RPE model suggests that perceptual signals are related to specific anatomically regionalised processes during exercise (Pandolf et al. 1984). These differentiated RPE are then combined in a process termed ‘perceptual signal integration’ to create RPE<sub>O</sub> (Robertson & Noble 1997). It is recognised that the reliability of exercise intensity is improved with mode-specific familiarisation during low and moderate intensity, self-regulated exercise guided by the RPE<sub>O</sub> (Eston & Williams 1988; Muller et al. 2004). However, the prescription and self-regulation of exercise may be enhanced in novice wheelchair users by using an RPE specific to the peripheral exertional signals experienced during hand-rim propulsion.

The purpose of this study was to: 1) establish the differentiated RPE (peripheral and central) and undifferentiated RPE (overall) during submaximal wheelchair propulsion in novice individuals; and 2) examine whether utilising the differentiated RPE from the exercising limbs can improve the self-regulation of wheelchair exercise when compared to traditional RPE<sub>O</sub> in the same novice group. It was hypothesised that RPE from the exercising muscle and joints would be greater than RPE<sub>C</sub> arising from the cardiorespiratory system during submaximal wheelchair propulsion. Furthermore, although the novice group would successfully self-regulate exercise based on RPE<sub>O</sub>, employing an RPE specific to the exercising muscle mass and joints would improve the accuracy of the self-regulation process.
4.3 Methods

4.3.1 Participants

Eighteen recreationally active, able-bodied males volunteered to participate in the study. The participants’ characteristics are shown in Table 4.1. Procedures for the current investigation were approved by the University’s Ethical Committee and performed in accordance with the Declaration of Helsinki. All participants provided written informed consent before testing commenced. Participants were physically active (>3 h·wk⁻¹) but not specifically upper-limb trained and had no prior experience of wheelchair propulsion. Thus, the cohort employed was homogenous in both training status and wheelchair experience. This able-bodied participant group provided an experimental population in which to preliminarily examine the current hypotheses.

Table 4.1 Participants’ characteristics

<table>
<thead>
<tr>
<th></th>
<th>Whole cohort (n=18)</th>
<th>Peripheral (n=9)</th>
<th>Overall (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23 ± 2</td>
<td>23 ± 2</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>77.7 ± 9.6</td>
<td>77.2 ± 6.3</td>
<td>78.1 ± 12.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181 ± 7</td>
<td>182 ± 7</td>
<td>180 ± 8</td>
</tr>
<tr>
<td>( \dot{VO}_{2\text{peak}} ) (L·min⁻¹)</td>
<td>2.91 ± 0.32</td>
<td>2.81 ± 0.17</td>
<td>2.93 ± 0.39</td>
</tr>
<tr>
<td>HR_{peak} (b·min⁻¹)</td>
<td>170 ± 11</td>
<td>172 ± 7</td>
<td>171 ± 15</td>
</tr>
</tbody>
</table>

Note. Data are mean ± SD.

4.3.2 Experimental design

The study utilised a repeated measures design with participants visiting the laboratory on three separate occasions. During the first session, participants completed a submaximal incremental test and a graded exercise test to exhaustion to determine \( \dot{VO}_{2\text{peak}} \). On a separate day, two 12-min intermittent exercise bouts consisting of three 4-min stages were completed at individualised imposed PO equating to ‘light’ (40% \( \dot{VO}_{2\text{peak}} \)) and ‘moderate’ (60% \( \dot{VO}_{2\text{peak}} \)) intensity exercise (Figure 4.1). On a third occasion, participants were assigned to either the overall group or peripheral group and
were required to self-regulate 12 min intermittent exercise according to either $\text{RPE}_0$ or $\text{RPE}_p$ reported during the corresponding imposed intensity trial (Figure 4.1). Session one and session two were separated by seven days. The main experimental trials of sessions two and three were separated by at least five days but no longer than 7 days.

**Figure 4.1** Experimental protocol for the imposed-intensity (session two) and RPE-regulated (session three) trials
4.3.3 Instrumentation

All testing was performed using a 15° cambered sports wheelchair with 0.66 m diameter wheels and 0.61 m hand-rims (Quattro, RGK, Burntwood, Staffordshire, England). The wheelchair was mounted on a wheelchair ergometer interfaced with a computer. The wheelchair ergometer consisted of a single roller (length, 1.14 m; circumference, 0.48 m). A flywheel sensor connected to the roller and interfaced to a computer calculated wheelchair velocity and displayed it visually on a computer monitor. Upon each visit participants performed two deceleration tests to allow PO to be calculated as previously described in section 3.3.3.

Tyre pressure was set at 100 psi for each participant and standardised for each session. The Borg 6-20 scale was used to attain participants differentiated RPE throughout all trials. Participants were given standardised instructions detailing the use of the Borg 6-20 scale and the associated verbal anchors at the beginning of each session (Borg 1998). To determine $RPE_C$, participants were asked to rate their perceived exertion for the heart, lungs and breathing (Borg 1998; Pandolf et al. 1984). To determine $RPE_P$, participants were asked to rate exertion only from the exercising muscle groups and joints (Borg 1998; Pandolf et al. 1984). $RPE_O$ was then reported as the combination of $RPE_P$ and $RPE_C$. The RPE scale was visible to participants for the duration of each trial.

4.3.4 Session 1: Preliminary measures

On arrival at the laboratory, body mass was measured to the nearest 0.1 kg, using double-beam seated scales (Marsden MPWS-300, Henley-on-Thames, UK). The degree of elbow extension elicited by each participant when sitting upright with their hands positioned at top dead centre of the wheel was measured using a goniometer and standardised to an optimal angle of 100–120°. A standardised 5-min warm up of no greater than 1.5 m.s$^{-1}$ was performed prior to all exercise sessions. Subsequently, participants performed an incremental exercise test consisting of five 4-min constant load exercise stages at ascending velocities, intended to elicit physiological responses covering a range from 40% to 80% $\dot{V}O_2$peak. Initial speeds were 1.2±0.2 m.s$^{-1}$ with subsequent velocity increments of 0.2 or 0.3 m.s$^{-1}$. HR was monitored continuously using radio telemetry (Polar PE 4000, Kempele, Finland). On-line respiratory gas
analysis was carried out throughout each 4-min stage via a breath-by-breath system (Cortex metalyser 3B, Cortex, Leipzig, Germany). Before each test, gases were calibrated according to the manufacturer’s recommendations using a 2-point calibration ($O_2 = 17.0\%$, $CO_2 = 5.0\%$ against room air) and volumes with a 3-L syringe at flow rates of 0.5–3.0 L.s$^{-1}$. The average respiratory data from the last 1-min of each stage was used to provide information of $\dot{V}O_2$. A small capillary blood sample was obtained from the earlobe at the start of the test and during a 1-min break between stages to determine $BLa^-\bar{\text{ }}$ using a YSI 1500 SPORT Lactate Analyser (YSI Inc, Yellow Springs, OH). Differentiated RPE were recorded in the last 15 s of each 4-min stage while the participant was still exercising.

After a 15-min rest period, a graded exercise test to exhaustion was performed to determine $\dot{V}O_2\text{peak}$. The test involved increments of 0.1 m.s$^{-1}$ every minute from an initial velocity of $1.7\pm0.6$ m.s$^{-1}$ at a freely chosen PF until volitional exhaustion. HR and expired air were measured continuously throughout the test and the final differentiated RPE was recorded as previously described. Breath-by-breath data allowed the highest 30 s rolling average $\dot{V}O_2$ value recorded during the exercise test to be taken as the $\dot{V}O_2\text{peak}$. For each participant a simple linear regression analysis was performed using the linear workload-$\dot{V}O_2$ relationship. The regression line created from the paired submaximal velocity and $\dot{V}O_2$ data was employed to interpolate individual velocities corresponding to a ‘light’ exercise intensity of 40% and a ‘moderate’ exercise intensity of 60% $\dot{V}O_2\text{peak}$.

4.3.5 Session 2: Imposed power

A standardised 5-min warm up was performed prior to the imposed intensity trial and standardised for the RPE-regulated trial as previously described. The imposed intensity bouts were performed at individualised exercise intensities corresponding to 40% and 60% $\dot{V}O_2\text{peak}$. Exercise intensities were presented in a counter-balanced order. Participants were informed of the velocity required and were asked to maintain it for 12 min of intermittent propulsion comprised of three 4-min stages separated by 3-min rest. The different intensity bouts were separated by 20 min rest. Participants had full vision of their velocity on the computer monitor throughout the whole session. $\dot{V}O_2$, VE, breathing frequency (BF) and HR were measured constantly during each bout and averaged over the final minute. Energy expenditure was obtained from $\dot{V}O_2$ and
associated respiratory exchange ratio (RER) by using the standard conversion table for
the energy equivalent of oxygen (Peronnet & Massicotte 1991). GE was calculated
according to principles of Woude et al. (1989) and defined as the ratio between external
energy produced and internal energy expended. PF were retrospectively calculated from
the velocity trace provided by the ergometer and averaged over the final 4-min bout of
each trial. Differentiated RPE were recorded and Bla− determined in the last 15 s of
each 4-min bout while the participant was still exercising. Collection of the RPE in the
final stages of each 4-min bout of exercise is a valid means of assessing perceived
exertion and is consistent with previous literature (Eston & Williams 1989), on the basis
that HR and VO2 can be considered to have reached a steady-state after 3 min of
continuous submaximal propulsion. The average recorded RPE during the 12 min
pushing at light and moderate intensities were taken as the anchor for the intensity of
the RPE-regulated bout.

4.3.6 Session 3: RPE-regulated

Participants were pair-matched for VO2peak and assigned to either the overall or
peripheral group, where they were required to self-regulate exercise intensity using
either RPEO or REP, respectively. Participants were informed of the average respective
RPE recorded during each imposed intensity trial and were instructed to reproduce a
workload equating to these RPE for each 4-min stage in the 12-min bouts. Participants
were blinded to their velocity and all physiological measurements but were informed of
time elapsed. BF, VO2, VE, HR, Bla−, PF and GE were measured in accordance with
the imposed intensity trials. PO was also recorded and averaged over each minute.
Participants were reminded of their target RPE prior to each 4-min stage.

4.3.7 Statistical analysis

All data was analysed using the statistical package IBM SPSS version 19 for
windows (SPSS inc, Chicago, IL). Using previously published experimental data by
Kang et al (2009), statistical package GPower 3.1.5 indicated a minimum sample size of
16 participants (eight participants per group) was required to determine similar
differences in PO between trials, with an effect size of 1.2, 90% power and an α of 5%.
Subsequently 18 participants were recruited.
Normal distribution of the outcome variables was confirmed by Shapiro-Wilk test ($W_{(10)} = 0.83 - 0.98$, $p = 0.07 - 0.94$). All descriptives are presented as mean ± SD with the exception of ordinal RPE data which are reported as median and quartile ranges. Differences in $\dot{V}O_{2\text{peak}}$ and age between groups were examined using Student’s dependent t-tests, as were paired values for $\dot{V}O_2$, $\%\dot{V}O_{2\text{peak}}$, PO, velocity, VE, BF, HR, $\%HR_{\text{peak}}$, BL a”, GE and PF averaged during the 12-min exercise bouts between the imposed and RPE-regulated trials. 95% CI diff are also provided. A 3-way (trial-by-intensity-by-group) mixed measures ANOVA was performed on all the variables above. In addition a 3-way (trial-by-group-by-time) mixed measures ANOVA was performed on the PO data from both the light and moderate intensity bouts to examine the responses across time. Non-parametric Friedman tests and Wilcoxon signed-rank tests were used to analyse differences in ordinal differentiated RPE data at both submaximal imposed intensities. Significance was set a priori at $p \leq 0.05$. A Bonferroni adjustment was performed on the alpha value when performing multiple comparisons. Effect sizes (ES) (Cohen’s d) are presented whereby 0.2 refers to a small effect, 0.5 a moderate effect and 0.8 a large effect (Cohen 1992).

4.4 Results

Participants’ peak physiological responses are shown in Table 4.1. Table 4.2 shows the differentiated RPE responses for the submaximal imposed intensity trials. Non-parametric difference tests found $\text{RPE}_P$ and $\text{RPE}_O$ to be greater than $\text{RPE}_C$ at both intensities. In turn, $\text{RPE}_P$ was greater than $\text{RPE}_O$ during moderate intensity propulsion only.

| Table 4.2 Differentiated RPE responses during submaximal wheelchair propulsion (n=18) |
|---|---|---|
| & $\text{RPE}_P$ & $\text{RPE}_C$ & $\text{RPE}_O$ |
| 40% $\dot{V}O_{2\text{peak}}$ | 10 (9,11) $\dagger$ | 9 (8,10) | 10 (8,12) $\dagger$ |
| 60% $\dot{V}O_{2\text{peak}}$ | 13 (13,14) $\dagger$ | 12 (11,13) | 13 (12,13) $\dagger$ |

Note. Data are median (quartiles). $\dagger$=significantly different from both $\text{RPE}_C$ and $\text{RPE}_O$ $\dagger$ = significantly different from $\text{RPE}_C$. ($p \leq 0.05$).
Age, VO$_{\text{2peak}}$ and body weight were consistent between groups. Comparisons between the imposed and RPE-regulated trials were made using paired sample t-tests and ES as shown in Table 4.3 and 4.4. Negative ES and significantly lower VO$_2$, VO$_{\text{2peak}}$ and BLα were present for the overall group during moderate intensity exercise when comparing the imposed and RPE-regulated trials. No significant differences were present between trials for the peripheral group at the same exercise intensity, with smaller ES and 95% CI$_{\text{diff}}$ compared to the overall group. In contrast, the overall group displayed smaller ES and 95% CI$_{\text{diff}}$ and no significant differences between the light intensity imposed and RPE-regulated trials. A significant over-production and larger ES were present for VO$_2$, %VO$_{\text{2peak}}$, HR, PO and BF in the peripheral group at the same intensity.

For the 3-way trial-by-intensity-by-group ANOVA, significant main effects for intensity ($p<0.001$) for VO$_2$, %VO$_{\text{2peak}}$, BLα, HR, %HR$_{\text{peak}}$, PF, BF, VE and PO indicated the manipulation of exercise intensity was successful, with all values greater in the moderate intensity trials than the light intensity trials. No difference in gross mechanical efficiency was found between the imposed and RPE-regulated bouts for either group at both intensities. Average efficiency for all participants was 6.3 ± 0.8 %. The 3-way time-by-trial-by group analysis confirmed PO was consistent across time for both the light (Figure 4.2) and moderate (Figure 4.3) intensity RPE-regulated trials.
Table 4.3 Physiological responses during 12 min imposed intensity and RPE-regulated wheelchair propulsion at 40% \( \dot{V}\text{O}_\text{peak} \)

<table>
<thead>
<tr>
<th></th>
<th>Imposed intensity</th>
<th>RPE-regulated</th>
<th>95% CI</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE&lt;sub&gt;p&lt;/sub&gt;</td>
<td>11 (10,12)</td>
<td>11 (10,12)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \dot{V}\text{O}_2 (\text{L} \cdot \text{min}^{-1}) )</td>
<td>1.14 ± 0.15</td>
<td>1.29 ± 0.13</td>
<td>-0.27 to -0.03</td>
<td>0.02†</td>
<td>1.15</td>
</tr>
<tr>
<td>%( \dot{V}\text{O}_2\text{peak} )</td>
<td>39 ± 4</td>
<td>45± 4</td>
<td>-10 to -1</td>
<td>0.02†</td>
<td>1.45</td>
</tr>
<tr>
<td>HR (b·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>91 ± 12</td>
<td>98 ± 5</td>
<td>-15 to 1</td>
<td>0.05†</td>
<td>0.83</td>
</tr>
<tr>
<td>% HR&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>54 ± 8</td>
<td>58 ± 4</td>
<td>-8 to 0</td>
<td>0.05†</td>
<td>0.65</td>
</tr>
<tr>
<td>BLa&lt;sup&gt;-&lt;/sup&gt; (mmol·L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.39 ± 0.42</td>
<td>1.88 ± 0.62</td>
<td>-0.75 to 0.18</td>
<td>0.19</td>
<td>0.54</td>
</tr>
<tr>
<td>PO (W)</td>
<td>26 ± 3</td>
<td>32 ± 4</td>
<td>-5 to 0</td>
<td>0.04†</td>
<td>0.40</td>
</tr>
<tr>
<td>Br Freq (1·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>22 ± 5</td>
<td>24 ± 5</td>
<td>-5 to 0</td>
<td>0.04†</td>
<td>0.40</td>
</tr>
<tr>
<td>VE (L·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>25.3 ± 2.1</td>
<td>27.8 ± 3.6</td>
<td>-1.6 to 0.4</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>GE (%)</td>
<td>6.7 ± 0.6</td>
<td>6.9 ± 0.7</td>
<td>-0.7 to 0.4</td>
<td>0.44</td>
<td>0.36</td>
</tr>
<tr>
<td>PF (p·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>26 ± 9</td>
<td>27 ± 10</td>
<td>-1.0 to 1.0</td>
<td>0.76</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE&lt;sub&gt;O&lt;/sub&gt;</td>
<td>9 (8,11)</td>
<td>9 (8,11)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \dot{V}\text{O}_2 (\text{L} \cdot \text{min}^{-1}) )</td>
<td>1.19 ± 0.19</td>
<td>1.20 ± 0.15</td>
<td>-0.18 to 0.15</td>
<td>0.40</td>
<td>0.10</td>
</tr>
<tr>
<td>%( \dot{V}\text{O}_2\text{peak} )</td>
<td>40 ± 3</td>
<td>42 ± 5</td>
<td>-5 to 1</td>
<td>0.20</td>
<td>0.33</td>
</tr>
<tr>
<td>HR (b·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>91 ± 12</td>
<td>95 ± 14</td>
<td>-10 to 1</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>% HR&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>53 ± 5</td>
<td>56 ± 6</td>
<td>-5 to 1</td>
<td>0.11</td>
<td>0.37</td>
</tr>
<tr>
<td>BLa&lt;sup&gt;-&lt;/sup&gt; (mmol·L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.27 ± 0.63</td>
<td>1.40 ± 0.72</td>
<td>-0.35 to 0.10</td>
<td>0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>PO (W)</td>
<td>26 ± 3</td>
<td>28 ± 4</td>
<td>-5 to 1</td>
<td>0.10</td>
<td>0.38</td>
</tr>
<tr>
<td>BF (1·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>23 ± 2</td>
<td>24 ± 2</td>
<td>-1 to 3</td>
<td>0.20</td>
<td>0.39</td>
</tr>
<tr>
<td>VE (L·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>26.2 ± 3.7</td>
<td>27.2 ± 5.8</td>
<td>-2.8 to 1.8</td>
<td>0.10</td>
<td>0.21</td>
</tr>
<tr>
<td>GE (%)</td>
<td>6.2 ± 0.4</td>
<td>6.1 ± 0.6</td>
<td>-0.5 to 0.4</td>
<td>0.79</td>
<td>-0.20</td>
</tr>
<tr>
<td>PF (p·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>23 ± 12</td>
<td>25 ± 13</td>
<td>-6 to 2</td>
<td>0.27</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Note. †= significant difference between imposed and RPE-regulated trials (\( p \leq 0.05 \)
Table 4.4 Physiological responses during 12 min imposed intensity and RPE-regulated wheelchair propulsion at 60% \( \dot{V}O_2^{\text{peak}} \)

