Mucosal immune and physiological responses to exercise in wheelchair athletes

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A Doctoral Thesis

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Abstract

Apart from motor and sensory function loss, an injury to the spinal cord can cause sympathetic dysfunction, which has been shown to affect immune responses. In this thesis, data from five experimental studies have been collected to compare physiological and psychophysiological exercise responses between wheelchair athlete subgroups with different disabilities (tetraplegic, paraplegic, and non-spinal cord-injured).

In two preparatory studies, physiological exercise responses to exhaustive (Chapter 4) and submaximal exercise (Chapter 5) were investigated in all three disability subgroups. Whilst reliability measures for peak oxygen uptake ($\dot{V}O_{2peak}$) were in a range observed previously in able-bodied athletes, the variation in tetraplegic athletes was larger when expressed relative to their $\dot{V}O_{2peak}$, questioning the use of this variable to track small changes in aerobic capacity in athletic populations. Submaximal physiological and psychophysiological exercise responses were found to be similar between disability subgroups when expressed as a percentage of $\dot{V}O_{2peak}$, justifying the protocol used in the laboratory study on mucosal immune function, which was based on the same percentages of $\dot{V}O_{2peak}$ for all disability subgroups.

The most extensive study of this thesis, detailed in Chapter 6, showed that single laboratory-controlled 60-min exercise sessions increase both salivary secretory immunoglobulin A (sIgA), a marker of mucosal immunity, and α-amylase, a marker of sympathetic activation in all three disability subgroups. However, the impaired sympathetic nervous system in tetraplegic athletes seemed to influence the fine-tuning of their sIgA response when compared with paraplegic and non-spinal cord-injured athletes, resulting in a larger exercise-induced increase of sIgA secretion rate when compared to paraplegic and non-spinal cord-injured athletes. Based on these results, the study detailed in Chapter 7 investigated sIgA responses in tetraplegic athletes during wheelchair rugby court training. Despite their disability, these athletes showed responses thought to be governed by the sympathetic nervous system, such as reductions of saliva flow rate as a result of strenuous exercise. Similarly, the responses observed in Chapter 8 imply a comparable trend of

chronic sIgA exercise responses in tetraplegic athletes as found in the able-bodied population, namely a decrease in sIgA secretion rate during periods of heavy training.

These are the first studies in wheelchair athlete populations to investigate mucosal immune responses. Interestingly, despite the disruption of their sympathetic nervous system, some responses in tetraplegic athletes are comparable with findings in able-bodied populations. It is possible that due to their highly trained nature, these tetraplegic individuals are able to compensate for their loss of central sympathetic innervation. This may be by way of adapted spinal reflex or parasympathetic nervous system activity, or increased sensitivity of receptors involved in autonomic pathways. Therefore, sympathetic nervous function in tetraplegic athletes may be qualitatively altered, but in parts still be functional.

Key words: Spinal cord injury, exercise testing, mucosal immune function, tetraplegia, paraplegia, autonomic innervation

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Preface

An overview of the publications and communications of the research conducted whilst writing this thesis is given below.

Publications

Leicht C.A., Tolfrey K., Lenton J.P., Bishop N.C. & Goosey-Tolfrey V.L. The verification phase and reliability of physiological parameters in peak testing of elite wheelchair athletes. *Eur.J.Appl.Physiol.*, accepted June 02 2012.

Leicht C.A., Bishop N.C. & Goosey-Tolfrey V.L. Submaximal exercise responses in tetraplegic, paraplegic and non spinal cord injured elite wheelchair athletes. *Scand.J.Med. Sci.Sports*, accepted May 23 2011.

Leicht C.A., Bishop N.C. & Goosey-Tolfrey V.L. (2011). Mucosal immune responses to treadmill exercise in elite wheelchair athletes. *Med.Sci.Sports Exerc., 43,* 1414-1421.

Leicht C.A., Bishop N.C. & Goosey-Tolfrey V.L. Mucosal immune responses during court training in elite tetraplegic athletes. *Spinal Cord*, accepted March 14 2012.

Leicht C.A., Bishop N.C., Paulson T.A.W., Griggs K.E. & Goosey-Tolfrey V.L. (2012). Salivary immunoglobulin A and upper respiratory symptoms during five months of training in elite tetraplegic athletes. *Int.J.Sports Physiol.Perform, 7*, 210-217.

Goosey-Tolfrey V.L. & Leicht C.A. Field based physiological testing of wheelchair athletes. *Sports Med.*, accepted July 10 2012.

Conference communications

Leicht C.A., Bishop N.C. & Goosey-Tolfrey V.L. Submaximal exercise responses in tetraplegic, paraplegic and non spinal cord injured elite wheelchair athletes. *VISTA conference* 2011, Bonn.

Leicht C.A., Bishop N.C. & Goosey-Tolfrey V.L. Impaired autonomic nervous system innervation has an impact on the mucosal immune response to exercise in wheelchair athletes (Poster). *International Society of Exercise and Immunology (ISEI) conference* 2011, Oxford. *Awarded with a commendation.*

Leicht C.A., Bishop N.C., Paulson T.A.W., Griggs K.E. & Goosey-Tolfrey V.L. High exercise loads depress salivary immunoglobulin A in elite tetraplegic athletes. *VISTA conference* 2011, Bonn.

Bishop N.C., Leicht C.A., & Gleeson M. Exercise, immune function and infection: issues for Olympic and Paralympic athletes. *International Convention on Science Education and Medicine in Science* 2012, Glasgow.