<table>
<thead>
<tr>
<th></th>
<th>Imposed intensity</th>
<th>RPE-regulated</th>
<th>95% CI_{diff}</th>
<th>( p )-value</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE_p</td>
<td>13 (13,15)</td>
<td>13 (13,15)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) (L·min(^{-1}))</td>
<td>1.64 ± 0.19</td>
<td>1.78 ± 0.26</td>
<td>-0.34 to 0.01</td>
<td>0.13</td>
<td>0.63</td>
</tr>
<tr>
<td>%( \dot{V}O_2^{\text{peak}} )</td>
<td>58 ± 3</td>
<td>62 ± 7</td>
<td>-7 to 0</td>
<td>0.07</td>
<td>0.67</td>
</tr>
<tr>
<td>HR (b·min(^{-1}))</td>
<td>107 ± 11</td>
<td>111 ± 9</td>
<td>-8 to 2</td>
<td>0.20</td>
<td>0.31</td>
</tr>
<tr>
<td>% HR_{peak}</td>
<td>66 ± 9</td>
<td>66 ± 7</td>
<td>-5 to 5</td>
<td>0.98</td>
<td>0.04</td>
</tr>
<tr>
<td>BLa(^{-}) (mmol·L(^{-1}))</td>
<td>2.56 ± 0.56</td>
<td>2.62 ± 0.73</td>
<td>-0.65 to 0.53</td>
<td>0.82</td>
<td>0.09</td>
</tr>
<tr>
<td>PO (W)</td>
<td>37 ± 2</td>
<td>39 ± 4</td>
<td>-4 to 0</td>
<td>0.08</td>
<td>0.55</td>
</tr>
<tr>
<td>BF (1·min(^{-1}))</td>
<td>29 ± 5</td>
<td>30 ± 4</td>
<td>3 to 4</td>
<td>0.70</td>
<td>0.22</td>
</tr>
<tr>
<td>VE (L·min(^{-1}))</td>
<td>37.1 ± 13.5</td>
<td>39.5 ± 8.5</td>
<td>-9.8 to 4.0</td>
<td>0.36</td>
<td>0.26</td>
</tr>
<tr>
<td>GE (%)</td>
<td>6.1 ± 0.7</td>
<td>6.1 ± 0.6</td>
<td>-0.1 to 0.3</td>
<td>0.35</td>
<td>0.01</td>
</tr>
<tr>
<td>PF (p·min(^{-1}))</td>
<td>31 ± 9</td>
<td>32 ± 13</td>
<td>-6 to 4</td>
<td>0.67</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE_o</td>
<td>13 (12,14)</td>
<td>13 (12,14)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \dot{V}O_2 ) (L·min(^{-1}))</td>
<td>1.76 ± 0.31</td>
<td>1.59 ±0.25</td>
<td>0.05 to 0.33</td>
<td>0.04†</td>
<td>-0.74</td>
</tr>
<tr>
<td>%( \dot{V}O_2^{\text{peak}} )</td>
<td>60 ± 3</td>
<td>53± 6</td>
<td>3 to 10</td>
<td>0.01†</td>
<td>-1.37</td>
</tr>
<tr>
<td>HR (b·min(^{-1}))</td>
<td>113 ± 19</td>
<td>108± 17</td>
<td>-3 to 13</td>
<td>0.18</td>
<td>-0.31</td>
</tr>
<tr>
<td>% HR_{peak}</td>
<td>66 ± 8</td>
<td>63 ± 8</td>
<td>-2 to 7</td>
<td>0.18</td>
<td>-0.36</td>
</tr>
<tr>
<td>BLa(^{-}) (mmol·L(^{-1}))</td>
<td>2.68 ± 0.90</td>
<td>2.21 ± 0.83</td>
<td>0.13 to 0.81</td>
<td>0.01†</td>
<td>-0.45</td>
</tr>
<tr>
<td>PO (W)</td>
<td>37 ± 3</td>
<td>35 ± 2</td>
<td>-1 to 5</td>
<td>0.11</td>
<td>-0.68</td>
</tr>
<tr>
<td>BF (1·min(^{-1}))</td>
<td>28 ± 3</td>
<td>28 ± 5</td>
<td>-4 to 3</td>
<td>0.85</td>
<td>0.00</td>
</tr>
<tr>
<td>VE (L·min(^{-1}))</td>
<td>39.0 ± 9.0</td>
<td>34.4 ± 6.5</td>
<td>-0.8 to 10.0</td>
<td>0.08</td>
<td>-0.59</td>
</tr>
<tr>
<td>GE (%)</td>
<td>5.9 ± 0.7</td>
<td>6.1 ± 0.8</td>
<td>-0.9 to 0.5</td>
<td>0.54</td>
<td>0.27</td>
</tr>
<tr>
<td>PF (p·min(^{-1}))</td>
<td>31 ± 10</td>
<td>32 ± 13</td>
<td>-5 to 3</td>
<td>0.51</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Note.** †= significant difference between imposed and RPE-regulated trials \((p \leq 0.05)\)
Figure 4.2 Minute-by-minute power output data during light intensity (40% VO₂peak) imposed and RPE-regulated trials. (a) = peripheral group; (b) = overall group.

Figure 4.3 Minute-by-minute power output data during moderate intensity (60% VO₂peak) imposed and RPE-regulated trials. (a) = peripheral group; (b) = overall group.
4.5 Discussion

The present study examined the hypothesis that differentiated RPE can provide a mode specific stimulus to improve the precision of self-regulated wheelchair exercise in novice users. In accordance with Lenton et al. (2008), RPE from the exercising muscle mass and joints was the dominant perceptual signal during submaximal wheelchair propulsion. Utilising these dominant RPE_\text{P} improved the precision of moderate intensity, self-regulated exercise (RPE = 13 ‘somewhat hard’) in this novice group, with an under-production in exercise intensity seen when incorporating both RPE_\text{P} and RPE_\text{C} to form undifferentiated RPE. However, the employment of RPE_\text{P} to self-regulate light intensity exercise (RPE = 9-11 ‘very light – fairly light’) resulted in a significant overproduction in exercise intensity which was not present when using undifferentiated RPE.

Differentiated RPE during wheelchair exercise

The perceptual dominance of RPE_\text{P} during manual hand-rim propulsion can be attributed to a combination of physiological and biomechanical factors. Oxygen availability is restricted during upper-limb exercise as a result of an impaired perfusion capacity of the upper-limb musculature (Calbet et al. 2005). Oxidative enzyme activity is also limited in previously untrained upper-limb muscles (Killerich et al. 2008). This impaired aerobic capability results in elevated lactate production and subsequent acidosis of exercising tissue during upper-limb versus lower-limb exercise of a comparable intensity (Helge et al. 2010), thereby elevating peripheral feelings of exertion (Hampson et al. 2001). Specific to wheelchair users, manual hand-rim propulsion is associated with neurologic and muscular pain in the wrist and shoulder joints due to high mechanical loads (Boninger et al. 2002). Novice users also exhibit a lower mechanical efficiency compared to experienced users as a result of inferior coordination, with technique parameters such as timing and stroke angle improving task efficiency with wheelchair experience (Woude et al. 2001). The greater energy-cost of producing a given workload and inefficiency in technique therefore contributes to the greater physical and muscular strain in novice users (Dallmeijer et al. 2004).

Differentiated RPE and self-regulated exercise

Perceptually-regulated exercise training has been employed to achieve gains in cardiovascular health and fitness (Parfitt et al. 2012). In this method, the RPE are
employed in ‘production’ mode, allowing individuals to self-regulate the intensity of exercise based on subjective exertional responses (Goosey-Tolfrey et al. 2010; Parfitt et al. 2012). The target RPE can be ‘estimated’ during prior exercise tasks of a known intensity (Chapter three; Goosey-Tolfrey et al. 2010) or clamped at a fixed RPE for a whole cohort (Parfitt et al. 2012). To date, RPE\(_O\) has traditionally been employed as the stimulus for self-regulated exercise. However, RPE\(_P\) is the dominant contributing factor to RPE\(_O\) during wheelchair propulsion (Lenton et al. 2008) and other modes of upper-limb exercise (Al-Rahamneh et al. 2011; Pandolf et al. 1984). As shown in Table 4.4, the present findings suggest a mode specific differentiated RPE, based on the aforementioned dominant peripheral signals, can improve the precision of moderate intensity, self-regulated wheelchair exercise in individuals unaccustomed to the demands of hand-rim propulsion. The significantly lower relative oxygen uptake, VE and BLa⁻ when self-regulating moderate intensity exercise based on RPE\(_O\) indicate lower levels of physiological strain compared to the target ‘imposed’ intensity trial. In a practical setting, an under-production in exercise intensity, as seen with undifferentiated RPE, may result in an insufficient training load being performed. Subsequently, targeted outcomes of training, whether functional or performance based, may not be attained. The current findings contrast with the successful self-regulation of moderate intensity wheelchair exercise reported in a group of experienced users employing undifferentiated RPE in Chapter three. However, experienced users have a greater familiarisation with the dominance in RPE\(_P\) during wheelchair propulsion. Therefore, a focus on these peripheral signals during self-regulated exercise may have facilitated the successful findings despite the employment of RPE\(_O\).

An unexpected finding of this study was the over-production in light intensity exercise, a method frequently applied for wheelchair training (de Groot et al. 2008; van den Berg et al. 2010b), when employing RPE\(_P\) (Table 4.3). Oxygen uptake, HR, BLa⁻, PO and BF were all significantly higher than the corresponding imposed intensity trial. The aforementioned factors regulating peripheral exertion, including mechanical work and muscle lactate production, were significantly lower for the light intensity exercise than the moderate intensity exercise. This over-production may therefore represent the insensitivity of novice users to small alterations in these peripheral signals, and the elevation in workload required to achieve perceptible changes whilst producing light exercise intensities. Since an over-production in prescribed exercise intensity may have
deleterious consequences on health, including over-use injury or cardiovascular strain, and may induce premature fatigue during exercise training, RPE₀ should be considered a more applicable tool for self-regulating low intensity training prior to further familiarisation in wheelchair propulsion. The effect of familiarisation on the accuracy of light intensity self-regulated wheelchair exercise utilising mode specific differentiated RPE requires investigation.

**Study limitations**

The application of a novice, able-bodied population in this study allowed for a cohort homogenous in training status and wheelchair experience in which to preliminarily examine the current hypotheses. Physiological responses in able-bodied, non-wheelchair user groups have been shown to comply with the overall trends shown by wheelchair users (Dallmeijer et al. 2004; Lenton et al. 2008; Woude et al. 1989). However, the sensorimotor and cardiovascular adaptations associated with a cervical level SCI require the verification of these findings in novice tetraplegic groups. In the current protocol, the preliminary testing and the imposed intensity exercise trial preceded the RPE-regulated trials. The ability of the participants to self-regulate exercise intensity may therefore have been facilitated by the performance of these previous sessions and the experience gained using RPE scales. This factor should be taken into consideration when considering the application of these findings. Further work is required to investigate the role of familiarisation training with RPE on the accuracy of self-regulated wheelchair exercise. Furthermore, the current work also only investigates constant load wheelchair propulsion and future work should extend these findings more practical long term, rehabilitation-based wheelchair training sessions and overground wheelchair propulsion.

**4.6 Conclusion**

In conclusion, RPEₚ provided the dominant perceptual signal during submaximal wheelchair exercise. When self-regulating exercise based on perceptual exertional signals, RPEₚ enabled a more precise self-regulation of moderate intensity wheelchair exercise in a novice user group than RPE₀. In contrast, RPE₀ provided a more accurate self-regulation tool during light intensity exercise and should be employed prior to familiarisation with differentiated RPE during light intensity wheelchair propulsion training.
5. Spinal cord injury level and the circulating cytokine response to strenuous wheelchair exercise

This chapter has been published in a modified form in *Medicine & Science in Sports & Exercise*:


Chapters three & four provided evidence that subjective RPE can provide a cost-effective tool for the regulation of wheelchair exercise.

The following chapter investigates the effect of SCI level on the response of inflammation-mediating plasma cytokines to acute strenuous wheelchair exercise.
5.1 Abstract

Purpose: A complete SCI above T6 results in the loss of sympathetic innervation of the adrenal medulla. This study examined the effect of a complete SCI above and below T6 on plasma concentrations of adrenaline, circulating IL-6 and other inflammatory cytokines in response to acute strenuous exercise.

Methods: Twenty-six elite male wheelchair athletes (8=C6-C7 tetraplegic (TETRA); 10=T6-L1 paraplegic (PARA); 8=non-spinal cord injured controls (NON-SCI)) performed a submaximal exercise test followed by a graded exercise to exhaustion on a motorised treadmill. Blood samples were taken pre-exercise, post-exercise and 30 min post-exercise (post30) and analysed for concentrations of IL-6, IL-10, IL-1ra, TNF-α, adrenaline and cortisol.

Results: Circulating IL-6 concentration was significantly elevated at post-exercise and post30 (~5-fold) in PARA and NON-SCI (p=0.003) whereas concentrations in TETRA did not change significantly from pre-exercise values. IL-10, IL-1ra and TNF-α were unaffected by exercise in all groups, however, both SCI groups presented elevated concentrations of IL-10 compared with NON-SCI (p=0.001). At post-exercise, plasma adrenaline concentrations were significantly higher than pre-exercise and post30 concentrations in PARA (~2-fold) (p=0.02) and NON-SCI (~3-fold). Plasma adrenaline concentrations were unchanged in TETRA throughout exercise, with concentrations significantly lower than PARA and NON-SCI at each time point. Plasma cortisol concentrations were significantly elevated in all groups at post-exercise and post30 compared with pre-exercise (p<0.001). Total exercise time was similar between groups (TETRA= 36±5; PARA= 35±5; NON-SCI= 38±6 min).

Conclusion: These findings suggest the sympathetic nervous system plays an important regulatory role in the circulating IL-6 response to exercise and has implications for the metabolic and inflammatory responses to exercise in individuals with injuries above T6.
5.2 Introduction

Marked (several-fold) elevations in circulating concentrations of the inflammation-responsive cytokine IL-6 immediately after strenuous exercise are associated with subsequent rises in the plasma concentrations of anti-inflammatory cytokines including IL-1ra, IL-10 and the soluble TNF receptors (Ostrowski et al. 1999; Steensberg et al. 2003). It is suggested this transient anti-inflammatory environment may be partly responsible for the positive effects of regular exercise in reducing circulating concentrations of IL-6 at rest via actions on adipocyte TNF-α and IL-6 release (Gleeson et al. 2011). Sustained low-level increases in plasma levels of TNF-α and IL-6 have been related to the future development of long-term conditions including CVD and type 2 diabetes (Gleeson et al. 2011).

Contracting skeletal muscle has been confirmed as the primary source of elevated concentrations of plasma IL-6 following exercise (Steensberg et al. 2000; Toft et al. 2011). The rise in cytosolic calcium ion concentration (Ca\(^{2+}\)) within contracting myofibres is believed to be a key stimulus for IL-6 release (Pederson & Febbraio 2008). In addition, a cross-talk between Ca\(^{2+}\)-dependent and stress-induced mitogen-activated protein kinase (MAPK)-dependent cell signalling pathways has also been shown to increase skeletal muscle IL-6 mRNA expression, particularly in the presence of low muscle glycogen (Chan et al. 2004; Pederson & Febbraio 2008). In contrast, the role of the sympathetic nervous system (SNS) in the regulation of the IL-6 response is less clear. Steensberg et al. (2001) reported a 29-fold increase in circulating IL-6 following a 2.5 h strenuous treadmill run in endurance trained, able-bodied participants. However, infusing adrenaline at rest to the same circulating concentrations as observed during exercise resulted in only a 6-fold increase in plasma IL-6 (Steensberg et al. 2001). Therefore, it has been suggested that the SNS plays only a minor role in exercise-induced elevations in plasma IL-6 concentration (Febbraio & Pederson 2002; Steensberg et al. 2001). In contrast both chemical, central and peripheral sympathetic denervation using 6-hydroxydopamine inhibited the plasma IL-6 response to exercise in rats (Yu et al. 2001). To confirm the contribution of the SNS to the exercise-induced IL-6 response in humans an in vivo model of attenuated SNS activity is needed. This would allow greater understanding of the mechanisms underlying the IL-6 response to acute exercise in humans.
An injury to the spinal cord results in the loss of central nervous system function below the level of the lesion, with the loss of function relative to the level and the completeness of the injury (Janssen et al. 2002). The neurons of the sympathetic nervous system are mainly located in the thoracic spinal cord (T1 – L1) and a complete injury above T6 results in the loss of sympathetic innervation of the adrenal medulla. The effect of the resulting decreased sympathetic outflow in tetraplegic individuals includes depressed circulating plasma adrenaline and noradrenaline concentrations at rest, during and after exercise (Schmid et al. 1998a; Schmid et al. 1998b). If it is the case that the SNS plays only a minor role in the IL-6 response to exercise, then individuals with complete injuries above T6 would be expected to demonstrate elevations in circulating IL-6 concentrations after exercise that are similar to those observed in individuals with an intact SNS (i.e. who are injured at or below T6, or who do not have a spinal injury). However, if this is not the case, a limited IL-6 response in those with injuries above T6 could have important health implications given the proposed metabolic and inflammatory roles of muscle-derived IL-6 in a population with already limited physical capacity.

Previously Kouda et al. (2012) have reported an absence of elevations in plasma levels of IL-6 and adrenaline immediately after 20 min ACE in individuals with cervical SCI. Non-SCI controls showed a small, yet significant increase in both measures. However, the short duration and moderate exercise workload of 60% \( \text{VO}_2\text{peak} \) may not have been a sufficient stimulus to induce elevations in plasma IL-6 concentrations within the SCI group. To our knowledge, there is no well-controlled research published that has investigated the effect of a SCI above and below T6 on the circulating cytokine response to an acute bout of strenuous exercise. Therefore, the purpose of this study was to determine the effect of complete cervical level SCI (i.e. above T6) compared with SCI at T6 or below on plasma concentrations of adrenaline, circulating IL-6 and other inflammatory cytokines in response to ~40 min of graded strenuous exercise. Specifically, the responses were assessed in trained wheelchair athletes with differing levels of spinal injury. A group of wheelchair athletes without a spinal injury were also included as a control. We hypothesised that those with SCI above T6 would have an attenuated adrenaline and IL-6 response compared with those with an intact SNS.
5.3 Methods

5.3.1 Participants

Twenty-six elite male wheelchair athletes (8=C6-C7 tetraplegic (TETRA); 10=T6-L1 paraplegic (PARA) and 8= wheelchair athletes without a SCI (NON-SCI)) volunteered to participate in the study. All participants were actively competing in international level wheelchair basketball or wheelchair rugby. A summary of the participants’ characteristics is presented in Table 5.1. All procedures were approved by the Loughborough University ethical advisory committee and performed in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to the exercise test. All participants were free from infectious symptoms and no incidences of pressure sores were reported.