Miscellaneous work accomplished besides thesis

Leicht C.A. & Goosey-Tolfrey V.L. A review of the physiological determinants of performance in upper body exercise in athletes with a disability. *English Institute of Sport (EIS)*, April 2011.

Leicht C.A., Smith P.M., Sharpe G., Perret C. & Goosey-Tolfrey V.L. (2010). The effects of a respiratory warm-up on the physical capacity and ventilatory response in paraplegic individuals. *Eur.J.Appl.Physiol., 110*, 1291-1298.

Paulson T.A.W., Bishop N.C., Leicht C.A. & Goosey-Tolfrey V.L. Perceived exertion as a tool to self-regulate exercise in individuals with tetraplegia. *Eur.J.Appl.Physiol.*, accepted May 11 2012.

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Abbreviations

AB	able-bodied
ACE	arm crank ergometer
ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
APM	age-predicted maximum
BLa	blood lactate concentration
BLa_{peak}	peak blood lactate concentration
C	cervical
CL	constant load
CV	coefficient of variation
ex	exercise
GXT	graded exercise test to exhaustion
HPA	hypothalamic pituitary adrenal
HR	heart rate
HR_{peak}	peak heart rate
IgA	immunoglobulin A
IM	intermittent
m.	*musculus* (muscle)
mRNA	messenger ribonucleic acid
MTP	maximum tolerated power
NON-SCI	non-spinal cord-injured
PARA	paraplegic
PBS	phosphate buffered saline
PO	power output
R^2	coefficient of determination
RER	respiratory exchange ratio
RER_{peak}	peak respiratory exchange ratio
RPE	rating of perceived exertion
SCI	spinal cord injured
SD	standard deviation
sIgA	salivary secretory immunoglobulin A
T	thoracic
TETRA	tetraplegic
TM	treadmill
URS	upper respiratory symptoms
VER	verification phase
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2max}$	maximum oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
WB	wheelchair basketball
WERG	wheelchair ergometer
WR	wheelchair rugby
WT	wheelchair tennis

The expressions above are written in full when mentioned first and then abbreviated for the rest of the thesis.

1

General introduction

Over the past decades, the body of evidence in exercise physiology in wheelchair athletes has grown steadily. The advancements in wheelchair design (Ardigo' *et al.*, 2005), combined with greater funding opportunities and sports professionalism have resulted in a greater number of wheelchair athletes performing on recreational (Hettinga *et al.*, 2010) and professional levels (Gold & Gold, 2007); likewise, the quality of the sports has improved. This has attracted researchers to investigate the underlying physiological mechanism responsible for the effects of successful rehabilitation and training programmes. Research suggests that employing similar training volumes and relative exercise intensities as suggested for the able-bodied (AB) population (American College of Sports Medicine, 2000) results in similar improvements of markers of physical capacity (Devillard *et al.*, 2007). From a sporting perspective, mapping physiological characteristics of different wheelchair athlete subgroups has shed more light on the physiological demands during competition. For example, wheelchair basketball was shown to be more physically demanding than wheelchair tennis, when comparing average oxygen uptake and heart rate (HR) between the sports (Croft *et al.*, 2010). It was further pointed out that due to the large

variability between wheelchair athletes, taking the severity of their disability (their functional ability) into account is crucial when comparing wheelchair athletes (de Lira *et al.*, 2010; Molik *et al.*, 2010). However, whilst the body of evidence is increasing in wheelchair athletes, it is still comparably small when compared with the research conducted in AB individuals. One aim of this thesis is therefore to extend the evidence, and to provide a sound base for laboratory-based testing procedures and assessments for different subgroups of wheelchair athletes.

Exercise immunology is a relatively new field, with a steady flow of research publications only starting some 30 years ago (Nehlsen-Cannarella *et al.*, 1991; Nieman *et al.*, 1990; Tomasi *et al.*, 1982). Exercise can either improve or suppress immune function, and moderate exercise tends to be beneficial, as opposed to hard and/or prolonged exercise, which can have adverse effects on health and immune parameters (Walsh *et al.*, 2011). However, in contrast to exercise physiology, exercise immunology is a field practically unexplored in wheelchair populations. The significance of more knowledge in this field for these populations may be of great practical relevance, as some wheelchair-bound populations are prone to respiratory dysfunction (Brown *et al.*, 2006) and pulmonary complications (Lucke, 1998) and may therefore benefit from a better understanding of their immune function.

1.1 Organisation of the thesis

In order to introduce the research area and ultimately, to be able to put the data collected in the experimental chapters into context, background information is presented in Chapter 2. The research of this thesis focuses on a very specific population, spinal cord injured (SCI) individuals. Therefore, the basic structure and tasks of the spinal cord is presented – together with the implications of a spinal cord injury. This is followed by an overview about the impacts of exercise on the SCI population, exercise being a topic at the very core of this thesis. The literature review concludes with information on exercise immunology in both SCI and AB populations. Methods employed in more than one of the experimental chapters are described in Chapter 3. The presentation of the five experimental chapters on exercise physiology and exercise immunology then forms the main part of this thesis. In the

final part, results are discussed as a whole, and the practical value of the novel findings and directions for potential future research are presented.