<table>
<thead>
<tr>
<th></th>
<th>TETRA</th>
<th>PARA</th>
<th>NON-SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>31±6</td>
<td>30±8</td>
<td>27±8</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>67.3±5.9†</td>
<td>72.3±13.5</td>
<td>84.8±10.7</td>
</tr>
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<td>Lesion level/ Disability</td>
<td>C6/7</td>
<td>T6–L1, Spina Bifida</td>
<td>Amputee, Club foot</td>
</tr>
<tr>
<td>ASIA impairment scale</td>
<td>A</td>
<td>A</td>
<td>-</td>
</tr>
<tr>
<td>TSI (yr)</td>
<td>11±6</td>
<td>19±7</td>
<td>11±5</td>
</tr>
<tr>
<td>WC sport</td>
<td>Rugby (n=8)</td>
<td>Basketball (n=10)</td>
<td>Basketball (n=8)</td>
</tr>
<tr>
<td>Training (h·wk⁻¹)</td>
<td>13±2</td>
<td>15±3</td>
<td>16±2</td>
</tr>
<tr>
<td>Time in sport (yr)</td>
<td>11±3</td>
<td>14±6</td>
<td>10±6</td>
</tr>
</tbody>
</table>

Note. † = Significant difference, TETRA vs. NON-SCI (p< 0.05). TSI = time since injury; WC = wheelchair

5.3.2 Experimental protocol and instrumentation

Participants reported to the laboratory between 0930 and 1130 having been fasted for at least 2 h. Participants were asked to refrain from strenuous physical activity and caffeine intake 24 h prior to exercise. On arrival participants completed a health, training and disability questionnaire, and body mass was obtained to the nearest 0.1 kg using double-beam seated scales (Marsden MPWS-300, UK). All exercise tests were
performed in the participants’ competition court sports wheelchair on a motorised treadmill (Appendix I) (HP Cosmos, Traunstein, Germany). The treadmill exercise protocol is shown in Figure 5.1.

![Figure 5.1 Experimental protocol](image)

Figure 5.1 Experimental protocol

Note. Measurement of: $O_2$ = oxygen uptake; $L$ = blood lactate; $x$ = differentiated RPE.

Following a 5-min warm up at 1.2 $m\cdot s^{-1}$, participants performed ~6 submaximal constant-load 4-min exercise stages at ascending speeds at a fixed gradient of 1.0%. The protocol was designed to elicit submaximal physiological responses covering a range from 40% to 80% $\dot{V}O_2$peak. This was followed by 15 min passive recovery. A graded exercise test to exhaustion (GXT) was then performed at a constant speed according to the protocol described by Leicht et al. (2013a; 2013b). Briefly, the gradient at the start of the GXT was 1.0% for all subgroups, with subsequent increases of 0.3% every minute for PARA and NON-SCI and 0.1% every 40 s for TETRA to account for the functional differences between groups and to ensure a minimum GXT duration of ~8 min (Leicht et al. 2013a; 2013b). After the GXT, participants recovered actively at a low intensity (1.2 $m\cdot s^{-1}$) at a 1.0% gradient) for 5 min. Participants then performed a verification test (VER), designed as a test to exhaustion at the same constant speed but 0.3% and 0.1% higher than the maximal gradient achieved during the GXT for NON-SCI/para and TETRA respectively (Leicht et al. 2013a; 2013b). The GXT and the VER were terminated when participants were unable to maintain the speed of the treadmill. Verbal encouragement was given throughout the test.
5.3.3 Data collection

Expired air was collected during the last minute of each submaximal stage and analysed using the Douglas bag technique. The concentration of oxygen and carbon dioxide in the expired air samples was determined using a paramagnetic oxygen analyser (Series 1400; Servomex Ltd, Sussex, UK) and an infrared carbon dioxide analyser (Series 1400; Servomex Ltd). Differentiated RPE were collected at the end of each submaximal stage as described in section 4.3.3. Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Kent, UK) and corrected to standard temperature and pressure (dry). Carbon dioxide output, VO2, VE, VE/VO2 and RER were calculated. Expired air was collected for at least the final 3 consecutive minutes of the GXT and for 2 min during the VER. BLa^- was analysed using a lactate analyser (YSI 1500 SPORT, YSI Incorporated, Yellow Springs, Ohio, USA), which was calibrated before each session using a lactate standard solution of 5 mmol·L^-1, provided by the manufacturer. A small capillary blood sample was obtained prior to exercise, following each submaximal exercise stage, immediately after the GXT and immediately after the VER. RPE_O were recorded upon termination of the VER. HR was continuously recorded at 5-s intervals using short-range radio telemetry (Polar PE 4000, Polar, Kempele, Finland). The higher of the two VO2_peak, HR_peak and peak blood lactate concentration (BLa^-peak) obtained in the GXT and the VER was taken as the peak value. A 4.9 ml blood sample was collected before (pre-exercise), immediately after the VER (post-exercise) and 30 min after exercise (post30) from an antecubital vein into a K3EDTA vacutainer. Participants were allowed to consume water ad libitum throughout the test. All participants had prior experience of the GXT and VER and were therefore familiar with the protocol.

5.3.4 Blood analyses

Blood samples were refrigerated until the final sample from each participant was collected and then spun down together in a refrigerated (4°C) centrifuge at 1500 g for 10 min. The separated plasma was then immediately stored at -80°C. Plasma concentrations of IL-6, IL-10, TNF-α, IL-1ra, cortisol and adrenaline were determined using quantitative sandwich-type enzyme-linked immunosorbant assay (ELISA) kits (IL-6, IL-10, TNF-α, IL-1ra: R&D systems, Abingdon, UK; cortisol: DRG instruments, Marburg, Germany; adrenaline: IBL international, Hamburg, Germany), according to
the manufacturers’ instructions (Appendix III). All samples were analysed in duplicate. The within assay co-efficient of variation for the analyses performed were as follows: adrenaline: 2.7%; cortisol: 1.3%; IL-6, 2.9%; IL-10: 2.5%; TNF-α: 3.9% and IL-1ra: 3.7%.

5.3.5 Statistical analysis

All data were analysed using the statistical package IBM SPSS for windows version 19 (SPSS inc, Chicago, IL). Using the data from Kouda et al. (2012) and Umemoto et al. (2011) as a foundation for our calculations we based our sample size calculation on a 2-fold increase in circulating IL-6 with a pooled SD of 80%. Using GPower 3.1.5, we calculated we would need 8 participants in each group to detect a similar change in plasma IL-6 concentration, with an effect size of 1.25, 90% power and an α of 5%.

Normal distribution of the outcome variables was confirmed for IL-1ra, TNF-α and cortisol. Data were analysed in a two factor (group x time of measurement) mixed measures ANOVA. Results for IL-6, IL-10, and adrenaline violated the assumptions of normal distribution and therefore the same statistical analysis was performed on the log-transformed data. Subsequently, where significant F-ratios were shown, separate one-way repeated measures ANOVA with tukey post-hoc tests were employed to determine changes across time within each group. Separate one-way between measures ANOVA with tukey post-hoc tests were employed to determine differences between groups at each time point. A Bonferroni adjustment was performed on the unadjusted alpha value when performing multiple comparisons. For comparisons where the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied. Pearson product moment correlation co-efficient were employed to examine the relationship between submaximal oxygen uptake and submaximal differentiated RPE. Data are presented as mean ± SD. Non parametric RPE data are presented as median and (quartiles). Significance was set a priori at p≤0.05.
5.4 Results

The peak physiological and perceived exertional responses for each group are provided in Table 5.2. Total exercise time was no different between groups (TETRA= 36±5; PARA= 35±5; NON-SCI= 38±6 min). The range of mean relative exercise intensities for TETRA, PARA and NON-SCI during the incremental submaximal exercise stages were 41%-83%, 33%-78%; 32%-81% \( \overline{\text{VO}_2}\text{peak} \) respectively. As shown in Table 5.3, a strong linear relationship was reported between all differentiated RPE and \( \overline{\text{VO}_2} \) in each group.

| Table 5.2 Peak physiological and exertional responses during wheelchair propulsion |
|---------------------------------------------|-----------------|-----------------|
| **VO\(_2\)\text{peak (L} \cdot \text{min}^{-1}\)** | TETRA | PARA | NON-SCI |
| 1.44±0.32 † | 2.85±0.87 ‡ | 3.75±0.33 |
| **HR\(_\text{peak (b} \cdot \text{min}^{-1}\)** | 127±10 † | 181±10 | 183±8 |
| **BLA\(_\text{peak (mmol} \cdot \text{L}^{-1}\)** | 5.39±0.96 † | 7.69±1.87 | 8.29±1.64 |
| **Overall RPE** | 20 (19,20) | 19 (19,20) | 20 (19,20) |
| **VE/\(\overline{\text{VO}_2}\)** | 37.7±4.9 | 36.2±3.7 | 34.1±3.5 |
| **RER** | 1.11±0.08 | 1.15±0.06 | 1.18±0.06 |
| **GXT time (min)** | 8.7±0.8 | 8.8±0.9 | 9.5±0.7 |
| **VER time (min)** | 2.0±0.0 | 1.9±0.4 | 1.9±0.3 |

**Note.** † = significant difference, TETRA vs. PARA and NON-SCI, \( p<0.05 \); ‡ = significant difference, PARA vs. NON-SCI, \( p<0.05 \).

| Table 5.3 Correlation co-efficient between submaximal differentiated RPE and submaximal oxygen uptake |
|---------------------------------------------|-----------------|-----------------|-----------------|
| **Group** | **RPE\(_P\)** | **RPE\(_C\)** | **RPE\(_O\)** |
| TETRA | 0.94 | 0.90 | 0.93 |
| PARA | 0.96 | 0.95 | 0.96 |
| NON-SCI | 0.95 | 0.91 | 0.96 |

**Note.** \( r \) = Pearson’s correlation co-efficient
A ~5-fold elevation in circulating IL-6 concentration was seen in PARA and NON-SCI at post30 (Fig 5.2a). Compared with pre-exercise values, plasma IL-6 concentrations were significantly elevated at post-exercise and post30 (trial*time interaction: \( p=0.003 \)) in PARA and NON-SCI only. In TETRA, plasma IL-6 concentrations did not change significantly from those at pre-exercise. Plasma IL-10 concentrations were unaffected by exercise in all groups (Figure. 5.2b). However, plasma IL-10 concentrations were higher in TETRA and PARA than in NON-SCI (main effect for group: \( p=0.001 \)). There were no significant interaction effects or main effects of group and time for plasma concentration of IL-1ra and TNF-\( \alpha \) (Figure 5.2c and 5.2d).

At post-exercise, plasma adrenaline concentrations increased significantly above pre-exercise values in PARA and NON-SCI only (Trial*time interaction: \( p<0.002 \) for interaction) (Figure 5.3a). Plasma adrenaline levels in PARA and NON-SCI were markedly higher than those in TETRA (main effect for group: \( p<0.001 \)), where values remained close to pre-exercise values throughout. Plasma cortisol concentration increased after exercise in all groups, with values at post-exercise and post30 (main effect for time: \( p<0.001 \)) significantly higher than at pre-exercise (Fig. 5.3b). There were no differences between groups at any time point.
Figure 5.2 Plasma cytokine responses to an acute bout of graded strenuous wheelchair exercise

**Note.** Time effects: ^ = NON-SCI and PARA significantly different from pre-exercise (p<0.05). Group effects: * = NON-SCI and PARA significantly different from TETRA (p<0.05). † = TETRA and PARA significantly different from NON-SCI (p<0.05). Data are mean ± SD.
Figure 5.3 Plasma adrenaline and cortisol responses to an acute bout of graded strenuous wheelchair exercise

Note. Time effects: ^ = All groups significantly different from pre-exercise (p<0.05). † = NON-SCI and PARA significantly different from pre and post30 exercise (p<0.05). Group effects: * = NON-SCI and PARA significantly different from TETRA (p<0.05). Data are mean ± SD.

5.5 Discussion

The present study examined the effect of SCI level on the circulating cytokine responses to an acute bout of strenuous wheelchair propulsive exercise in trained wheelchair athletes. The main finding was that those with a disrupted SNS (TETRA) demonstrated an attenuated IL-6 response to strenuous exercise compared with the ~5-fold elevation in circulating IL-6 concentrations observed at post30 in those with an intact SNS (SCI at or below T6; PARA and NON-SCI). In accordance with previous literature (Schmid et al. 1998a; Schmid et al. 1998b), both PARA and NON-SCI exhibited significant increases in plasma adrenaline concentration at post-exercise, whereas values remained close to pre-exercise in TETRA. This would indicate that, in contrast to previous suggestions, the SNS contributes to the exercise-induced plasma IL-6 response. The attenuated adrenaline, and therefore (peripheral) SNS response is in contrast to the significant activation of the HPA axis as evidenced by marked elevations in plasma cortisol concentration after exercise observed in each group. Our findings therefore support the view that the HPA axis plays only a minor role in exercise-induced elevations in plasma IL-6 (Gleeson et al. 2011).
The magnitude of the IL-6 response is dependent on exercise intensity and duration where intensity directly reflects the contractile activity within the active muscle mass (Pederson & Febbraio 2008). The increases in plasma concentrations of IL-6 observed here in PARA and NON-SCI are greater than those seen in previous work investigating the myokine response to 20 min ACE (Kouda et al. 2012) and similar to those seen following 2 h ACE (Umemoto et al. 2011). The overall magnitude of the IL-6 response in the present study may have been attenuated due to the rest period employed prior to the GXT; however, this was the same for all groups and would not affect the between groups analysis. Comparisons between cervical SCI and thoracic SCI or NON-SCI groups should acknowledge the differences in absolute workload evident when performing exercise at the same relative intensity. In this study, all participants performed exercise to exhaustion. Although absolute intensity would be lower in TETRA as a result of a lower functional capacity, this group still performed ~35 min of exercise with post-exercise RPE of 20 and post-exercise blood lactate, VE/\( \dot{V} \)O\(_2\) and RER values reflecting the demanding nature of the protocol. The HR_{peak} of ~130 b·min\(^{-1}\) (compared to values of ~180 b·min\(^{-1}\) in PARA and NON-SCI) is considered ‘maximal’ in this group and is a result of the attenuated sympathetic drive in this population, with increases in HR during exercise the result of withdrawal of parasympathetic tone (Valent et al. 2007a).

In the present study, despite performing exercise of the same relative intensity and duration, available muscle mass was lower in TETRA than PARA and NON-SCI as a consequence of their higher spinal cord lesion level. This difference in active muscle mass could therefore be argued to account for the attenuated IL-6 response. However, data from published literature suggests that even a small muscle mass is capable of releasing IL-6. Using a model of whole-body exercise Helge et al. (2011) recently reported that IL-6 release relative to lean limb mass is greater from the upper-limb compared with the lower-limb despite a lower oxygen demand and glycogen utilization during upper-limb exercise. Furthermore, it has previously been proposed that when performing the same absolute intensity, the stimulus for IL-6 release is stronger for a small muscle mass than a larger muscle mass as the force production per unit of muscle is higher (Steensberg et al. 2000). This, together with the data of Helge et al. (2011) and the maximal nature of the exercise protocol, support the attenuated IL-6 response.
observed here in TETRA cannot be attributed merely to differences in absolute workload.

The current study describes the circulating IL-6 response to exercise, although it has been shown that post-exercise increases in plasma IL-6 can be almost solely attributed to muscle IL-6 release (Steensberg et al. 2000; Toft et al. 2011). To date, evidence for the effect of disrupted SNS function on exercise-induced plasma IL-6 responses in individuals with a cervical level SCI is potentially limited by the low absolute intensity (10 W) and short duration of the exercise performed (Kouda et al. 2012). Infusion of physiological concentrations of adrenaline in vivo has been shown to induce a 6-fold increase in plasma concentrations of IL-6 in resting humans (i.e. independent of muscular contraction). However, exercise that elicited the same physiological concentrations of adrenaline resulted in a 29-fold increase in IL-6 (Steensberg et al. 2001), leading to the suggestion that any role of the SNS in the IL-6 release from muscle during exercise is only minor. In contrast, sympathectomised animals show complete attenuation of the plasma IL-6 response to exercise (Yu et al. 2001), and the comparable response observed in individuals with a high level SCI supports the involvement of the SNS in the release of IL-6 from contracting skeletal muscle.

Nevertheless, the exact mechanism whereby the SNS regulates the synthesis of IL-6 within, or release of IL-6 from, contracting skeletal muscle can only be proposed; potential roles for the SNS include the activation of myofibre IL-6 protein synthesis and/or the activation of the transporter mechanisms responsible for IL-6 release. For example, Frost et al. (2004) reported a 40-fold increase in IL-6 mRNA expression and a 15-fold increase in skeletal muscle IL-6 protein content following the infusion of adrenaline in rats at rest, with the effect blunted by infusion of α-adrenergic and a β_{1/2} -adrenergic antagonists. Furthermore, adrenaline infusion has been shown to initiate a 2-fold increase in the activity of Jun N-terminal kinase (JNK), a regulator of IL-6 mRNA expression, with this effect inhibited by the inclusion of α- and β-adrenergic receptor blockades (Napoli et al. 1998). Elsewhere contraction-mediated JNK signalling has been speculated as a primary mechanism for the increased IL-6 expression in contracting muscle over Ca^{2+} and other MAPK dependent signalling pathways (Whitham et al. 2012). Therefore a synergy between contraction induced and SNS activated signalling pathways for muscle IL-6 expression following exercise may be
considered. It may also be proposed that β-adrenoreceptor stimulated vasodilation may increase the perfusion of contracting skeletal muscle, thereby facilitating IL-6 release and the subsequent elevation in circulating concentrations in those with intact SNS activation.

In contrast to previous literature this study did not observe any post-exercise elevations in the circulating concentrations of the anti-inflammatory cytokines IL-10 or IL-1ra (Ostrowski et al. 1999; Steensberg et al. 2003). These elevations usually follow the peak in IL-6 (Pederson & Febbraio 2008). With this in mind, our findings may simply reflect the short duration of the post-exercise sampling period employed in the study protocol. Plasma IL-6 concentrations in PARA and NON-SCI were highest at the final blood sample (Post-30). Therefore, a lag in any IL-6-induced anti-inflammatory cytokine release is likely. Additional samples 1 to 2 h after exercise would have been beneficial to identify any anti-inflammatory cytokine response later in the post-exercise period.