1.2 Experimental chapters: Objectives and outline

The principal aim of this thesis was to investigate the impact of exercise on a range of physiological and psychophysiological parameters in wheelchair athletes. The objectives were:

- To determine the reliability of peak oxygen uptake ($\dot{V}O_{2peak}$) and related parameters obtained in a peak testing protocol
- To compare submaximal exercise responses between subgroups of wheelchair athletes
- To analyse the impact of acute laboratory exercise bouts on mucosal immune responses
- To analyse the impact of acute field-based exercise bouts on mucosal immune responses
- To examine the impact of exercise on chronic resting immune function

A summary of the studies to address these objectives is depicted in Figure 1.1. The first three studies focused on the comparison between disability subgroups, tetraplegic (TETRA), paraplegic (PARA), and non-spinal cord-injured (NON-SCI) wheelchair athletes. **Study 1** (Chapter 4) investigated the reliability of peak variables obtained in treadmill ergometry in the three subgroups. Further to this, the added value of including a verification phase in a peak testing protocol was examined. **Study 2** (Chapter 5) analysed submaximal exercise responses and investigated whether the same exercise intensity (expressed as $\%\dot{V}O_{2peak}$) evokes similar physiological and psychophysiological responses in the three subgroups. The results of these first two studies were used to help designing the protocol of study 3. **Study 3** (Chapter 6) is the most extensive study of this thesis, investigating the impacts of single laboratory-controlled training sessions upon mucosal immunity and comparing the results between the three subgroups. Based on the outcomes of study 3, further studies examined the field of exercise immunology in TETRA athletes in settings outside the laboratory, i.e. in a wheelchair rugby training and competition

environment. Whilst **study 4** (Chapter 7) investigated the impact of all-out on-court wheelchair rugby training sessions on mucosal immune function, **study 5** (Chapter 8) investigated the relationship of markers of the mucosal immune system and training load in TETRA athletes over a five-month training and competition period.

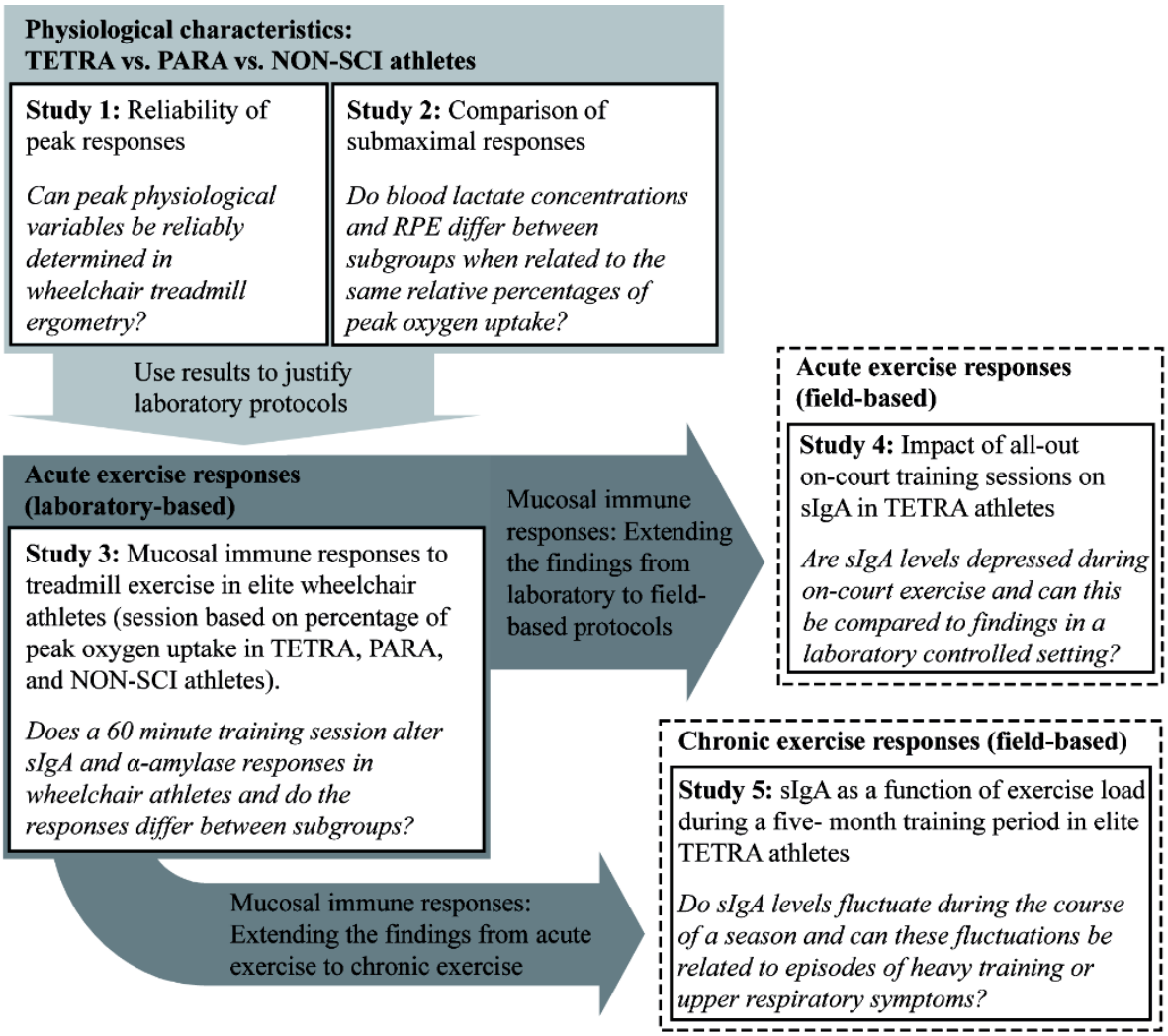

Figure 1.1 Overview of the experimental studies of this thesis.

Main questions are given in italic script. **TETRA**, tetraplegic; **PARA**, paraplegic; **NON-SCI**, non-spinal cord-injured; **RPE**, rating of perceived exertion; **sIgA**, salivary secretory immunoglobulin A.

2

Literature review

2.1 The spinal cord

With its one billion neurons, the spinal cord is today recognised by neuroscientists as a key structure of the central nervous system (Guertin & Steuer, 2009). Neurons leaving and entering the spinal cord transmit information to (e.g., flex muscles, digest food, or increase HR) and from (e.g., feel temperature or pain) the body. The innervation of muscles in humans is organised in a segmental fashion: Upper limbs are innervated by the cervical spinal cord, the trunk by the thoracic spinal cord, and lower limbs by the lumbar and sacral region (Marieb & Hoehn, 2007) (Figure 2.1A). Muscles within a body segment further follow this organisation; for example, *m. trapezius* (trapezius muscle) is innervated by spinal nerves C3 – C4, *m. biceps brachii* (elbow flexor) is innervated by C5 – C6, and *m. triceps brachii* (elbow extensor) by C6 – C8 (Marieb & Hoehn, 2007).

An analysis of the cross-section of the spinal cord (Figure 2.1B, Figure 2.2) shows its rough division into two areas, known as grey and white matter. This distinction is down to the histological structure of these areas as the grey matter is dense in cell bodies

responsible for the darker appearance. Motor neurons are located in the ventral horn of the grey matter, sensory neurons in the dorsal horn, and sympathetic neurons in between those two structures, the lateral horn. The white matter mainly consists of axons, the part of a neuron containing no cell body. Ascending and descending tracts within the white matter connect to higher levels of the nervous system, as well as interconnect different segments of the spinal cord (Marieb & Hoehn, 2007).

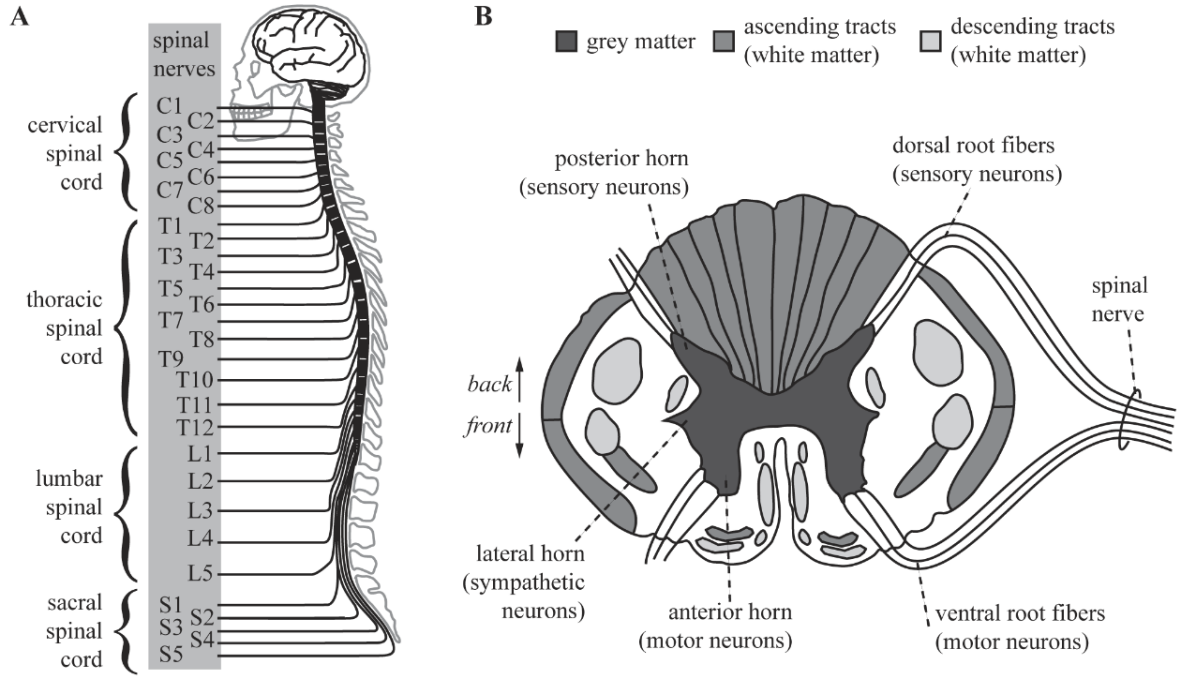

Figure 2.1 Sections of the spinal cord.

A, longitudinal section; **B**, cross-section of the spinal cord with major ascending and descending tracts. Drawn from information from Marieb & Hoehn (2007).

The spinal cord is a two-way conduction pathway between the brain and effective organs, such as muscles or skin. However, the spinal cord can also govern actions independently of higher levels of the nervous system, for example by reflex activity (Marieb & Hoehn, 2007). A popularly known reflex is the patella tendon reflex, where tapping the patella tendon just below the knee cap sends a sensory signal to the spinal cord, which then activates motor neurons causing the leg to kick (Figure 2.2A). Other reports (Vissing *et al.*,

1991) describe reflex activity of the sympathetic nervous system caused by muscle contractions (Figure 2.2B). Furthermore, there is evidence that the spinal cord is a command centre involved in the control and modulation of several complex functions, such as the perception of pain (Melzack & Wall, 1965) or locomotion (Guertin & Steuer, 2009).