Previous studies have shown higher resting plasma IL-6 concentrations in sedentary individuals with spinal injuries compared with those without SCI and this has been related to risk of future development of chronic long-term inflammatory conditions (Kouda et al. 2012; Umemoto et al. 2011). In contrast, in the present study, resting plasma concentrations of IL-6 and the pro-inflammatory cytokine TNF-α did not differ between the groups at rest. This may reflect the highly trained nature of the participants in this study. However, one unexpected finding from this study is the higher circulating concentrations of IL-10 in TETRA and PARA compared with NON-SCI. The principal function of IL-10 appears to be to limit and ultimately terminate inflammatory responses, and its principle source appears to be regulatory T cells (Moore et al. 2001). With this in mind, these data may suggest a strong anti-inflammatory adaptation in these athletes. On the other hand, IL-10 also has inhibitory effects on toll-like receptor expression and macrophage antigen presentation (Maynard & Weaver 2008; McCoy et al. 2010) among other inhibitory immune cell actions and thus has the potential to suppress immunity if produced in excess. A SCI is also associated with depressed immune cell functions (Campagnolo et al. 2008; Yamanaka et al. 2010), and therefore the elevated resting levels of IL-10 may be related to the increased frequency of infections experienced by those with a SCI.
5.6 Conclusion

In conclusion, the findings from this study suggest the SNS plays an important regulatory role in the circulating IL-6 response to strenuous exercise. Our findings support a role for the SNS as an important modulator of the release and/or synthesis of IL-6 from contracting skeletal muscle. This has important health implications for individuals with cervical level spinal injuries as exercise-induced elevations in plasma IL-6 have previously been related to the initiation of an anti-inflammatory environment in the hours post-exercise.
6. Plasma cytokine and perceived exertional responses to submaximal arm-crank ergometry: The effect of exercise intensity and the addition of lower-limb cycling

Chapter five provided evidence that strenuous upper-limb exercise results in a significant increase in plasma IL-6 concentrations in thoracic SCI and non-SCI participants.

The following chapter investigates the IL-6 and anti-inflammatory cytokine response to two different intensities of submaximal ACE and hybrid exercise involving upper and lower-limb exercise in non-SCI participants.
6.1 Abstract

**Purpose:** To investigate the effect of exercise intensity and the addition of lower-limb cycle ergometry on the anti-inflammatory cytokine and differentiated RPE response to submaximal ACE.

**Methods:** On three separate occasions, 12 healthy, recreationally active non-SCI males (age = 23±5 yr; body mass = 79.5±9.2 kg; \( \dot{V}O_{2\text{peak}} = 2.48±0.35 \text{ L} \cdot \text{min}^{-1} \)) performed three 30-min exercise trials in a counter-balanced order. Participants performed: 1) moderate intensity (60% \( \dot{V}O_{2\text{peak}} \)) ACE only (M-ACE); 2) light intensity (40% \( \dot{V}O_{2\text{peak}} \)) ACE only (L-ACE); and 3) light intensity ACE with the addition of lower-limb cycling (HYB) to match total PO in M-ACE. Differentiated RPE were recorded throughout exercise. Blood samples were collected at rest, immediately post-exercise, and 1 and 2 h post-exercise. Plasma concentrations of IL-6, IL-10, IL-1ra, adrenaline and cortisol were determined by enzyme linked immunoassay.

**Results:** Plasma IL-6 concentrations were significantly \((p<0.05)\) elevated (~1.5-fold) immediately post and 1 h post-exercise following all trials. However, plasma IL-6 concentrations 2 h post-exercise in M-ACE were significantly \((p<0.05)\) higher than in L-ACE and HYB, showing a 3-fold elevation above plasma concentrations at rest. The greater elevation in plasma IL-6 observed 2 h post M-ACE was associated with a significant \((p<0.05)\) elevation in plasma concentrations of the anti-inflammatory cytokine IL-1ra. No response was seen in IL-10, cortisol and adrenaline for any trial. All differentiated RPE (RPE\(_P\), RPE\(_C\) and RPE\(_O\)) were significantly \((p<0.05)\) higher during M-ACE than HYB, and higher in HYB than L-ACE. RPE\(_P\) was greater than RPE\(_C\) and RPE\(_O\) throughout each trial.

**Conclusion:** The current findings suggest 30 min moderate intensity ACE alone can initiate an anti-inflammatory cytokine response independent of sympathetic nervous system activation in non-SCI participants. A longer duration of light intensity ACE or hybrid exercise may result in a greater plasma IL-6 response and in turn, an anti-inflammatory cytokine response. As evidenced during submaximal wheelchair ergometry (Chapter four), RPE\(_P\) is the dominant perceptual signal in undifferentiated RPE during light and moderate intensity ACE.
6.2 Introduction

Contracting skeletal muscle releases the myokine IL-6 in a dose and time-dependent manner (Peake et al. 2004; Scott et al. 2011; Starkie et al. 2001; Toft et al. 2011). Elevated plasma IL-6 concentrations are associated with an elevation in circulating concentrations of the inflammation-suppressing cytokines IL-1ra, IL-10 and the soluble TNF receptor (Ostrowski et al. 1999; Peake et al. 2005; Scott et al. 2011). Experimental evidence from lower-limb exercise suggests an exercise intensity of 65-75% \( \text{VO}_{2\text{peak}} \) must be achieved to initiate elevations in plasma IL-1ra and IL-10 concentrations (Peake et al. 2004; Peake et al. 2005; Scott et al. 2011). This transient anti-inflammatory environment may be partly responsible for the positive effects of regular exercise in reducing adipocyte driven chronic low-grade inflammation (Gleeson et al. 2011).

Following a motor complete SCI, participation in ADL and physical exercise are dependent predominantly on the small muscle mass of the arms and shoulders (Figoni 2009; Janssen et al. 1994). Exercise prescription (mode and dose) must prevent over-use of the upper-limbs whilst achieving exercise intensities sufficient to maintain or improve physical capacity and cardiovascular health (Figoni 2009; Woude et al. 2001). Substantial elevations in plasma IL-6 concentrations have been observed following 2 h ACE at 60% \( \text{VO}_{2\text{peak}} \) (~6-fold) and 40 min strenuous, intermittent wheelchair propulsion (~5-fold) in trained thoracic SCI and non-SCI participant groups (Chapter five; Umemoto et al. 2011). Of interest is the anti-inflammatory cytokine responses to upper-limb exercise at an intensity and duration that is consistent with current guidelines for exercise prescription in SCI populations (>20 min moderate intensity, 3 x week) (Figoni 2009; Ginis et al. 2011). As described in the previous chapter, Kouda et al. (2012) observed a 1-fold elevation in plasma IL-6 concentrations following 20 min ACE at 60% \( \text{VO}_{2\text{peak}} \) in non-SCI participants only. No work to date has examined the anti-inflammatory cytokine response to moderate intensity upper-limb exercise (exercising at an RPE of 12-15) in non-SCI or thoracic level SCI populations.

Both intracellular (e.g. mechanical stimuli, cytosolic calcium concentrations and the depletion of glycogen stores within contracting myofibres) and extracellular (SNS activation) signalling mechanisms are related to the magnitude of IL-6 signalling and release following acute exercise (Chan et al. 2004; Keller et al. 2006; Scott et al. 2011;
Welc & Clanton 2013; Whitham et al. 2012). Previously, Markovitch et al. (2008) reported no myokine or anti-inflammatory cytokine response to 30 min walking exercise at 50% \( \text{VO}_2\text{peak} \). In contrast, a greater IL-6 release was observed from the upper than from the lower-limb during submaximal exercise despite a lower \( \text{VO}_2 \) (Helge et al. 2011). Higher carbohydrate (CHO) metabolism and \( \text{BLA}^- \) are frequently observed in upper-limb exercise versus lower-limb exercise of a comparable absolute and relative exercise intensity (Ahlborg & Jensen-Urstad 1991; Kang et al. 1997). Untrained muscles of the upper-limb also exhibit an impaired capacity for oxygen extraction (Calbet et al. 2005) and oxidative metabolism (Killerich et al. 2008). The greater force production per unit area observed in contractions in smaller muscle groups is suggested to augment the calcium stimulus for IL-6 production/release (Steenberg et al. 2000). However, the catecholamine response to exercise usually requires an exercise intensity of ~60 to 70% \( \text{VO}_2\text{peak} \). Whether the IL-6 response to light intensity upper-limb exercise can initiate an anti-inflammatory response requires investigation. This would have important implications for wheelchair propulsion and ACE, as light to moderate exercise intensities are typically prescribed during the acute stages of SCI rehabilitation to limit upper-extremity over-use (de Groot et al. 2003). Low intensity wheelchair training also has positive effects on physical capacity (van den Berg et al. 2010b) and wheelchair propulsion technique (de Groot et al. 2008).

The addition of lower-limb FES-evoked contractions to voluntary upper-limb ACE in persons with a SCI has been shown to increase \( \text{VO}_2 \) and cardiorespiratory stress compared to arm-exercise alone (Hasnan et al. 2013; Hettinga & Andrews 2008). Elsewhere, prolonged low intensity, whole body exercise has been shown to improve metabolic health independent of aerobic fitness in non-SCI populations (Helge et al. 2010). Additional lower-limb contractions performed concurrently with upper-limb exercise may confer a greater total calcium stimulus and therefore cytokine response than ACE alone.

The primary aim of this study was to compare the plasma IL-6 and anti-inflammatory cytokine (IL-1ra and IL-10) response to 30 min: 1) moderate intensity (60% \( \text{VO}_2\text{peak} \) ACE; 2) light intensity (40% \( \text{VO}_2\text{peak} \)) ACE; and 3) light intensity (40% \( \text{VO}_2\text{peak} \)) ACE with the addition of lower-limb cycling to match the total PO in the moderate intensity ACE trial (Figure 6.1). It was hypothesised that 30 min moderate intensity ACE would result in a significant elevation in plasma IL-6 and anti-
inflammatory cytokine (IL-1ra and IL-10) concentrations. Despite the lower relative exercise intensity, it was also hypothesised that the same duration light ACE would result in a significant IL-6 response albeit of a smaller magnitude, and that this response would be augmented by the addition of lower-limb cycling. A secondary aim was to compare the differentiated RPE between the three trials. Due to the peripheral limitations experienced during upper-limb exercise, it was hypothesised that RPE_p would be the dominant perceptual signal in all trials. Further, the addition of lower-limb cycling to concurrent ACE would increase oxygen uptake compared to light intensity ACE alone but not affect the differentiated RPE response.

**Figure 6.1** Example of the experimental protocol

### 6.3 Methods

#### 6.3.1 Participants

Twelve recreationally active, non-SCI males (age = 23±5 yr; body mass = 79.5±9.2 kg; $\dot{V}O_{2\text{peak}} = 2.48\pm0.35$ L·min$^{-1}$) volunteered to participate in the study. Procedures for the current investigation were approved by the University’s Ethical Committee and performed in accordance with the Declaration of Helsinki. All
participants provided written informed consent before testing commenced. Participants were moderately physically active (>30 min, 3 d/wk) but not specifically upper-limb trained.

6.3.2 Experimental design

The study utilised a counter-balanced, repeated measures design with participants performing four exercise sessions. During the first visit, participants completed a submaximal incremental test and a graded exercise test to exhaustion to determine $\text{VO}_2\text{peak}$ on an arm-crank ergometer. On three separate occasions at the same time of day, each participant performed 30 min light intensity ACE, moderate intensity ACE and hybrid exercise (light ACE plus lower-limb cycling). Main trials were separated by at least 7 d. All trials were completed within 28 d.

6.3.3 Instrumentation

All exercise trials were performed on electric-magnetically braked ergometers designed for upper and lower-limb exercise (Appendix I) (Lode, Lode B.V. Medical Technology, Groningen, The Netherlands). The Borg 6-20 scale was used to determine participants differentiated RPE ($\text{RPE}_P$; $\text{RPE}_C$; $\text{RPE}_O$) throughout all trials as described in section 4.3.3.

6.3.4 Preliminary measures

On arrival at the laboratory, body mass was measured to the nearest 0.1 kg using double-beam seated scales (Marsden MPWS-300, Henley-on-Thames, UK). Participants rested in a seated position for 15 min before resting $\text{VO}_2$ was estimated for 10 min using online respiratory gas analysis via a breath-by-breath system (Cortex metalyser 3B, Cortex, Leipzig, Germany). Subsequently, participants positioned themselves on the exercise bike and seat height was adjusted to allow lower-limb cycle ergometry (LCE) with a slight flexion of the knee (Figure 6.1). ACE ergometer height was adjusted to ensure crank axis level with sternum and distance allowing flexion of the elbow during cycling (Figure 6.1). Ergometer setup was standardised between trials. Following a standardised warm-up of 20 W for 5 min, participants performed an incremental ACE only exercise test consisting of five 4-min constant load exercise stages at ascending POs, intended to elicit physiological responses covering a range from 40% to 80% $\text{VO}_2\text{peak}$. Initial POs were 20±5 W with subsequent velocity
increments of 20 W. Crank rate was maintained between 70-80 rpm. HR was monitored continuously using radio telemetry (Polar PE 4000, Kempele, Finland). On-line respiratory gas analysis was carried out throughout each 4-min stage via a breath-by-breath system (Cortex metalyser 3B, Cortex, Leipzig, Germany). Before each test, gases were calibrated according to the manufacturer’s recommendations using a 2-point calibration (O2 = 17.0 %, CO2 = 5.0 % against room air) and volumes with a 3-L syringe at flow rates of 0.5–3.0 L·s⁻¹. The average respiratory data from the last 1-min of each stage was used to provide information of VO₂. A small capillary blood sample was obtained from the earlobe at the start of the test and during a 1-min break between stages to determine BLa¯ using a YSI 1500 SPORT Lactate Analyser (YSI Inc, Yellow Springs, OH). Differentiated RPE were recorded in the last 15 s of each 4-min stage while the participant was still exercising.

After a 15-min rest period, a graded exercise test to exhaustion was performed to determine VO₂peak. The test involved increments of 10 W every minute from an initial PO of 40±9 W at a freely chosen crank rate above 60 rpm until volitional exhaustion. Expired air and HR were measured continuously throughout the test and the final differentiated RPE was recorded as previously described. Breath-by-breath data allowed the highest 30 s rolling average VO₂ value recorded during the exercise test to be taken as the VO₂peak. For each participant a simple linear regression analysis was performed using the linear workload-VO₂ relationship. The regression line created from the paired submaximal velocity and VO₂ data was employed to interpolate individual PO corresponding to a ‘light’ exercise intensity (40% VO₂peak) and a ‘moderate’ exercise intensity of (60% VO₂peak).

6.3.5 Main experimental trials

40% VO₂peak ACE only (L-ACE) and 60% VO₂peak ACE only (M-ACE)

A standardised 5-min upper-limb warm up was performed prior to all main trials as previously described. The ergometer was then set at the imposed PO and participants were asked to maintain a cadence of 70-80 rpm for 30 min. VO₂ and HR were measured constantly during each bout and averaged over the final minute. Differentiated RPE were recorded and BLa¯ was determined every 10 min.
**40% \( \dot{V}O_2 \text{peak} \) ACE plus \( \Delta \) LCE (HYB)**

Participants performed a 5-min LCE warm-up at an intensity of 20 W concurrent with the standardised ACE warm-up. Both ergometers were set at the imposed PO and participants were asked to maintain a cadence of 70-80 rpm for ACE and 80-90 rpm for LCE for the 30 min bout. The difference (\( \Delta \)) in PO between the L-ACE and M-ACE was calculated for each participant. This \( \Delta \) PO was the imposed intensity of lower-limb cycling added to concurrent light intensity ACE during HYB. Total PO was therefore matched between M-ACE and HYB. \( \dot{V}O_2 \), HR, differentiated RPE and BLa\(^-\) were determined in accordance with L-ACE and M-ACE. In addition, RPE\( \text{p} \) for the lower-limb were recorded during HYB.

6.3.6 Blood analyses

A 7.5 ml blood sample was collected before (pre-exercise), immediately after exercise (post-exercise), 1 h post-exercise (post+1) and 2 h post exercise (post+2) from an antecubital vein into a K\(_3\)EDTA vacutainer. Blood samples were treated and plasma analysis performed as described in section 5.3.4. The within assay co-efficient of variation for the analyses performed were as follows: adrenaline: 3.0%; cortisol: 2.5%; IL-6: 5.4%; IL-10: 5.0% and IL-1ra: 6.2%.

6.3.7 Statistical analysis

All data were analysed using the statistical package IBM SPSS for windows version 20 (SPSS inc, Chicago, IL). Normal distribution of the outcome variables was confirmed for all data. Non-parametric Friedman tests and Wilcoxon signed-rank tests were used to analyse differences in ordinal differentiated RPE data within each trial. Non-parametric Kruskal-Wallis H tests and Mann-Whitney-U tests were used to analyse differences in ordinal differentiated RPE data between each trial. Plasma cytokine and stress hormone data were analysed in a two factor (group x time of measurement) mixed measures ANOVA. Subsequently where significant F-ratios were shown, separate one-way repeated measures ANOVA with tukey post-hoc tests were employed to determine changes across time within each group. Separate paired students t-tests were employed to determine differences between groups at each time point. A Bonferroni adjustment was performed on the unadjusted alpha value when performing multiple comparisons. For comparisons where the assumption of sphericity was
violated, a Greenhouse-Geisser correction was applied. All physiology data were analysed by paired students t-tests. Data are presented as mean ± SD. Non-parametric RPE data are presented as median and (quartiles). Significance was set a priori at 
p≤0.05.

6.4 Results

A comparison of the physiological responses across the exercise intensities and modalities of the main trials is provided in Table 6.1. The mean intensity for the lower-limb cycling component of HYB was 32±5 W. Following student dependent t-tests, all variables except RER were found to be significantly higher for M-ACE than L-ACE. In addition, HR, %HRpeak and BLa⁻ were significantly higher in M-ACE than HYB, with all HR values higher in HYB than L-ACE.

Table 6.1 Physiological responses to 30 min L-ACE, M-ACE and HYB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L-ACE</th>
<th>M-ACE</th>
<th>HYB</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO (W)</td>
<td>38±11 †</td>
<td>70±16</td>
<td>70±16</td>
</tr>
<tr>
<td>VO₂ (L·min⁻¹)</td>
<td>0.96 ± 0.16 †</td>
<td>1.50 ± 0.26</td>
<td>1.56 ± 0.31</td>
</tr>
<tr>
<td>%VO₂ peak (ACE)</td>
<td>40 ± 3 †</td>
<td>62 ± 7</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>HR (b·min⁻¹)</td>
<td>109 ± 14 †</td>
<td>139 ± 10</td>
<td>127 ± 16 ‡</td>
</tr>
<tr>
<td>%HR peak</td>
<td>60 ± 8 †</td>
<td>77 ± 8</td>
<td>70 ± 9 ‡</td>
</tr>
<tr>
<td>RER</td>
<td>0.96 ± 0.05</td>
<td>1.01 ± 0.06</td>
<td>0.94 ± 0.05</td>
</tr>
<tr>
<td>BLa⁻ (mmol·l⁻¹)</td>
<td>1.66 ± 0.83 ‡</td>
<td>3.05 ± 1.37</td>
<td>1.59 ± 0.54 ‡</td>
</tr>
</tbody>
</table>

Note. † = Significantly different from M-ACE and HYB; ‡ = significantly different from M-ACE. Data are mean ± SD.
The differentiated RPE responses for all main trials are shown in Figure 6.2. RPE\textsubscript{P} was significantly ($p<0.04$) higher than RPE\textsubscript{C} and RPE\textsubscript{O} at each time point, within each trial. In addition, RPE\textsubscript{O} was significantly higher than RPE\textsubscript{C} at each time point in M-ACE and at 20 and 30 min in L-ACE. An effect of time on differentiated RPE was present in L-ACE only, with RPE\textsubscript{P} significantly different between each time point and RPE\textsubscript{C} at 10 min significantly lower than at 20 and 30 min. All differentiated RPE were significantly higher in M-ACE than HYB, with HYB higher than L-ACE at each time point.