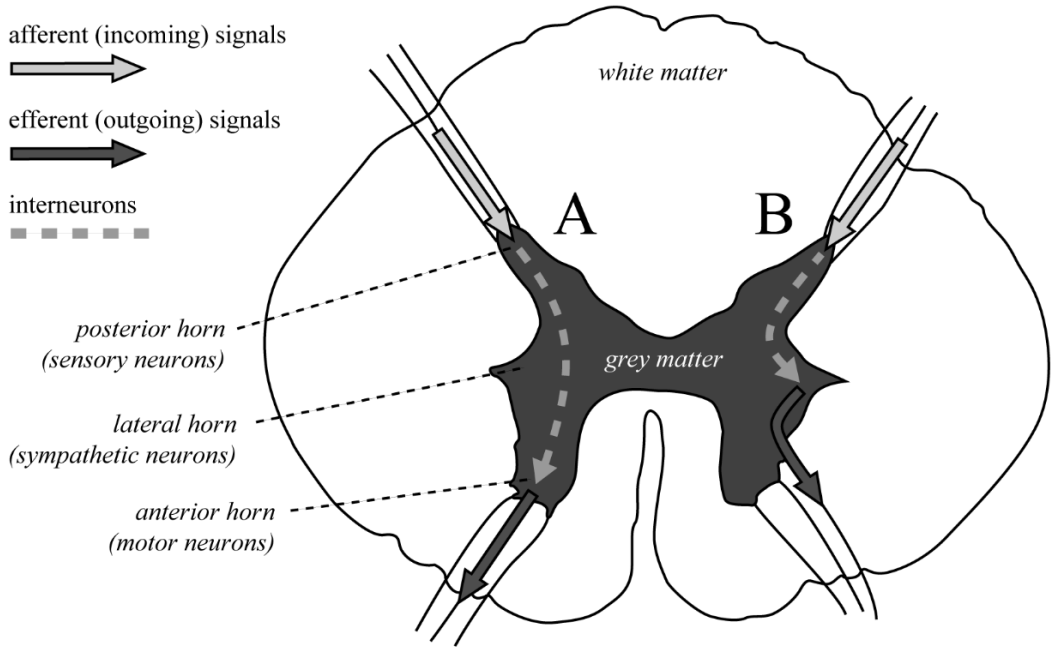

Figure 2.2 The anatomical basis for reflex activity.

Cross-section of the spinal cord. Sensory signals triggering a motor (**A**) or an autonomic (**B**) response. Drawn from information from Marieb & Hoehn (2007).

2.2 Physiological adaptations following a spinal cord injury

An injury to the spinal cord can be caused by trauma or disease (e.g., cancer). Independent of the cause of spinal damage, more than half of the survivors will experience varying degrees of motor, sensory or autonomic function loss (Jacobs & Nash, 2004). Cervical spinal cord injuries cause function loss in all four extremities (tetraplegia), while thoracic or lumbar spinal cord injuries cause function loss in the legs and trunk muscles and organs innervated below the level of lesion (paraplegia), with the degree of dysfunction

approximately proportional to the level of lesion (Bhambhani, 2002; Baydur *et al.*, 2001). A spinal cord injury is commonly described by indicating the place most caudal ("away from the head") with normal motor and sensory function (for example T10 for a low paraplegia) together with the completeness of injury: An examination of Figure 2.1B makes clear that if an incomplete lesion only affects the ventral (front) part of the spinal cord, some ascending dorsal (back) tracts may be left intact. An individual with such a lesion may experience complete motor dysfunction, but still be able to recognise sensory signals, such as temperature, pressure, or pain (Freeman Somers, 2010).

It is important to note that even a complete lesion of the spinal cord does not necessarily abolish all neural function below the level of lesion. Whilst a complete lesion interrupts all signals coming from or going to higher levels of the nervous system, spinal reflexes (as shown in Figure 2.2) below the level of lesion are preserved (Corbett *et al.*, 1971), if spinal nerves are not damaged (Jacobs & Nash, 2004). Therefore, a sensory stimulus may lead to muscle spasms (hence the name spastic paralysis), and sensory inputs can actively be used to access lost function, such as tapping the bladder (suprapubic tapping) in order to activate the muscles required for its emptying (Hansen *et al.*, 2004; Amarenco *et al.*, 2001). On the other hand, if spinal nerves are damaged, they cannot transmit sensory and/or motor signals and reflex activity is lost. This is the case if the lesion is below the level of ~T10 – T12, where the spinal column contains spinal nerves only (Figure 2.1A). These injuries will lead to a condition called flaccid paralysis, as muscles cannot be activated by sensory stimuli, which explains the greater loss of lower extremity muscle mass in individuals with flaccid rather than spastic paralysis (Jacobs & Nash, 2004).

2.2.1 Influence on physical performance

As a consequence of the loss of motor innervation below the lesion, less active muscle mass is available during arm exercise in comparison with that seen in AB individuals, who can use trunk and leg muscles for stabilisation (Hutzler *et al.*, 1998; van Loan *et al.*, 1987). Further to the loss of functional muscle mass, the physical capacity in SCI individuals is limited because of lost sympathetic control (Hoffman, 1986). Multiple regression procedures indicate that 48 – 80% of the variance in physical capacity (expressed as $\dot{V}O_{2peak,}$ aerobic and anaerobic power output, or isometric upper body strength) of SCI

individuals can be explained by lesion level and completeness of the spinal cord injury, activity level, gender, age, body mass, and time since injury, with lesion level being the most important determinant (Janssen *et al.*, 2002). Therefore, maximum oxygen uptake ($\dot{V}O_{2max}$) and other related physiological measures such as peak power output, ventilation rate and blood lactate concentration (BLa) are inversely related to lesion level – meaning that the higher the level of injury, the lower the peak responses and vice versa (Bhambhani, 2002). The lower aerobic capacity in SCI individuals is further magnified as $\dot{V}O_{2max}$ during upper body exercise in AB individuals is about 30% lower when compared with leg exercise (Nagle *et al.*, 1984). Anaerobic capacity and strength parameters show similar lesion level-dependent decreases as aerobic capacity (Janssen *et al.*, 2002; Hutzler *et al.*, 1998). This relationship is also reflected when comparing sporting classification with peak power output (Figure 2.3). This is of particular importance, as most wheelchair sports and everyday tasks require substantial anaerobic power (Hutzler *et al.*, 1998).