Following a two factor mixed measures ANOVA, plasma IL-6 concentrations showed a significant main effect for time ($p=0.02$) and time\textsuperscript{*}trial interaction ($p=0.045$). Post-hoc analysis showed plasma IL-6 concentrations were significantly ($p<0.05$) elevated immediately post-exercise (~1-fold) and at post+1 (~1.5-fold) in each trial. At post+2, plasma IL-6 concentrations in M-ACE were significantly elevated above rest (3-fold) ($p=0.03$) and significantly higher than L-ACE and HYB ($p<0.05$) (Figure 6.3\textit{a}). A significant main effect for time ($p=0.01$) and time\textsuperscript{*}trial interaction ($p=0.02$) were also present for plasma IL-1ra concentrations. Plasma IL-1ra was unaffected by exercise in L-ACE and HYB. In contrast, a significant elevation was seen at post+2 in M-ACE (1-fold) with values significantly higher than at pre-exercise ($p=0.005$) (Figure 6.3\textit{b}). At post+2, plasma IL-1ra were also significantly higher than L-ACE ($p=0.03$) but not HYB ($p=0.51$). No significant main effects were observed for plasma IL-10 concentrations (Figure 6.2\textit{c}).

A significant effect for time ($p<0.001$) but non-significant trial\textsuperscript{*}time interaction ($p>0.05$) were present for plasma cortisol concentrations. In all trials, plasma cortisol concentrations were significantly lower at post+1 and post+2 than at pre-exercise ($p<0.005$) (Figure 6.4\textit{a}). No significant main effects were observed for plasma adrenaline concentrations (Figure 6.4\textit{b}).
Table 6.2 Differentiated RPE responses during 30 min L-ACE, M-ACE and HYB

<table>
<thead>
<tr>
<th></th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPE(_P)</td>
<td>RPE(_C)</td>
<td>RPE(_O)</td>
</tr>
<tr>
<td>L-ACE</td>
<td>10 (8,12)(^\dagger)</td>
<td>9 (7,12)</td>
<td>9 (8,9)</td>
</tr>
<tr>
<td>M-ACE</td>
<td>13 (12,16)(^\dagger^*)</td>
<td>12 (10,13)(^*)</td>
<td>13 (11,15)(^\dagger^*)</td>
</tr>
<tr>
<td>HYB</td>
<td>12 (11,13)(^\dagger^\wedge)</td>
<td>11 (9,12)(^\wedge)</td>
<td>11 (10,13)(^\wedge)</td>
</tr>
</tbody>
</table>

Note. \(^\dagger\) = RPE\(_P\) significantly different from RPE\(_O\) and RPE\(_C\) within time and trial; \(^\dagger^*\) = RPE\(_O\) significantly different from RPE\(_C\) within time and trial; \(^*\) = M-ACE significantly different from HYB within time and RPE; \(^\wedge\) = HYB significantly different from L-ACE within time and RPE. (\(p<0.05\)).
Figure 6.2 Plasma cytokine responses to 30 min L-ACE, M-ACE and HYB

Note. Time effects: † = All groups significantly higher than pre-exercise; ‡ = M-ACE significantly higher than pre-exercise. Group effects: Σ = M-ACE significantly higher than L-ACE and HYB; μ = M-ACE significantly higher than L-ACE, (p<0.05).
Figure 6.3 Plasma adrenaline and cortisol responses to 30 min L-ACE, M-ACE and HYB

Note. Time effects: † = all groups significantly lower than pre-exercise ($p<0.05$)

6.5 Discussion

This study investigated the effect of exercise intensity and the addition of lower-limb cycling (32±5 W) on plasma cytokine and differentiated RPE responses to 30 min submaximal ACE. A small (~1.5-fold) increase was observed in plasma IL-6 concentrations immediately post and 1 h post-exercise in each trial. Importantly, 30 min M-ACE resulted in a significantly greater elevation in plasma IL-6 concentrations (3-fold) 2 h post-exercise compared to L-ACE and HYB. The greater IL-6 response was associated with a significant increase in plasma concentrations of IL-1ra 2 h post exercise following M-ACE. Plasma IL-1ra concentrations remained unchanged following L-ACE and HYB. RPE$_P$ was the dominant perceptual signal throughout each trial independent of intensity or mode. Interestingly, the addition of lower-limb cycling resulted in higher RPE$_P$ and RPE$_C$ compared to L-ACE only.

Exercise intensity and the anti-inflammatory cytokine response

The transient elevation in plasma anti-inflammatory cytokine (IL-1ra and IL-10) concentrations is one mechanism whereby acute exercise may exert a down-regulatory effect on adipose-driven, chronic low-grade inflammation (Gleeson et al. 2011). Contraction-induced elevations in the myokine IL-6 are considered the primary initiator of this anti-inflammatory response, with contributions from SNS and HPA-axis
activation at vigorous exercise intensities (Gleeson et al. 2011). To date, the anti-inflammatory cytokine response to upper-limb exercise has received little attention. Experimental work employing long duration (>2.5 h), vigorous running exercise (Ostrowski et al. 1999) or infusion of IL-6 to supraphysiologic (140 pg·ml⁻¹) concentrations (Steensberg et al. 2003) have observed the greatest elevations (>30-fold) in plasma IL-1ra and IL-10. In contrast, more modest plasma IL-1ra (150-200 pg·ml⁻¹ & 200-250 pg·ml⁻¹) plasma IL-6 responses (1.5-4 pg·ml⁻¹ & 6-8 pg·ml⁻¹) have been observed following 60 min moderate (60-65% VO₂peak) and vigorous intensity (75-80% VO₂peak) treadmill running, respectively (Peake et al. 2004; Scott et al. 2011). Elevated plasma IL-10 concentrations (15 pg·ml⁻¹) were observed following vigorous intensity but not moderate intensity exercise (Peake et al. 2005). Subsequently a threshold of 65-75% VO₂peak has been proposed to attain the greatest anti-inflammatory cytokine response following acute exercise (Scott et al. 2011). The present findings suggest 30 min ACE at 60% VO₂peak is sufficient to initiate an IL-1ra response in accordance with previous lower-limb exercise studies. This plasma IL-1ra response was present despite a smaller absolute plasma IL-6 concentration and independent of plasma SNS and HPA-axis activation. In accordance with Peake et al. (2005), no IL-10 response was observed following moderate intensity exercise.

The myokine IL-6 is proposed as a stress-sensing hormone with paracrine and endocrine functions, including the stimulation of fat metabolism in skeletal muscle (Welc & Clanton 2013; Wolsk et al. 2010). As outlined in Chapter five, the production and release of IL-6 is responsive to a range of intracellular (mechanical stimuli, calcium concentration; glycogen depletion) and extracellular (SNS activation) dependent signalling pathways. Higher CHO metabolism and BLA® are frequently reported during upper-limb versus lower-limb exercise (Ahlborg & Jensen-Urstad 1991; Helge et al. 2010). The generation of force per unit area of muscle is also considered to be higher in small muscle groups (Steensberg et al. 2000). Hence, at an equivalent PO, upper-limb exercise may provide a greater stimulus for IL-6 release. Helge et al. (2011) observed a higher release of IL-6 from upper-limb versus lower-limb during submaximal whole body exercise despite a lower VO₂. Further, plasma IL-6 was unchanged following 40 min low intensity LCE (~80 W) (Mendham et al. 2011). In contrast, 30 min ACE at an equivalent PO in the present study resulted in a 3-fold elevation in plasma IL-6 concentration and a 1-fold elevation in plasma IL-1ra concentrations.
Exercise of a light intensity is typically prescribed during the acute stages of SCI rehabilitation to limit peripheral fatigue and the risk of upper-limb over-use injury (de Groot et al. 2003; de Groot et al. 2008). In support of our hypothesis, L-ACE resulted in a significant yet small IL-6 response (1.5-fold) but no anti-inflammatory cytokine response. During HYB, the total PO of lower-limb cycling (32±5 W) was higher than the achievable PO previously observed during FES-evoked exercise in persons with a SCI (Hunt et al. 2007). The addition of low intensity lower-limb cycling resulted in a significant elevation in VO₂ and HR above L-ACE despite the low absolute PO. These findings have important implications for elderly, overweight and osteoarthritic populations who may benefit from elevating cardiorespiratory stress whilst minimising strain on the exercising joints. Despite the elevation in cardiovascular stress, no increase in the cytokine or stress hormone response was observed in HYB compared to L-ACE alone. These findings are in accordance with previous work exploring low intensity lower-limb exercise in non-SCI participants (Markovitch et al. 2008; Mendham et al. 2008). However, the voluntary recruitment of mainly slow-twitch muscle during low intensity LCE results in the oxidation of fat as the primary energy source (Helge et al. 2010). This contrasts significantly to the recruitment of fast twitch, highly fatigable type 11 fibres during involuntary FES-evoked contractions, which rely heavily on glycolytic energy production pathways (Hunt et al. 2007; Martin et al. 2012). Whether FES-evoked exercise may provide a sufficient stimulus to initiate an IL-6 response in paralysed skeletal muscle and augment the anti-inflammatory cytokine response observed during upper-limb exercise requires investigation.

An interesting finding from the current study was the lack of difference in IL-6 response immediately post and 1 h post exercise between all trials. Previously Scott et al. (2011) compared the cytokine response to exercise at 55%, 65% and 75% VO₂peak, and observed no effect of exercise intensity on the magnitude of the IL-6 response after 20 or 30 min of exercise. Along with exercise intensity, exercise duration can also modulate the cytokine response to acute exercise. Therefore a longer duration of L-ACE and HYB may result in an IL-6 response of similar magnitude to M-ACE and also result in IL-1ra response independent of SNS activation.

As a naturally occurring antagonist to pro-inflammatory signalling of IL-1α and IL-1β, plasma IL-1ra is an important modulator of systemic inflammation and innate immune responses (Dinarello et al. 2011). IL-1β is associated with impaired insulin
secretion in pancreatic β–cell and the development of type 2 diabetes (Banerjee & Saxena 2013). Exogenous IL-1ra supplementation improves insulin secretion and glycaemic control (Larsen et al. 2009). To date, the absolute concentration of IL-6 required for the initiation of an IL-1ra response in vivo following exercise remains unknown. However, the findings from the current study suggest an elevation in plasma IL-6 concentrations to around 1 pg·ml\(^{-1}\) may be sufficient to initiate an IL-1ra response. IL-10 is a potent modulator of both adaptive and innate immunity (Maynard & Weaver 2008). In contrast to IL-1ra, vigorous intensity exercise is required to elevate plasma IL-10 concentrations (Peake et al. 2005). The SNS is a known modulator of IL-10 secretion in vitro (Platzer et al. 2000). Therefore, elevations in plasma IL-10 following exercise may be more closely related to SNS activation rather than acute IL-6 response.

Previously 30 min moderate intensity (50-60% \(\dot{V}O_{2\text{peak}}\)) walking has been found to elicit little effect on pro or anti-inflammatory cytokine concentrations (Markovitch et al. 2008), yet 12 weeks structured training at the same intensity resulted in improved resting low-grade inflammatory state (Thompson et al. 2010). Chronic low-grade inflammation shows an inverse relationship with cardiovascular disease risk (Libby & Ridker 2004) and physical activity and fitness levels (Buchholz et al. 2009; Shanley et al. 2013). Reductions in low-grade inflammatory biomarkers, despite only small increases in physical activity levels, suggest other anti-inflammatory mechanisms may be responsive to exercise, independent of exercise intensity (Gleeson et al. 2011). Short-term aerobic and resistance exercise training results in a reduction in the concentration of circulating inflammatory monocytes (Timmerman et al. 2008). Further, higher levels of physical activity are associated with a lower monocyte TNF-α response to bacterial stimulation and a lower monocyte toll-like receptor expression (Timmerman et al. 2008). Regular physical activity may also prevent adipose tissue macrophage infiltration and reverse macrophage differentiation from inflammatory to anti-inflammatory phenotypes (Kawanashi et al. 2010). Well controlled research is required to understand changes in monocyte and macrophage phenotypes in response to long-term upper-limb exercise training.

Cortisol is a stress hormone secreted from the adrenal cortex in response to HPA axis activation (Derr et al. 2006). Typically, exercise intensities of >60% \(\dot{V}O_{2\text{peak}}\) are required to initiate a HPA axis response (Maresh et al. 2006). In contrast to the significant increases observed in Chapter five, plasma cortisol concentrations
significantly decreased from pre and post exercise to 2 h post exercise in the present study. A predictable diurnal variation in cortisol secretion exists, characterised by a peak upon wakening in the early morning and a nadir in the late afternoon (Derr et al. 2006). The resting cortisol concentrations observed in the present study (600 nmol-L) therefore reflect the timing of the pre-exercise samples, with participants reporting to the lab in the early morning (between 0730 & 0830) following an overnight fast. This contrasts to the pre-exercise concentrations observed in chapter five (300 nmol-L), where samples were taken in the later morning or early afternoon. The higher resting concentrations and subsequent decline in all trials may therefore have masked any effect of exercise intensity or exercise modality on plasma cortisol responses.

Differentiated RPE during ACE and hybrid exercise

RPE_p has been found to be the dominant perceptual signal during submaximal wheelchair propulsion in novice and experienced users (Chapter four; Lenton et al. 2008). Differentiated RPE therefore provides a more accurate indicator of peripheral strain and fatigue during wheelchair propulsion exercise than undifferentiated RPE alone. During a graded exercise test to exhaustion, Al-Rahamneh et al. (2011) observed significantly higher RPE_p than RPE_C during ACE. To date little is known about the differentiated RPE response to submaximal ACE. As in Chapter four, RPE_p was the dominant perceptual signal during both light and moderate intensity ACE. RPE_p was higher than both RPE_C and RPE_O throughout each trial. This has important implications for monitoring the intensity of ACE, as using RPE_O alone may underestimate the perception of effort during submaximal and maximal exercise.

An interesting finding from the current work was the differentiated RPE responses to HYB. RPE_C was shown to be sensitive to cardiovascular adjustments of increased HR compared to L-ACE alone. In addition, the performance of L-ACE with concurrent lower-limb cycling in the present study resulted in a significantly higher RPE_p response than performing L-ACE alone. During high intensity, whole body exercise vasodilation in the active muscle mass exceeds the ability to augment $\dot{Q}$ (Calbet et al. 2004). Subsequently, blood pressure is maintained by vasoconstriction within the exercising muscle mass and a reduced blood flow to the upper-limb (Calbet et al. 2004). In contrast, Goodman et al. (2007) observed no cardiovascular adjustments when only low intensity lower-limb cycling (20-30% $\dot{V}O_{2peak}$) was added to concurrent
submaximal ACE. Therefore, the greater $RPE_P$ observed in HYB than L-ACE may indicate the stabilisation of the upper-limb and trunk during the concurrent exercise task rather than vasoconstriction induced reduction in peripheral blood flow.

### 6.6 Conclusion

In conclusion, moderate intensity ACE resulted in a significant IL-6 and IL-1ra response in non-SCI participants. The IL-1ra response occurred independent of SNS activation. Both light intensity ACE and hybrid exercise resulted in a small, significant elevation in plasma IL-6 but not plasma IL-1ra concentrations. A longer duration of L-ACE or HYB exercise may result in a greater elevation in IL-6 and a subsequent anti-inflammatory cytokine response. In accordance with Chapter four, $RPE_P$ was the dominant perceptual signal during submaximal ACE. Differentiated RPE should be employed when using subjective perceived exertion to monitor the intensity of ACE.
7. Exercise mode and the circulating cytokine response in persons with a thoracic spinal cord injury: A feasibility study comparing hand cycling and hybrid exercise

This chapter has been accepted for publication in a modified form in the Journal of Rehabilitation Research & Development:


In Chapter six, 30 min moderate intensity ACE but not hybrid exercise resulted in a significant anti-inflammatory cytokine (IL-1ra) response in non-SCI participants.

The following chapter investigates: 1) whether the same anti-inflammatory response will be observed following 30 min hand cycling of the same relative intensity in untrained participants with a thoracic level SCI, and 2) whether concurrent FES-evoked lower-limb cycling can augment the anti-inflammatory cytokine response to upper-limb exercise alone.
7.1 Abstract

**Purpose:** To compare the plasma response of inflammation-mediating cytokines to acute hand cycling exercise with (HYB) and without (HC) the addition of FES-evoked lower-limb cycling in persons with a thoracic level SCI.

**Methods:** Five community-based individuals with motor-complete SCI (4=male and 1=female; Age=44±15 years; Body mass=66.6±14.3 kg; ASIA impairment scale A) and at least 3 months FES-evoked cycling experience volunteered to participate. On separate occasions, participants performed 30 min HYB and HC at a fixed workload (60% PO\textsubscript{peak} obtained during HC only). Blood samples were collected at rest, immediately post-exercise, and 1 and 2 h post-exercise. Plasma concentrations of IL-6, IL-10, IL-1ra, adrenaline and cortisol were determined by enzyme linked immunoassay.

**Results:** Plasma IL-6 concentrations were significantly ($p<0.04$) elevated (~2.5-fold) 1 h and 2 h post-exercise following HYB. A small (0.5-fold) non-significant increase in IL-6 was observed following HC, with concentrations significantly higher in HYB 2 h post-exercise ($p<0.02$). Although not statistically significant ($p=0.15$), a ~1-fold increase in IL-10 concentration was seen in HYB 2 h post exercise. Plasma IL-1ra was unaffected by exercise in both trials. During both trials, increases in adrenaline ($p<0.04$) and cortisol ($p=0.08$) were observed immediately post-exercise.

**Conclusion:** Initial findings from this feasibility study suggest paralysed skeletal muscle releases the cytokine IL-6 in response to electrically evoked contractions. Hybrid exercise may offer a method of maximising the anti-inflammatory potential of acute exercise in individuals with a thoracic level SCI. The current findings require verification in a larger cohort.
7.2 Introduction

Lower-limb paralysis and immobilisation following a SCI result in an increase in adiposity and atrophy of skeletal muscle (Goosey-Tolfrey & Sutton 2012). Persons with a SCI are exposed to an elevation in risk factors for chronic disease including; altered metabolic regulation, physical inactivity and chronic inflammation (Cowan & Nash 2010). Cardiovascular mortality rates are therefore higher than those for non-SCI groups, with the onset of disease occurring earlier in life (Cowan & Nash 2010). Following release from contracting skeletal muscle, the myokine IL-6 is associated with subsequent elevations in plasma concentrations of the anti-inflammatory cytokines IL-1ra, IL-10 and the soluble TNF receptors (Pederson 2012; Scott et al. 2011). It is proposed that this transient anti-inflammatory environment may contribute to the down-regulation of pro-inflammatory pathways driving the development of insulin resistance and atherosclerotic plaque (Gleeson et al. 2011).

In non-SCI and thoracic level SCI groups, elevations in plasma IL-6 concentrations have been observed following moderate intensity, submaximal ACE (Kouda et al. 2012; Umemoto et al. 2011), wheelchair basketball performance (Kinoshita et al. 2013) and maximal, treadmill based wheelchair propulsion (Chapter five). In Chapter six, a significant anti-inflammatory cytokine response (IL-1ra) was observed in response to 30 min moderate intensity ACE in non-SCI participants (60% VO₂peak). The relatively low physical strain, range of accessible equipment and beneficial effects on physical capacity make hand cycling a popular exercise mode for rehabilitation practitioners and sports competitors alike (Arnet et al. 2012; Hettinga et al. 2010). No work to date has examined the circulating plasma cytokine response to acute hand cycling exercise.