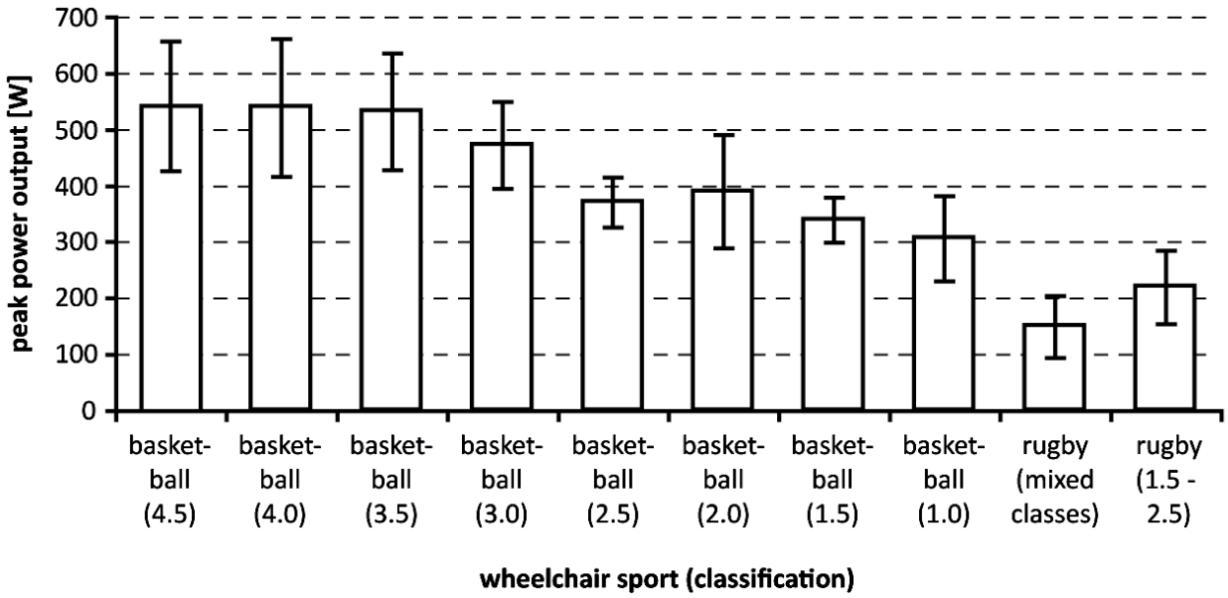

Figure 2.3 Peak power output dependency on sport and sporting classification.

Data from Wingate tests using arm crank ergometry. Note that the disability of wheelchair rugby is more severe than the disability of wheelchair basketball players, and that classification takes the severity of disability into account. It must be acknowledged that wheelchair basketball and rugby are not restricted to individuals with a spinal cord injury (further details on sports and classification are given in Chapter 2.3.3). Compiled from various sources (Molik *et al.*, 2010; Goosey-Tolfrey *et al.*, 2006; Morgulec *et al.*, 2005).

2.2.2 Cardiovascular adaptations

The distribution of blood during upper body exercise in SCI individuals is impaired because of a lack of sympathetic vasoconstriction below the lesion and a loss of motor innervation of the leg muscles, resulting in muscle pump inactivity. This causes an inability to redistribute blood below the lesion and as a consequence, a lower ventricular filling pressure during upper body exercise. The resulting lower stroke volume as a consequence of lower ventricular filling is compensated by an increase in HR (Janssen & Hopman, 2005). Further, due to their lower physical capacity, the cardiac output at the same relative workload is lower in PARA individuals when compared with AB individuals (Hopman *et al.*, 1993). However, using aids to improve blood redistribution (i.e. by applying counter-pressure to the legs with an anti-G suit – normally used by fighter pilots – or by exercising in the supine position to promote venous return) do not improve $\dot{V}O_{2max}$ (Hopman *et al.*, 1998). It has therefore been suggested that the limitation in $\dot{V}O_{2max}$ in the SCI population is located peripherally (i.e. the small muscle mass and the oxygen extraction by the muscle) rather than centrally (Janssen & Hopman, 2005). This is in line with findings in the AB population, where skeletal blood flow is thought to be the limiting factor in arm ergometry (Reybrouck *et al.*, 1975).

Further to the above adaptations, the autonomic innervation of the heart in individuals with lesion levels above the fourth thoracic (T4) segment is impaired (Jacobs & Nash, 2004), resulting in a lowered maximal HR of about 100 – 135 beats per minute (Goosey-Tolfrey *et al.*, 2006; Campbell *et al.*, 2004; Jacobs & Nash, 2004; Coutts *et al.*, 1983). As a consequence of the above mentioned lower ventricular filling, a decreased left ventricular heart mass (~25% reduction in comparison with AB individuals) and smaller left ventricular dimensions are found consistently in tetraplegic (TETRA) individuals (Janssen & Hopman, 2005; Kessler *et al.*, 1986). Furthermore, the linear relationship of HR and oxygen uptake ($\dot{V}O_2$) does not appear to exist in all TETRA individuals, hence the use of HR for training prescription in this population has been questioned (Valent *et al.*, 2007b).