The application of electrical currents to restore some functional movement, including standing and cycling, in paralysed skeletal muscle is termed functional electrical stimulation (Ragnarsson 2008). Intensive FES-evoked cycling and resistance training improves metabolic health while increasing the size and strength of lower limb lean body mass (Martin et al. 2012; Ragnarsson 2008). The addition of FES-evoked cycling to concurrent upper-limb exercise, termed ‘hybrid’ exercise, has been shown to elicit greater cardiorespiratory stress compared to arm exercise alone (Hasnan et al. 2013). Via the activation of a larger muscle mass, hybrid exercise may be more
effective in reducing the risk of chronic diseases including CVD and type 2 diabetes (Bakkum et al. 2012).

To the authors’ knowledge, no previous work has examined the IL-6 and anti-inflammatory cytokine response to FES-evoked muscle contractions in paralysed skeletal muscle. The aim of this feasibility study was to compare the plasma cytokine responses to an acute bout of hand cycling and hybrid exercise at a fixed workload. It was hypothesised that 30 min moderate intensity voluntary hand cycling exercise would result in a significant IL-6 and anti-inflammatory cytokine response in a group of persons with a thoracic level SCI. Further, this response would be augmented following hybrid exercise due to the greater volume of muscle mass recruited via FES-evoked lower-limb contractions.

### 7.3 Methods

#### 7.3.1 Participants

Five community-based participants with motor complete SCI (4=male and 1=female; ASIA impairment scale A; T5-T7) volunteered to participate in the study (Table 7.1). Participants were required to be at least 1 y post injury and exclusion criteria included the incidence of pressure sores, a pacemaker or lower limb metal implants, and evidence of hypertension or previous dysreflexic responses to FES-evoked exercise. All procedures were approved by the local ethical advisory committee and performed in accordance with the Declaration of Helsinki. At the time of providing written informed consent, all participants were required to have undertaken at least 2 months (2-3 sessions per week) supervised FES-evoked cycling training at a private rehabilitation centre. All participants had undergone full medical screening by an independent physician prior to undertaking individual FES-evoked training. Experience of hand cycling was not a pre-requisite for inclusion.
Table 7.1 Participants’ characteristics and peak physiological responses to hand cycling GXT

<table>
<thead>
<tr>
<th>Part. No.</th>
<th>Age (yr)</th>
<th>Body Mass (kg)</th>
<th>Height (m)</th>
<th>Injury level</th>
<th>TSI (yr)</th>
<th>$\dot{V}O_2$ (L·min$^{-1}$)</th>
<th>RER</th>
<th>PO (W)</th>
<th>HR (b·min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>55</td>
<td>1.63</td>
<td>T5</td>
<td>4</td>
<td>1.91</td>
<td>1.11</td>
<td>90</td>
<td>178</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>80</td>
<td>1.75</td>
<td>T5/6</td>
<td>3</td>
<td>1.50</td>
<td>1.07</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>72</td>
<td>1.75</td>
<td>T6</td>
<td>25</td>
<td>1.90</td>
<td>1.21</td>
<td>80</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>78</td>
<td>1.72</td>
<td>T6</td>
<td>5</td>
<td>1.31</td>
<td>1.10</td>
<td>50</td>
<td>134</td>
</tr>
<tr>
<td>5*</td>
<td>42</td>
<td>48</td>
<td>1.50</td>
<td>T6</td>
<td>3</td>
<td>0.88</td>
<td>1.07</td>
<td>50</td>
<td>164</td>
</tr>
<tr>
<td>Mean</td>
<td>44</td>
<td>66.6</td>
<td>167</td>
<td>-</td>
<td>8</td>
<td>1.50</td>
<td>1.11</td>
<td>64</td>
<td>157</td>
</tr>
<tr>
<td>SD</td>
<td>15</td>
<td>14.3</td>
<td>11</td>
<td>-</td>
<td>10</td>
<td>0.43</td>
<td>0.07</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

Note. All participants presented motor and sensory complete injury (ASIA impairment scale A); * denotes female participant.

7.3.2 Experimental design

The study utilised a repeated measures design. During the first visit a synchronous, hand cycling only, graded exercise test to exhaustion was performed to determine $\dot{V}O_2$peak and POpeak. During the second and third visits, participants performed 30 min hand cycling exercise with and without concurrent lower-limb FES-evoked cycling at the same absolute PO (60% hand cycling POpeak) in a counter-balanced order. Main trials were separated by at least seven but no more than fourteen days.

7.3.3 Instrumentation

All exercise tests were performed on an eight-gear hybrid exercise bicycle (Appendix I) (Berkelbike BV, St Michielsgestel, The Netherlands) mounted on a cycle force magnetic flow ergotrainer (T1682; Tacx, Wassenaar, the Netherlands). A deceleration test to calculate rolling resistance was performed prior to each session as described in the manufacturer’s operating manual. Whilst in a seated position, feet were strapped to adjustable aluminium ankle-calf supports to prevent movement about the ankle and constrain limb movement to the sagittal plane. The power measured at the
front wheel was the result of the combined torque produced about the arm and leg cranks. The bicycle offers voluntary synchronous hand cycling with passive or FES-evoked asynchronous leg cycling movements. For FES-evoked cycling, an external 6-channel stimulator (Appendix I) (Impuls, Berkelbike BV, St Michielsgestel, The Netherlands) provides electrical stimulation via self-adhesive electrodes (Tens, Nottingham, UK) placed bilaterally over the neuromuscular points of the quadriceps, hamstring and gluteus muscles. The stimulator receives information about pedal position and crank velocity from the crank encoder (Appendix I) to control the cyclic stimulation pattern. The maximum stimulation intensity is 145 mA with a frequency of 35Hz. Stimulation intensity can be manually adjusted via 7.5 mA increments/decrements. Seat angle, seat position and crank height were individually manipulated and standardised to ensure the crank axle was positioned at shoulder height and sufficient degree of flexion remained around the knee joint. Tyre pressure was set at 60 psi and controlled before each session using a manual type pump with psi gauge (Topeak sport bike pump, Halfords, UK). The Borg 6-20 scale was used to attain participants differentiated RPE (RPEP; RPEC; RPEO) as described in section 4.3.3.

7.3.4 Preliminary measures

On arrival participants’ body mass was obtained to the nearest 0.1 kg using double-beam seated scales (Marsden MPWS-300, UK). Participants rested in a seated position for 15 min before resting \( \text{VO}_2 \) was measured breath-by-breath for 10 min using an online respiratory gas analysis system (Cortex metalyser 3B, Cortex, Leipzig, Germany). To allow familiarisation with the hybrid cycle and gearing system, all participants performed 10 min unloaded hand cycling with passive leg movements. Following familiarisation, a 5-min warm-up was performed at 10 W. The graded exercise test to exhaustion began at a PO of 10 W for 2 min. Subsequently, 10 W increments were added every minute until PO could not be maintained or the participants requested to stop. As some participants were unable to manually adjust the gearing system, the experimenter altered gearing selection upon request. The highest 30-s rolling average \( \text{VO}_2 \) value was defined as \( \text{VO}_{2\text{peak}} \). PO\(_{\text{peak}}\) was determined as the highest PO for a completed stage of the graded exercise test.
7.3.5 Main experimental trials

Hand cycling exercise only (HC)

Following a 5-min standardised warm up of 10 W, hand cycling exercise was performed at an imposed exercise intensity of 60% $P_{\text{Opeak}}$ for 30 min. The participants self-selected their gearing during the first 3 min of exercise. Gearing was subsequently standardised across trials. $\dot{V}O_2$ and HR (Polar PE 4000, Kempele, Finland) were measured continuously throughout the 30-min trial. A small capillary blood sample was obtained from the earlobe before exercise and every 10 min during exercise to determine BLa⁻ using a YSI 1500 SPORT Lactate Analyser (YSI Inc, Yellow Springs, OH). The lactate analyser was calibrated with a lactate standard of 5 mmol·l⁻¹. Differentiated RPE was recorded every 10 min.

Hand cycling exercise with concurrent FES-evoked lower-limb cycling (HYB)

Self-adhesive electrodes were placed bilaterally over the lower-limbs as previously described. Low intensity stimulation (60 mA) was provided during the standardised warm up. Stimulation amplitude was manually increased 7.5 mA every 5 min during the main trial from an initial intensity of 60 mA. An incremental stimulation protocol was employed to negate the premature fatigue of lower-limb muscles and maintain a consistent recruitment of muscle fibres throughout the 30-min trial. $\dot{V}O_2$, HR, differentiated RPE and BLa⁻ were recorded as in HC.

7.3.6 Blood analyses

A 7.5 ml blood sample was collected before (pre-exercise), immediately after (post-exercise), 1 h post (post+1) and 2 h post exercise (post+2) from an antecubital vein into a $K_3$EDTA vacutainer. Blood samples were treated and plasma analysis performed as described in section 5.3.4. The within assay co-efficient of variation for the analyses performed were as follows: adrenaline: 2.7%; cortisol: 3.6%; IL-6: 6.5%; IL-10: 8.3% and IL-1ra: 4.3%.
7.3.7 Statistical analysis

All data were analysed using the statistical package IBM SPSS for windows version 20 (SPSS inc, Chicago, IL). Normal distribution of the outcome variables was confirmed for all data. Non-parametric Friedman tests and Wilcoxon signed-rank tests were used to analyse differences in ordinal differentiated RPE data within each trial. Non-parametric Mann-Whitney-U tests were used to analyse differences in ordinal differentiated RPE data between each trial. Plasma cytokine and stress hormone data were analysed in a two factor (group x time of measurement) mixed measures ANOVA. Where significant F-ratios were shown, separate one-way repeated measures ANOVA with tukey post-hoc tests were employed to determine changes across time within each trial. Separate paired students t-tests were employed to determine differences between groups at each time point. A Bonferroni adjustment was performed on the unadjusted alpha value when performing multiple comparisons. For comparisons where the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied. All physiology data were analysed by paired students t-tests. Data are presented as mean ± SD. Significance was set a priori at $p \leq 0.05$. Effect sizes (Cohen’s d) are presented whereby 0.2 refers to a small effect, 0.5 a moderate effect and 0.8 a large effect (Cohen 1992).

7.4 Results

The participants’ peak physiological responses are shown in Table 7.1. A comparison between physiological responses to the HC and HYB trials is provided in Table 7.2. During 30 min exercise at a fixed workload $\dot{V}O_2$, $\%\dot{V}O_{2peak}$, RER and $BLa^-$ were significantly higher in HYB than HC. No difference was found between trials for HR or $\%HR_{peak}$.
Table 7.2 Physiological responses to 30 min hand cycling and hybrid exercise. Data are mean ± SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HC</th>
<th>HYB</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO (W)</td>
<td>39 ± 12</td>
<td>39 ± 12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(\dot{V}O_2) (L·min(^{-1}))</td>
<td>0.86 ± 0.14</td>
<td>1.00 ± 0.15</td>
<td>&lt;0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>%(\dot{V}O_2)peak</td>
<td>60 ± 15</td>
<td>70 ± 18</td>
<td>&lt;0.01</td>
<td>0.62</td>
</tr>
<tr>
<td>HR (b·min(^{-1}))</td>
<td>104 ± 16</td>
<td>105 ± 11</td>
<td>0.91</td>
<td>0.14</td>
</tr>
<tr>
<td>%HR(_\text{peak})</td>
<td>66 ± 3</td>
<td>68 ± 10</td>
<td>0.81</td>
<td>0.14</td>
</tr>
<tr>
<td>RER</td>
<td>0.94 ± 0.05</td>
<td>1.04 ± 0.12</td>
<td>&lt;0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>BLa(^{-}) (mmol·l(^{-1}))</td>
<td>1.94 ± 0.65</td>
<td>3.94 ± 1.56</td>
<td>&lt;0.01</td>
<td>1.30</td>
</tr>
</tbody>
</table>

The differentiated RPE responses for HC and HYB trials are shown in Figure 7.1. A significant (p<0.03) increase in differentiated RPE was present during HC, with all RPE higher at 20 and 30 min than 10 min. No differences were seen in differentiated RPE across time during HYB. Although non-statistically significant (p=0.07), a tendency was shown for RPE\(_P\), RPE\(_C\) and RPE\(_O\) to be lower in HYB than HC (Figure 7.1).

When performing the two factor mixed measures ANOVA, a significant effect for time (p=0.04) and significant time*trial interaction (p=0.013) were observed for plasma IL-6 concentrations. Plasma IL-6 was significantly elevated at post+1 (~2.5-fold) post+2 (~2.5-fold) following HYB, with values significantly (p<0.05) higher than at pre-exercise and immediately post-exercise. A small (0.5-fold) non-significant (p=0.15) increase in IL-6 was present at post+1 and post+2 following HC. In contrast, concentrations were significantly higher in HYB than HC at post+2 (p<0.02) (Figure 7.2a). No significant effects for time (p>0.05) or time*trial interactions (p>0.05) were present for IL-10. Plasma IL-1ra was unaffected (p>0.05) by exercise in both trials (Figure 7.2b). Although not statistically significant, there was a tendency for IL-10 concentrations to rise in HYB. A mean ~1-fold increase in IL-10 concentration was present at post+2 (Figure 7.2c).

A significant effect for time (p=0.05) and non-significant time*trial interaction (p>0.05) for adrenaline showed a significant effect of exercise in both trials.
Immediately post-exercise, plasma adrenaline concentrations were significantly elevated ($p<0.05$) above pre-exercise concentrations and then returned to baseline levels at post+1 (Figure 7.3a). Plasma cortisol concentrations showed a small but non-significant (main effect for time: $p=0.08$; time*trial interaction: $p=0.97$) increase in both HC and HYB (Figure 7.3b).

**Figure 7.1** Differentiated RPE responses during 30 min moderate intensity HC and HYB exercise

**Note.** *Time effects:* † = HC significantly different from 10 min. Data are median (quartiles). ($p<0.05$).
Figure 7.2 Plasma cytokine responses to 30 min moderate intensity HC and HYB exercise

Note. Time effects: † = HYB significantly different from pre-exercise and post-exercise; Group effects: ‡ = HYB significantly greater than HC. Data are mean ± SD. (p<0.05).
Figure 7.3 Plasma adrenaline and cortisol responses to 30 min moderate intensity HC and HYB exercise

Note. Time effects: † = Both trials significantly different from all other time points. Data are mean ± SD. (p<0.05).

7.5 Discussion

The present study investigated the plasma cytokine response to an acute bout of moderate intensity (60% \( \text{VO}_{2\text{peak}} \)) hand cycling exercise with and without concurrent FES-evoked lower-limb cycling at a fixed PO. The addition of FES-evoked cycling to voluntary hand cycling resulted in a significantly greater IL-6 response (2.5-fold) than performing hand cycling exercise alone (0.5-fold). The elevation in IL-6 following HYB was associated with an increase in plasma IL-10 concentrations 2 h post exercise. In accordance with previous literature (Hasnan et al. 2013), submaximal \( \text{VO}_2 \) was significantly higher during HYB compared with HC.

The magnitude of the skeletal muscle-derived IL-6 response is dependent on both the intensity and duration of exercise, where intensity indirectly reflects contractile activity within the active muscle (Pederson 2012). The production and release of IL-6 is regulated by a synergy of signalling pathways responsive to mechanical stimuli, intramuscular calcium concentrations, muscle glycogen stores and the SNS (Chapter five). In contrast to the findings of Chapter six, a small significant elevation in SNS-mediated plasma adrenaline concentrations was observed immediately post-exercise.
following HC. Despite this SNS response, the 0.5-fold increase in plasma IL-6 observed was similar to the IL-6 response observed following 30 min at equivalent PO (~40 W) in non-SCI participants. As in the light intensity trial (40% VO₂peak) of the previous chapter, these findings suggest the absolute PO performed (30-50 W) by the untrained cohort may have been too low to initiate a significant IL-6 and IL-1ra response. It is therefore proposed that intracellular pathways (calcium concentration, mechanical stimuli) may provide the primary signalling mechanism for the IL-6 response to submaximal exercise. As proposed in Chapter five, the SNS may play a greater role of in the IL-6 response as exercise intensity increases.

Voluntary hand cycling drove simultaneous passive lower-limb cycling during HC, as the participants’ feet were attached to the foot pedals to standardise limb movement and position between trials. Ter Woerds et al. (2006) previously reported no alteration in arterial leg blood flow in persons with a SCI during passive cycling. It can therefore be assumed that passive movements made no contribution to the metabolic response in HC. In contrast to voluntary muscle contractions, the recruitment of motor units during electrical stimulation progresses from large motor units to small motor units (Martin et al. 2012). The small elevation (~0.1 L·min⁻¹) in VO₂ during HYB therefore represents the recruitment of highly fatigable fast-twitch muscle fibres with a low oxidative capacity (Martin et al. 2012). The significantly greater BLa⁻ and RER also observed during HYB highlight the reliance on anaerobic carbohydrate metabolism during FES-evoked cycling. As well as a mediator of inflammation, the myokine IL-6 is proposed as an energy sensing hormone that exerts autocrine and paracrine effects on skeletal muscle lipolysis to maintain substrate ability during exercise (Pederson 2012). High rates of glucose metabolism and a subsequent lowering of muscle glycogen stores may therefore have contributed to the greater IL-6 response via stress induced mitogen activated protein kinase signalling, as previously described by Chan et al. (2004).

In contrast to the intensity-dependent plasma cytokine response reported by Scott et al. (2011), no IL-1ra response was seen with either exercise mode. However, the greater IL-6 response observed following hybrid exercise was associated with a small elevation in plasma IL-10 concentrations. IL-10 is principally released by regulatory T cells and acts to down-regulate inflammatory processes via inhibitory effects on pro-inflammatory cytokine expression and immune cell activation (Gleeson et al. 2011;
Moore et al. 2001). Previously, the IL-10 response to vigorous exercise has been associated more with the SNS than acute elevations in IL-6 (Peake et al. 2005). Whether the post-exercise elevation following HYB was IL-6 dependent or the consequence of a blood flow and SNS-mediated elevation in circulating IL-10 secreting immune cells requires further investigation. In contrast to Kjaer et al. (1996), an interesting finding of the present study was that SNS and HPA-axis activation were unaffected by the addition of FES-evoked cycling. The lack of humoral and reflex activation of the SNS and HPA-axis may be explained by the lower stimulation intensities and subsequent lower level of muscle recruitment and concentration of circulating metabolites (BLa⁻ = 4 vs. 8 mmol·l⁻¹) in the present study (Kjaer et al. (1996). These findings have important implications when examining the cardiovascular responses to FES-evoked exercise.

In the current study, differentiated RPE appeared lower during HYB than HC despite the high anaerobic component of FES-evoked cycling and the significant elevation in BLa⁻. This finding is in agreement with Laskin et al. (1993) who, despite a greater \( \dot{VO}_2 \), reported a lower overall perception of effort during hybrid rowing involving FES-evoked leg movements than arms-only rowing. An increase in differentiated RPE was also observed across time during HC but not HYB in the present work. Afferent feedback relaying sensory information detailing localised chemical and mechanical stress during exercise is considered a primary driver of effort perception (Noble & Robertson 1997). The accumulation of H⁺ ions in contracting muscle is associated with lactate production during exercise, which in turn reduces muscle pH and induces metabolic acidosis (Hampson et al. 2001.) However, afferent innervation is lost in muscle groups below the lesion level following a sensory complete SCI. The absence of change in RPE\(_P\) or RPE\(_O\) during HYB despite the elevated rates of lactate production and associated reduction in muscle pH confirms afferent feedback as primary driver of effort perception during exercise and physical stress.

Another interesting finding is that no perceptual dominance was found between RPE\(_P\) and RPE\(_C\) during either trial. In Chapter four and Chapter six RPE\(_P\) was found to be significantly higher than RPE\(_C\) during submaximal wheelchair propulsion and ACE, respectively. This perceptual dominance is driven by afferent signals from the exercising muscles and joints, including the reduction in tissue pH and mechanical...
strain, which are frequently higher than central perceptual signals, including BF and VE. The lack of dominance in RPE\textsubscript{P} during HC is contrary to both previous chapters, despite an absolute PO equal to the light intensity trial in Chapter six. These findings may therefore reflect the more efficient application of force during asynchronous hand cycling compared to ACE and wheelchair propulsion, and the greater cardiorespiratory strain. The familiarisation to upper-limb exercise in this community based SCI cohort may also mean perceptions of peripheral strain are lower than novice non-SCI participants despite the lower physical capacity.

It is important to note that the current findings are derived from a small sample of participants. The effect sizes observed in the physiological data were sufficient to identify significant differences between the trials. In contrast, the more variable cytokine responses may have affected the possibility of finding significant differences with the small participant group investigated. The magnitude of the cytokine response to FES-evoked exercise may also be influenced by the magnitude of age and/or SCI-related skeletal muscle atrophy of the current cohort. The effect of stimulation intensity (high vs. low) and mode of FES-evoked contraction (cycling vs. isometric) on plasma myokine and cytokine responses requires investigation in a larger cohort homogenous for age and time since injury. Emphasis should also be placed on maximising the anti-inflammatory response to voluntary upper-limb exercise, whilst considering the effect of relative and absolute exercise intensities on upper limb over-use in populations with low physical capacity.

7.6 Conclusion

These initial findings suggest paralysed skeletal muscle releases the myokine IL-6 in response to electrically evoked contractions. Moderate intensity (60\% \text{PO}_{\text{peak}} obtained during HC only) hybrid exercise was associated with an elevation in plasma concentrations of the anti-inflammatory cytokine IL-10; an effect not present when performing hand cycling exercise alone in an untrained cohort. Hybrid exercise may offer a method of maximising the anti-inflammatory potential of acute exercise in individuals with a thoracic SCI responsive to FES-evoked contractions. In response to voluntary upper-limb exercise alone, the absolute intensity (W) rather than relative (\% \text{PO}_{\text{peak}}) may be an important determinant in the magnitude of the anti-inflammatory cytokine response.
8. General Discussion

8.1 Overview of experimental chapters

In addition to regular rehabilitative care, the promotion of regular exercise should be encouraged as this may significantly improve physical capacity (Dallmeijer et al. 1999b; de Groot et al. 2003; Valent et al. 2008). In chronic SCI populations, regular participation in sport or exercise has been shown to reduce risk factors for CVD (Dallmeijer et al. 1997; Buchholz et al. 2009) and enhance performance in ADL (Dallmeijer 1999a). The primary objectives of the current thesis were to:

1. Investigate the use of subjective ratings of perceived exertion (RPE) as a tool for the self-regulation of wheelchair exercise in novel participant groups, including individuals with tetraplegia and individuals novice to wheelchair propulsion.

2. Examine a) the acute response of inflammation-mediating plasma cytokines to traditional and novel exercise modalities available to people with a SCI, and b) how these responses may be affected by autonomic impairment and the small muscle mass activated during traditional upper-limb exercise modalities.

The main findings from the five experimental chapters of the current thesis are summarised in Table 8.1.

In Chapters three and five, significant linear relationships were observed between differentiated RPE and workload independent of SCI injury level. Despite impaired sensorimotor and cardiovascular function, trained participants with a complete cervical level SCI (Chapter three) successfully self-regulated acute exercise using subjective RPE (RPEo 11-14; 70% VO2peak). No differences in physiological or PO responses were observed during imposed intensity and self-regulated wheelchair propulsion in the trained population group. Subsequently, Chapter four employed the same experimental design in a non-SCI participant group novice to wheelchair propulsion and the demands of upper-limb exercise. Differentiated RPEp was the dominant perceptual signal during both light (40% VO2peak) and moderate (60% VO2peak) intensity wheelchair propulsion. It was therefore investigated whether a differentiated RPE specific to the peripheral
Table 8.1 General summary of experimental protocols and main findings

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Modality (ME %)</th>
<th>Population</th>
<th>Intensity (%VO₂peak)</th>
<th>Time (min)</th>
<th>Absolute PO (W)</th>
<th>Overall RPE range</th>
<th>Main findings</th>
</tr>
</thead>
</table>
| 3       | WERG (8-10)    | Trained TETRA | 70                  | 20ᵃ       | 43 ± 9          | 11-14            | ▪ Linear differentiated RPE- VO₂ relationship  
▪ Successful self-regulation of exercise |
| 4       | WERG (5-7)     | Novice NON-SCI | 40 60 (3 x 4)       | 12ᵇ       | 26 ± 3          | 8-12             | ▪ RPEₚ dominant perceptual signal at both intensities  
▪ Self-regulation using RPEₚ more accurate at moderate than light intensities in novice users |
| 5       | WERG (-)       | Trained NON-SCI, PARA & TETRA | Graded submax (40-80) followed by max | 40ᵇ       | n/a-            | 17-20            | ▪ Linear differentiated RPE-VO₂ relationships for all groups  
▪ SNS plays regulatory role in IL-6 response to strenuous exercise |
| 6       | ACE (9-13)     | Novice NON-SCI | 40 60 30ᵃ           | 38±11 70±16 | 8-12 12-15      | ▪ Significant IL-1ra response to 60% ACE  
▪ Longer duration of 40% may elicit an anti-inflammatory response  
▪ Addition of lower-limb cycling increased cardiorespiratory stress but not cytokine response |
| 7       | HYB (10-14)    | Novice NON-SCI | 60                   | 70±16     | 10-15           |                  |              |
|         | HYB (7-13)     | Recreational PARA | 60 70 30ª           | 39±12     | 12-15           | ▪ Absolute intensity important in determining magnitude of anti-inflammatory response to upper-limb exercise alone  
▪ FES-evoked contractions initiate IL-6 response in paralysed skeletal muscle |

Exercise protocol: ᵃ = continuous; ᵇ = discontinuous.
strain experienced during wheelchair propulsion would improve the self-regulation of exercise in this novice group. The application of RPE_P prevented the small under-estimation of propulsion intensity observed when employing RPE_O at moderate exercise intensities. In contrast, RPE_P resulted in a significant over-estimation of light intensity exercise. Therefore it is recommended that RPE_O should be employed for the self-regulation of light intensity exercise prior to further familiarisation with differentiated RPE.

Limited work to date has explored the effects of SCI level and the small muscle mass activated during traditional upper-limb exercise modes on the proposed health protective, anti-inflammatory benefits of acute exercise. Chapter five extended previous experimental literature to support the role of the sympathetic nervous system in the plasma response of the inflammation-mediating myokine IL-6 to strenuous exercise. A large elevation in plasma IL-6 concentration was observed following strenuous wheelchair propulsion in both non-SCI and thoracic SCI participants. In contrast, the impaired sympathetic function observed following a cervical level SCI was associated with a reduced IL-6 response. Unfortunately the post-exercise sampling period was too short to identify any elevations in the anti-inflammatory cytokines IL-1ra or IL-10.

Despite the large elevations in IL-6 reported in Chapter five, submaximal exercise intensities are typically recommended during sub-acute rehabilitation and for community based individuals. Chapters six and seven explored the acute IL-6 and anti-inflammatory cytokine response to submaximal exercise employing traditional and novel exercise modalities. A significant elevation in plasma IL-6 and the anti-inflammatory cytokine IL-1ra were observed following 30 min moderate intensity ACE in Chapter six. Light intensity ACE resulted in a significant but smaller plasma IL-6 response and no IL-1ra response. In Chapter seven, 30 min moderate intensity hand cycling exercise in a recreationally active thoracic SCI participant group resulted in an IL-6 response of a similar magnitude to that observed during light intensity ACE in the previous chapter. As shown in Table 8.1, a comparable absolute PO was observed during moderate intensity hand cycling (Chapter seven) and light intensity ACE (Chapter six) in recreationally active thoracic SCI and non-SCI participants respectively. It is therefore proposed that the absolute intensity of upper-limb exercise is important in determining the IL-6 and anti-inflammatory cytokine response to submaximal exercise independent of SNS activation.
In Chapter six, the addition of low intensity leg cycling increased HR and VO$_2$ but not the plasma cytokine response to light intensity ACE in a non-SCI group. The voluntary muscle fibre recruitment required in non-SCI participants to perform the low PO (~15-30 W) observed during FES-evoked cycling presumably provided an insufficient stimulus for an IL-6 response. A novel finding of the current thesis is that FES-evoked concentrations in trained participants resulted in a significant increase in plasma IL-6 concentrations not observed following hand cycling exercise alone. The magnitude of the IL-6 response was associated with elevated plasma concentrations of the anti-inflammatory cytokine IL-10. The findings from the current feasibility study suggest that the involuntary recruitment of paralysed skeletal muscle may augment the anti-inflammatory potential of acute exercise.

8.2 Contribution to scientific understanding and application of findings

8.2.1 RPE and self-regulated exercise

In both sub-acute (Pelletier et al. 2013) and community based populations (Kehn & Kroll 2009), the perceived benefits of exercise are understood. However, participation in physical activity is lower in people with a SCI than in non-SCI counterparts (Ginis et al. 2010). A number of barriers to exercise participation challenge the ability to maintain a physically active lifestyle following a SCI, including a lack of accessible facilities and low self-esteem (Kehn & Kroll 2009). Clinicians and rehabilitation supervisors are in the best position to encourage and guide exercise prescription, however, only 18%-40% of patients report receiving exercise advice (Cowan et al. 2009). A lack of exercise knowledge and access to traditional tools (PO, VO$_2$ or HR) for the regulation of exercise may provide an additional barrier to exercise prescription for many healthcare professionals. The impaired sympathetic drive and subsequent HR response to exercise, particularly in persons following a cervical level SCI, mean traditional tools for the regulation of exercise intensity may also not be suitable in some SCI populations (Valent et al. 2007a; Leicht et al. 2012).

RPE has received a growing amount of attention as an easy to administer and cost-effective tool for the self-regulation of exercise intensity. Target RPE can either be ‘estimated’ during a preliminary exercise test of a prescribed workload (Goosey-Tolfrey et al. 2010) or set at a fixed intensity (e.g. ‘somewhat hard’) across a whole cohort (Parfitt et al. 2012). ACSM guidelines currently consider there insufficient evidence to
employ RPE as a primary method of regulating exercise. However, Parfitt et al. (2012) recently reported a significant improvement in cardiorespiratory fitness, cholesterol and BMI in 16 previously sedentary participants following an 8-week perceptually regulated exercise training programme at an overall RPE of 13. In trained participants with a thoracic level SCI, Goosey-Tolfrey et al. (2010) and Muller et al. (2004) reported the successful self-regulation of acute hand cycling and wheelchair propulsion exercise respectively. RPE has also previously been used as a supplementary method of regulating exercise training during 9 months combined aerobic and resistance exercise training where HR data was unavailable (Hicks et al. 2003).

The utility of RPE as a tool for the monitoring of submaximal exercise intensity in populations with SCI has, however, previously been questioned (Cowan et al. 2012; Lewis et al. 2007). Cowan et al. (2012) reported a weak relationship between submaximal $\dot{V}O_2$ and RPE in 12 community and rehabilitation-based participants with paraplegia during a graded ACE test to exhaustion. Similarly, Lewis et al. (2007) challenged the relationship between RPE and both HR and $\dot{V}O_2$ in a group of paraplegic and tetraplegic participants of similar training status following a discontinuous, graded ACE test. The absence of RPE data from submaximal, steady-state exercise conditions (Cowan et al. 2009) and lack of individual assessment of RPE-physiological relationships across exercise intensities (Lewis et al. 2007) may have influenced these findings.

Independent of SCI injury level, significant linear RPE-workload relationships were observed during steady-state, submaximal wheelchair propulsion in Chapters three and five. Thus, the impaired sympathetic drive and greater sensorimotor loss experienced in participants with tetraplegia appeared to have little influence on the subjective RPE response to acute exercise. As previously observed in persons with thoracic level SCI (Goosey-Tolfrey et al. 2010; Muller et al. 2004), participants with a cervical level SCI in Chapter three also successfully self-regulated an acute bout of exercise using RPE. It must be noted that the trained participants had a large amount of experience in wheelchair propulsion. Therefore, the findings from this chapter can only support the application of RPE for the self-regulation of wheelchair propulsion in trained SCI populations.
It could be argued that the greatest benefit of self-regulating exercise using RPE may be gained from rehabilitation and community-based populations, where access to exercise prescription tools may be limited. However, little work to date has examined the self-regulation of exercise in participants untrained in upper-limb exercise or novice to upper-limb exercise modalities. In accordance with previous literature (Lenton et al. 2008), differentiated RPE_p was the dominant perceptual signal during light and moderate intensity wheelchair propulsion in Chapter four and ACE in Chapter six. High mechanical shoulder load and low GE, particularly during wheelchair propulsion, may have contributed to elevated peripheral strain in the untrained, non-SCI participants. Utilising traditional RPE_O may therefore under-estimate the subjective peripheral strain during upper-limb exercise in novice groups.

It was hypothesised that employing a differentiated RPE specific to the peripheral strain experienced by novice wheelchair users may improve the self-regulation of exercise in this population group. In support of this hypothesis, the application of differentiated RPE_p for the self-regulation of moderate intensity wheelchair propulsion in Chapter four prevented the under-estimation of exercise intensity seen when employing RPE_O. Differentiated RPE may therefore provide a more specific tool for the self-regulation of moderate intensity exercise in novice wheelchair users. However, the current findings are only based on a cohort of 18 non-SCI participants and therefore require verification in a larger cohort. Interestingly, this hypothesis did not hold true when self-regulating light intensity wheelchair propulsion. A significant over-estimation in exercise intensity was seen when employing RPE_p at PO’s equivalent to 40% VO2peak. This over-estimation could be attributed to the insensitivity of the novice wheelchair-user group to small changes in the strength of peripheral exertional signals.

Low intensity exercise training is considered an effective method of improving physical capacity (van den Berg et al. 2010b) and wheelchair propulsion skills (de Groot et al. 2002; de Groot et al. 2008) following a SCI whilst minimising upper-limb strain and, the risk of over-use injuries. Therefore, the long-term effects of low intensity wheelchair propulsion in chronic SCI populations are an important area of research (Scheer et al. 2013). Previously, Muller et al. (2004) reported a higher CV for the self-regulation of low intensity versus high intensity wheelchair racing training using subjective perceived exertion. Eston & Williams (1988) also suggested a higher reproducibility of exercise intensity was found at an RPE >17, and that structured
familiarisation should be performed prior to the self-regulation of lower exercise intensities. In contrast to these findings, Chapter four provides encouraging support for the self-regulation of light intensity wheelchair propulsion using overall RPE. Further work is required to assess the efficacy of RPE-regulated wheelchair propulsion training in populations with a SCI.

As shown in Table 8.1, an interesting observation from the current thesis is that both light intensity exercise (RPEo 8-12) and moderate intensity exercise (RPEo 12-15) resulted in a typical range of overall RPE. These findings were present independent of training status, disability status and exercise mode. Therefore, it could be considered that relative exercise intensity is transferable across upper-limb exercise modes and participant groups.

8.2.2 Interleukin-6 and the anti-inflammatory effect of exercise

The relative risk of CVD and all-cause mortality displays an inverse, dose-dependent relationship with levels of physical activity and cardiorespiratory fitness (Blair & Morris 2009). Physical inactivity is associated with the development of inflamed adipose tissue and chronic, low-grade elevations in circulating concentrations of the adipose-derived inflammatory mediators TNF-α, IL-6, IL-1β and hepatic CRP. Chronic low-grade inflammation is considered predictive of future cardiovascular disease (Libby & Ridker 2004) and contributes to an insulin resistant state via impaired downstream signalling of the insulin receptor (Tilg & Moschen 2008). Injury to the spinal cord results in prolonged physical inactivity and reduced energy expenditure and therefore predisposes individuals to an elevated risk of obesity-related chronic disease. Low-grade inflammation in chronic SCI is greater than age-matched non-SCI controls (Gibson et al. 2008; Kouda et al. 2012; Wang et al. 2007). The suppression of inflammatory profiles could provide a therapeutic countermeasure to CVD risk following a SCI (Nash et al. 2011).

Regular exercise is reported to reduce the risk of CVD, in part because exercise exerts a range of anti-inflammatory effects (Gleeson et al. 2011). Using cross-sectional analysis, resting plasma IL-6 and CRP concentrations show a positive inverse association with activity frequency and perceived physical fitness in non-SCI populations (Shanley et al. 2013). In chronic SCI populations, positive relationships have been observed between markers of low-grade inflammation and both peak aerobic
capacity (Manns et al. 2005) and time spent participating in leisure time physical activity (Buchholz et al. 2009).

The current thesis explored the skeletal muscle derived IL-6 response to exercise and the associated cascade of plasma anti-inflammatory cytokines. IL-6 is proposed as a stress-sensing hormone, responsive to a complex of intracellular and extracellular signalling pathways (Welc & Clanton 2013). Previous literature regarding the primary stimuli for IL-6 release from contracting skeletal muscle has been equivocal, with the SNS (Frost et al. 2004), intracellular Ca$^{2+}$ (Holmes et al. 2004), depleted muscle glycogen stores (Chan et al. 2004) and mechanical stimuli (Whitham et al. 2012) all receiving support. By employing participant groups with impaired (TETRA) and in-tact (PARA & non-SCI) autonomic nervous system function this thesis was the first to explore the contribution of the SNS on the IL-6 response to strenuous exercise in vivo. As described in Chapter five, the SNS appears to have a contributing role in the signalling pathway of the acute IL-6 response in following strenuous exercise in non-SCI and PARA. The magnitude of the IL-6 in these groups was comparable to those observed following 2 h moderate intensity ACE (Umemoto et al. 2011) and 60 min moderate intensity treadmill running (Peake et al. 2004; Scott et al. 2011). In TETRA, signalling pathways responsive to mechanical stimuli and intracellular Ca$^{2+}$ appeared to contribute to a small (0.5-fold) IL-6 response. Subsequently, a synergy between contraction induced and SNS-activated signalling pathways for muscle IL-6 expression appears responsible. Both may differentially activate Jun N-terminal kinase and stress-activated protein kinase signalling pathways to up-regulate IL-6 expression, with mechanical stimuli more dominant at submaximal intensities and the SNS contributing as exercise intensity and duration increases.