2.2.3 Adaptations of the respiratory system

Respiratory muscles are, functionally and morphologically, skeletal muscles. Therefore, it is not surprising that the respiratory system is affected in SCI individuals. Both forced vital capacity and strength of the respiratory muscles are decreased depending on the lesion level in SCI individuals (Mueller *et al.*, 2008; Baydur *et al.*, 2001; Silva *et al.*, 1998; Hopman *et al.*, 1997). While low level PARA individuals are comparable with AB individuals with respect to respiratory function (forced vital capacity or forced expiratory flow in 1 s) and upper body strength, respiratory function in tetraplegia is markedly reduced (Haisma *et al.*, 2006). Dysfunctional muscles of inhalation prevent deep breaths, whereas dysfunctional muscles of exhalation result in impaired cough, which can lead to an accumulation of secretions that may arise from respiratory infections (Brown *et al.*, 2006). This poses a major problem in this population, as the most common cause of death is related to respiratory illnesses (Brown *et al.*, 2006; Frankel *et al.*, 1998). Hence, a number of studies have investigated methods to train the respiratory system in SCI individuals, which has been shown to be successful to improve respiratory muscle parameters, such as maximum inspiratory and/or expiratory strength, vital capacity and maximum voluntary ventilation (Goosey-Tolfrey *et al.*, 2010a; Verges *et al.*, 2009; Litchke *et al.*, 2008; van Houtte *et al.*, 2006; Liaw *et al.*, 2000; Derrickson *et al.*, 1992).

2.2.4 Autonomic function loss

The autonomic nervous system governs functions of the body that are beyond conscious control, such as secretion of sweat, hormones or enzymes, or the control of HR and digestion. It can roughly be divided into the parasympathetic and the sympathetic nervous system. Whilst the parasympathetic nervous system is involved in building up reserves and fortifying the body against times of needs and stress, such as slowing down HR or promoting digestion, the sympathetic nervous system is involved in "fight and flight" responses, such as accelerating HR or increasing blood pressure (Cannon, 1927). Most importantly in the case of a spinal cord injury, the neurons of the sympathetic nervous system are mainly located in the thoracic spinal cord (T1 – L1), whereas the bulk of parasympathetic neurons originates from the brain stem (Marieb & Hoehn, 2007). This means a complete cervical spinal cord injury leads to a complete disruption of sympathetic

signals sent from higher command centres. The resulting decreased sympathetic outflow in TETRA individuals has been documented previously: Symptoms include depressions of peak heart rate (HR_{peak}) as described above (Goosey-Tolfrey *et al.*, 2006; Campbell *et al.*, 2004; Jacobs & Nash, 2004; Coutts *et al.*, 1983), depressed adrenaline and noradrenaline plasma concentrations at rest and after exercise (Schmid *et al.*, 1998b; Schmid *et al.*, 1998c), a depression of the increase in adrenaline and noradrenaline plasma concentrations during exercise (Yamanaka *et al.*, 2010; Schmid *et al.*, 1998b; Schmid *et al.*, 1998c), or decreased sympathetic neuron activity (Stjernberg *et al.*, 1986).

2.3 Physical activity in the spinal cord injured population

Among SCI individuals, participation in sport is comparable with that in the AB population. In a pilot study conducted in the United Kingdom, 47% SCI individuals participated in physical activities, 20% in sport, 27% in recreation (Tasiemski *et al.*, 2000). An older research article describes participation in sport as the most popular leisure activity in a third of the studied SCI population (Sutton *et al.*, 1982). These values are similar to those observed in the AB population in the United Kingdom (Cochrane & Davey, 2008), Israel (Fogelman *et al.*, 2004) and the USA (Caspersen & Merritt, 1995), where about 30 – 40% of the population are considered to be regularly active.

Nonetheless, it has been noted by others that SCI individuals are one of the most physically inactive segments of society (Dearwater *et al.*, 1985): Physical activity levels are comparably low in free-living adults with chronic paraplegia (Buchholz *et al.*, 2003). This is partly due to various barriers for sport in SCI individuals, such as lack of accessible facilities and personal assistance, or unaffordable equipment (Kehn & Kroll, 2009). However, overcoming these barriers and participating in physical activity can be of great value for SCI individuals.

2.3.1 Benefits from physical activity

Participation in physical activity and sports has a significant impact on both aerobic and anaerobic capacity (Dallmeijer *et al.*, 1999; Hutzler *et al.*, 1998). Muscles which are

innervated above the lesion level retain their function and, therefore, remain as trainable as in the AB population. Hence, muscular strength significantly increases after resistance and circuit training in SCI individuals (Jacobs, 2009; Hicks *et al.*, 2003; Jacobs *et al.*, 2001). Muscular adaptations following strength training are thought to include improved motor unit activation, increased activity of anaerobic enzymes and increased muscular cross-sectional area (Jacobs, 2009).

Similar to the AB population, SCI individuals improve endurance parameters following a minimum of four weeks of training (Table 2.1). Underlying mechanisms for these improvements include increased capillary and mitochondrial density, oxidative enzyme activity, intramuscular substrate stores and a shift in the muscle fibre type distribution (Jacobs, 2009). Training has a positive impact on both maximal (Table 2.1) and submaximal parameters, such as HR, BLa or rating of perceived exertion (RPE), which are lower at given workloads following training at moderate intensities (Hooker & Wells, 1989). Due to the positive effects on endurance parameters, it was concluded that the amount of muscle mass in SCI individuals seems to be sufficient to elicit volume loading of the heart so as to stress central haemodynamic mechanisms (Devillard *et al.*, 2007). Importantly, the function of non-specifically trained muscles can improve as a result of general conditioning. For example, an improved respiratory function in SCI individuals was observed due to benefits on accessory inspiratory muscles after upper body training (Devillard *et al.*, 2007).