To date, no previous work has examined the anti-inflammatory cytokine response to upper-limb exercise. In addition, only Kouda et al. (2012) have investigated the IL-6 response to a duration and intensity of exercise consistent with current guidelines. As shown in Chapter six, 30 min moderate intensity ACE resulted in a significant elevation in plasma IL-6 (3-fold) and IL-1ra (1-fold) independent of SNS activation. When compared to treadmill running exercise of a similar absolute PO (Mendham et al. 2011), upper-limb exercise results in a greater stimulus for IL-6 and subsequent IL-1ra responses independent of SNS activation. The current findings support the observations
of Helge et al. (2011) where a greater IL-6 response was found from the exercising muscle mass of the upper-limb than lower-limb despite lower oxygen uptake and similar substrate utilisation. Contributing factors may include a greater mechanical force per unit area of muscle and greater elevations in intracellular Ca$^{2+}$. In contrast, only a small (0.5-fold) elevation in IL-6 was observed in Chapter seven following 30 min, moderate intensity hand cycling in recreationally active SCI participants despite a significant elevation in plasma adrenaline concentrations. The IL-6 response was of a similar magnitude to the response observed following 30 min light intensity ACE in non-SCI participants at a similar absolute PO (Table 8.2). Thus, the current findings suggest that absolute PO, and the associated Ca$^{2+}$ and mechanical stimuli dependent signalling, are the key determining factors in the IL-6 and anti-inflammatory cytokine response to submaximal upper-limb exercise.

Elsewhere, Umemoto et al. (2011) reported a significantly greater plasma IL-6 and plasma adrenaline response to 2 h ACE in non-SCI controls than thoracic SCI participants. In the current thesis, $\dot{V}O_{2\text{peak}}$ and achievable PO$_{\text{peak}}$ were considerably lower in recreationally active SCI (Chapter seven) than non-SCI participants (Chapter six). Contributing factors may include lower trunk stability in the SCI group, as all participants had injuries at T5/T6; upper-limb pain or simply lower cardiorespiratory fitness and strength. Differences in absolute PO when exercising at the same relative exercise intensity should therefore be considered when interpreting acute cytokine responses between population groups. Further work is required to determine the optimal PO for initiating an anti-inflammatory cascade following acute exercise. When comparing plasma cortisol data across chapters, it is important to acknowledge the effect of sample collection times on the findings. In Chapter six, samples were taken in the early morning following an overnight fast. This led to significantly higher resting concentrations (600 nmol·L$^{-1}$) than Chapter five and seven (300 nmol·L$^{-1}$), due to the diurnal variation in cortisol secretion (Derr et al.2006). These elevated resting concentrations may therefore have masked any subsequent effect of exercise intensity on the post-exercise plasma concentrations in Chapter five.

The addition of FES-evoked leg contractions to voluntary upper-limb exercise recruits a greater muscle mass than upper-limb exercise alone (Bakkum et al. 2012). Subsequently, hybrid exercise increases oxygen uptake and cardiorespiratory strain
during acute exercise (Hasnan et al. 2013). However, skeletal muscle shows a dramatic decline in size and quality following paralysis, with the most marked decreases observed in the first year following injury (Castro et al. 1999). FES-evoked training has been shown to have a range of peripheral effects, including increased muscle CSA and contractile properties, that may off-set this decline (Baldi et al. 1998). Although highly inefficient with respect to external PO, FES-evoked cycling exercise also causes a large metabolic strain (Perret et al. 2010) and has positive effects on glucose tolerance and low-grade inflammation (Griffin et al. 2009). A novel finding of the current thesis was that paralysed skeletal muscle has the potential to release IL-6 in response to involuntary contractions. Although only taken from a small participant group experienced in FES-evoked exercise, the findings of this pilot work suggest hybrid exercise may confer an additional anti-inflammatory benefit to performing upper-limb exercise alone.

It should be noted that IL-6 is a controversial cytokine. The complexity of the human system means the exact mechanisms of IL-6 action are very difficult to elucidate in vivo. The repeated elevation in plasma anti-inflammatory cytokines following exercise is common-place. Yet to date little is known about the role of IL-1ra and IL-10 in down-regulating adipose or endothelial inflammatory processes in the post-exercise period. The acute plasma cytokine responses examined in the current thesis alone cannot account for the reduction in chronic low-grade inflammation and CVD risk observed following only small increases in physical activity. Adverse lipid profiles combine with plasma CRP concentrations to confer a greater relative risk for CVD than inflammatory markers alone (Libby & Ridker 2004). Therefore, the modulation of traditional risk factors for CVD including blood lipid profiles and blood pressure with exercise interventions remains important. Macrophage infiltration and the subsequent activation of inflammatory signalling pathways within adipose tissue is considered the primary driver of chronic low-grade inflammation (Cancello & Clement 2006). In an animal model, regular exercise reduced macrophage infiltration and induced phenotypic changes in adipose-derived macrophages cells during high-fat feeding (Kawanishi et al. 2010). Macrophages switched from inflammatory cytokine secreting M1 cells to anti-inflammatory cytokine secreting M2 cells following 16 weeks exercise training (Kawanishi et al. 2010). In contrast, little is currently known about the effects of exercise on macrophage phenotypes in human models. However, the reduced
expression of intracellular adhesion molecules responsible for the binding of inflammatory cells to adipose and endothelial tissue may confer a protective benefit of exercise against macrophage infiltration (Gleeson et al. 2011). Physically active humans also display a lower percentage of circulating activated inflammatory monocytes (CD14\textsuperscript{low} CD16\textsuperscript{+}), and exercise training in elderly males and females results in a reduction in monocyte pro-inflammatory cytokine (TNF-\alpha) secretion following bacterial stimulation (Tinnerman et al. 2008). Both factors could contribute to a reduction in chronic low-grade inflammation following prolonged improvements in physical activity levels.

8.2.3 Application of research findings

The current thesis aimed to document SCI specific ‘tools’ and ‘benefits’ to support the prescription of exercise in both rehabilitation and community-based population groups with a SCI. In chronic SCI populations, those involved in regular sport or physical activity display a reduction in risk factors for chronic disease, including improved blood lipid profiles and the maintenance of insulin sensitivity. The current thesis suggests that the reliance on the upper-limbs during acute exercise does not impair the anti-inflammatory cytokine response, provided the absolute PO of the exercise task is sufficient. The promotion of exercise during rehabilitation and in active community-based populations is important therefore to 1) achieve gains in physical capacity, and 2) to maximise the anti-inflammatory potential of regular exercise and reduce the risk of chronic disease development. In clinical, recreational and sporting environments, RPE may provide a cost-effective tool for supporting the regulation of exercise intensity. In individuals inexperienced in the demands of upper-limb exercise, the perceptual dominance in RPE\textsubscript{P} during submaximal WERG and ACE suggest differentiated RPE should always be employed during upper-limb exercise modalities to monitor levels of upper-limb strain.

Spinal cord injured population groups are heterogeneous with respect to motor/sensory function and physical capacity. Peak physical capacity following injury can be very low, especially in those with a cervical level SCI. Subsequently, inverse relationships are commonly reported between physical capacity and performance in ADL (Janssen et al. 1994) and risk factors for chronic disease, including blood lipid profiles (de Groot et al. 2007) and chronic inflammation (Manns et al. 2005). However,
prolonged exercise training can significantly improve peak aerobic and anaerobic capacities independent of SCI level (Goosey-Tolfrey et al. 2006; Hutzler 1998). The findings from a highly trained group of participants with a cervical level SCI show no evidence of elevated markers of chronic low-grade inflammation. Therefore, the reduced of IL-6 response to acute exercise observed in Chapter five did impair the anti-inflammatory potential of regular exercise in this group. Previously, Dallmeijer et al. (1997) observed an improved lipid profile in persons with a cervical level SCI actively involved in regular recreational sports activity. Therefore other mechanisms of anti-inflammatory action, including reduced visceral adiposity, and improvements in blood lipid profiles are important targets for exercise interventions in this population. Where available, the addition of regular FES-evoked exercise may confer additional health protective effects in individuals with a thoracic or cervical level SCI who are responsive to FES-evoked contractions. Exploring the potential of isometric or prolonged low intensity stimulation may negate the need for expensive hybrid exercise equipment while still maximising the anti-inflammatory potential of paralysed skeletal muscle.

8.3 Future research directions

The present thesis has taken a transitional approach to address the primary research objectives and investigate a number of original hypotheses. Both athletic and recreationally active SCI participants and novice non-SCI participants have been employed to simulate potential mechanisms that may influence the application of this research in the wider SCI population. In order for these findings to effectively support exercise prescription practice, a number of further research questions have arisen that require attention. These questions will be outlined below.

Self-regulated wheelchair exercise: Subjective RPE

Whilst the volume of evidence supporting the application of RPE as a primary method of self-regulating exercise is growing, the majority of experimental research in this area has been performed in controlled laboratory environments. Future work should explore the efficacy of repeated bouts of self-regulated wheelchair exercise using RPE in more practically relevant environments; particularly in rehabilitation and community settings. Randomised controlled trials comparing the effects of self-regulated exercise training against traditional exercise prescription techniques are required in untrained participant groups, with the aim of improving wheelchair propulsion technique or
enhancing physical capacity. It must also be acknowledged that the demands of wheelchair exercise on an ergometer may not adequately replicate those experienced during overground manual wheelchair propulsion. Therefore, it is important to understand how the ability to self-regulate exercise intensity may be transferred across experimental settings. Additional work investigating the efficacy of self-regulating regular ACE and hand cycling exercise training is also required.

**The anti-inflammatory cytokine response: Effect of exercise modality**

At present, the anti-inflammatory responses to regular exercise are poorly understood. The transient anti-inflammatory environment driven by an elevation in plasma IL-6 concentrations is one mechanism whereby acute exercise may provide a protective effect against chronic inflammation. Yet, prior to this thesis, no work has investigated the anti-inflammatory cytokine response to upper-limb exercise. Chapter five employed participants trained in wheelchair propulsion to specifically investigate the effect of SCI injury level, and specifically the impairment in SNS function, on the acute IL-6 response to strenuous exercise. Subsequently, the current thesis chose to extend previous research by exploring the anti-inflammatory cytokine response to submaximal exercise involving both traditional (ACE and hand cycling exercise) and novel (hybrid exercise) modalities. ACE and hand cycling are more mechanically efficient exercise modes than wheelchair ergometry, and are therefore associated with a greater absolute PO at the same relative exercise intensity. However, manual wheelchair propulsion is the most accessible mode of daily ambulation and is frequently employed for recreational sports participation. Therefore, an avenue of further research would be to compare the acute plasma cytokine response to wheelchair propulsion and ACE/hand cycling at the same absolute PO. Specifically, it would be interest to investigate whether the lower GE observed during wheelchair propulsion may increase the intracellular and/or the extracellular stimuli responsible for driving the anti-inflammatory cytokine response. An extension of this work would be to compare the effect of both exercise intensity and duration on the acute anti-inflammatory cytokine response. Findings from studies employing able-bodied participants have proposed that the performance of discontinuous, high intensity may confer a greater benefit to health than traditional continuous, submaximal exercise. It is currently unknown whether these propositions translate to upper-limb exercise and, particularly, in populations with a SCI.
The priorities of the future studies are:

i. The self-regulation of 3 weeks overground wheelchair propulsion training in novice and experienced participants using differentiated ratings of perceived exertion.

ii. The effect of exercise duration on the inflammation-mediating cytokine response to light-intensity ACE and wheelchair propulsion.

iii. The effect of exercise modality (wheelchair propulsion vs. ACE) on the inflammation-mediating cytokine response at the same absolute and relative exercise intensities.

The future research questions outlined above will help extend the findings of the current thesis into exercise prescription practice. As previously mentioned, no work has yet examined the feasibility of prescribing and regulating a wheelchair based, training intervention based solely on RPE. Wheelchair propulsion training plays a pivotal role in both early rehabilitation (wheelchair skills) and recreational sports participation. Therefore, this is a highly important field of research that requires immediate attention. Given the difficulties of working with SCI population groups, particularly in a rehabilitation setting, it is imperative that exercise protocols are initially investigated in non-SCI or trained SCI individuals in order to adopt the most appropriate design for future studies.

Light intensity exercise training has been shown to have beneficial effects on physical capacity (de Groot et al. 2003; van den Berg et al. 2010b) and wheelchair skills (de Groot et al. 2008). The findings from Chapter six suggest light intensity ACE may initiate a similar inflammation-mediating cytokine response to moderate intensity ACE given a sufficient duration of exercise. To aid exercise prescription practice, further work is required to establish the optimal duration of light intensity exercise with respect to plasma cytokine responses. A range of exercise modalities are also available to rehabilitation therapists and practitioners. In order for the health benefits of exercise training to be maximised, a secondary outcome would be to document the effect of exercise modality on the plasma cytokine responses at intensities traditionally employed for rehabilitation and training. It is hoped that the findings of the current thesis will be used to enhance future research in this area, particularly given the importance of exercise training in persons with a SCI.
9. References


Swain, D.P. & Leutholtz, B.C. (1997). Heart rate reserve is equivalent to% VO₂ reserve, not to% VO₂max. Medicine & Science in Sports & Exercise, 29(3), 410-414.


10. Appendices

The following information is contained in the appendices:

Appendix I – Exercise equipment.

Appendix II – Borg 6-20 scale with instructions for use.

Appendix III – Generic overview of analytical techniques for i) blood lactate concentration and ii) plasma cytokine and hormone concentrations.
Appendix I

i) Custom made, single drum wheelchair ergometer – Bromakin Wheelchairs Ltd, Loughborough, UK

ii) HP cosmos treadmill – HP, Traunstein, Germany.
iii) Lode Angio arm-crank ergometer – Lode BV, Groningen, the Netherlands.

iv) Lode Corival leg cycle ergometer – Lode BV, Groningen, the Netherlands.
v) Berkelbike hybrid bicycle -Berkelbike BV, St Michielsgestel, the Netherlands).

(A) = stimulator; (B) angle crank encoder (B); (C) flow ergo trainer.

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Appendix II

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>No exertion at all</td>
</tr>
<tr>
<td>7</td>
<td>Extremely light</td>
</tr>
<tr>
<td>8</td>
<td>Very light</td>
</tr>
<tr>
<td>9</td>
<td>Light</td>
</tr>
<tr>
<td>10</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>11</td>
<td>Hard (heavy)</td>
</tr>
<tr>
<td>12</td>
<td>Very hard</td>
</tr>
<tr>
<td>13</td>
<td>Extremely hard</td>
</tr>
<tr>
<td>14</td>
<td>Maximal exertion</td>
</tr>
</tbody>
</table>

Borg RPE scale

The Borg 6-20 scale (Borg 1998)
Prior to each study, all participants were familiarised with the Borg 6-20 scale according to the standardised instructions described by Borg (1998). Instructions were provided as follows:

‘While exercising we want you to rate your perceptions of exertion, i.e., how heavy and strenuous the exercise feels to you. Look at this rating scale; we want you to use this scale from 6 to 20, where 6 means ‘‘no exertion at all’’ and 20 means ‘‘maximal exertion’’.

9 - corresponds to ‘‘very light’’ exercise. For a healthy person it is like pushing slowly at his or her own pace for some minutes.

13 – on the scale is ‘‘somewhat hard’’ exercise, but it still feels ok to continue.

17 – ‘‘very hard’’ is very strenuous. A healthy person can still go on, but he or she has to really push themselves, and the person is very tired.

19 – on the scale is an extremely strenuous exercise level. For most people this is the most strenuous exercise they have ever experienced.

Try to appraise your feeling of exertion as honestly as possible, without thinking about what the actual physical load is. Don’t underestimate it, but don’t overestimate it either. It’s your own feeling of effort and exertion that’s important, not how it compares to other people’s. What other people think is not important either. Look at the scale and the expressions and then give a number.

Any questions?’

In studies where differentiated RPE were employed, participants were instructed to report feeling of exertion for their exercising muscles and joints (peripheral RPE) separately to their feeling of exertion from their heart rate and breathing (central RPE). A combined RPE was then collected (overall RPE) as a summation of both peripheral and central ratings.
Appendix III

i) Blood lactate analysis

Capillary blood samples for the analysis of BLa were taken from the earlobe, using a safety lancet (Sarstedt Ltd, Leicester, UK) and a heparinised capillary tube. In all studies, BLa was analysed using a lactate analyser (YSI 1500 SPORT, YSI Inc, Yellow Springs, OH), which was calibrated according to the manufacturer’s instructions before each test using a lactate standard of 5mmol·l⁻¹.

The YSI 1500 uses sensor technology for the determination of lactate from whole capillary blood. Briefly, the sensor consists of a three-layer membrane, with immobilised L-lactate oxidase in the middle layer. As substrate diffuses through the membrane, it contacts the lactate oxidase and is rapidly oxidised, producing hydrogen peroxide (H₂O₂). The hydrogen peroxide is, in turn, oxidised at the anode of the YSI sensor, resulting in an electrical current proportional to concentration of lactate in the sample of whole blood.

Co-efficient of variation for the YSI 1500 lactate analyser was calculated as 5.0%.

ii) Plasma cytokine and hormone analysis

All blood samples were taken from an antecubital vein into a K₃EDTA vacutainer. Blood samples were refrigerated until the final sample from each participant was collected and then spun down together in a refrigerated (4ºC) centrifuge at 1500 g for 10 min. The separated plasma was then immediately stored at -80ºC.

Plasma concentrations of IL-6, IL-10, TNF-α, IL-1ra, cortisol and adrenaline were determined using commercially available quantitative sandwich-type enzyme-linked immunosorbant assay (ELISA) kits. All ELISAs were performed according to the manufacturer’s instructions. Briefly, each ELISA consisted of the following steps:

- Plasma samples and standard samples of a known concentration are pipetted into a 96 well plate pre-coated with the specific capture antibody for the protein being measured.
- During an incubation period, any protein within the plasma is bound by the immobilised antibody in the well.
• The unbound substances are washed away, before a second detection anti-body is added to each well, followed by a further incubation period.

• The wash step is then repeated to remove any unbound anti-body reagent, and a substrate and amplifier solution is added to the wells to bind to the antibody-sample complex. This step initiates a reaction and brings about a colour change in the sample.

• Finally, the reaction is stopped and the absorbance of each well is recorded using a micro plate reader. Sample concentrations are determined by relation to a standard curve generated by plotting the absorbance of standards’ samples against the standards’ known concentration.

All samples were analysed in duplicate. Within each study, all samples from one participant were analysed on the same micro plate. The within assay co-efficient of variation for the analyses performed are provided within the ‘blood analyses’ section of each experimental chapter.

For more detailed instructions for each specific assay, refer to manufacturer’s instructions:

• **IL-6, IL-10, TNF-α, IL-1ra**: R&D systems, Abingdon, UK.

• **Cortisol**: DRG instruments, Marburg, Germany.

• **Adrenaline**: IBL international, Hamburg, Germany.