Improved fitness has an influence on everyday life: Physical capacity is inversely related with physical strain during activities of daily living, indicating that a low physical capacity can lead to high strain levels and concomitant fatigue or even make it impossible to perform certain activities (Janssen *et al.*, 2002). This observation is emphasised in individuals with higher lesion levels, implying that improving physical fitness in this group is of particular importance (Janssen *et al.*, 1994).

Finally, physical activity has positive impacts on social and psychological aspects of SCI individuals. Members of sport clubs find contact with a community of people with common interests and similar disabilities, which facilitates the handling of their disability and development of camaraderie (Goodwin *et al.*, 2009). Both quality of life and community

integration are higher among SCI individuals who perform sports when compared with their sedentary counterparts (McVeigh *et al.*, 2009).

Table 2.1 Effects of exercise training on endurance performance.

Participants	**N**	**Participant activity level**	**Exercise type and intensity**	**Weekly sessions**	**Duration of training**	**Performance gain**	**Reference**
TETRA and PARA (review analysing 69 studies)	1-90 (mean: 12)	not stated	60-80% HR_{peak}, 60-65%$\dot{V}O_{2peak}$	2-3	4-12 wk	increases in $\dot{V}O_{2peak}$ (extent not stated)	Hicks *et al.*, 2011
TETRA and PARA (review analysing 25 studies)	1-20 (mean: 10)	recreationally trained and untrained	40-90% HR reserve, HR_{peak}, PO_{peak}, $\dot{V}O_{2peak}$	3 or more x 20-120 min	4-32 wk	+18% $\dot{V}O_{2peak}$	Valent *et al.*, 2007a
various (review analysing 20 studies)	not stated	not stated	not stated	not stated	8-12	+10-20% $\dot{V}O_{2peak}$	Jacobs & Nash, 2004
PARA	9	not stated	arm cranking at 70-85% HR_{peak}	3 x 30 min	12 wk	+12% $\dot{V}O_{2peak}$	Jacobs, 2009
PARA	7	physically active	wheelchair exercise, 1 min at MTP, 4 min at ventilatory threshold	3 x 45 min	6 wk	+16% $\dot{V}O_{2peak}$	Bougenot *et al.*, 2003
PARA	5	physically active	wheelchair exercise at 50-80% MTP	3 x 30 min	4 wk	+19% $\dot{V}O_{2peak}$	Tordi *et al.*, 2001
TETRA	8	physically inactive	50-60% HR reserve	3 x 15-35 min	8wk	+99% $\dot{V}O_{2peak}$	DiCarlo, 1988
PARA	10	recreationally trained	arm cranking at 80% HR_{peak}	5 x 30 min	8 wk	+15% $\dot{V}O_{2peak}$	Taylor *et al.*, 1986

TETRA, tetraplegic; **PARA**, paraplegic; **HR**, heart rate, **$\dot{V}O_{2peak}$**, peak oxygen uptake; **PO**, power output; **MTP**, maximum tolerated power; **RPE**, rating of perceived exertion.

A lower occurrence of depression in SCI individuals was noted in those who perform sport on a regular basis, independently of the mode of exercise (i.e. wheelchair basketball, racing, tennis) and the type of disability (Muraki *et al.*, 2000). Moreover, increases in energy, self-confidence and quality of life are reported by SCI individuals participating in structured exercise training programmes, while health and fitness are identified as reasons for continued participation in exercise (Kehn & Kroll, 2009).

2.3.2 Training recommendations

The recommendations for endurance and strength training in SCI individuals do not differ substantially from the advice offered to the AB population: Three to five weekly exercise sessions of 20 – 60 min in duration and at an intensity of 50 – 80% $\dot{V}O_{2peak}$ is the recommended exercise prescription for individuals with paraplegia using various modes of exercise, such as arm cranking, wheelchair propulsion, swimming, wheelchair sports, circuit resistance training, electrically stimulated cycling, or electrically stimulated walking (Hicks *et al.*, 2011; Jacobs & Nash, 2004). Indeed, exercise programmes employing this exercise frequency and intensity lead to improvements in markers of physical capacity, such as $\dot{V}O_{2peak}$ (Table 2.1), power, mechanical efficiency, or the submaximal HR response (Devillard *et al.*, 2007).

It is possible that the impact of training interventions differs between individuals with different disabilities. For example, an individual with very little functional muscle mass (e.g., high level TETRA) may require more time to recover than an individual with comparably greater functional muscle mass (e.g., low level PARA) from a given exercise bout. Further research is therefore warranted to determine optimal training interventions for different disability subgroups. Because the body of evidence is rather small in this area, training recommendations are given at a considerably basic level. It is generally recommended that arm exercise programmes should follow those of leg exercises (Colivicchi *et al.*, 2002). Whereas $\dot{V}O_{2peak}$ is independent of the mode of exercise (i.e. arm cranking versus exercise on a wheelchair) (Price & Campbell, 1999; Arabi *et al.*, 1997; Tropp *et al.*, 1997), differences in efficiency between various propulsion methods exist: Arm cranking exercise is more efficient than wheelchair pushing, resulting in a higher maximal power output (Hintzy *et al.*, 2002; Price & Campbell, 1999; Tropp *et al.*, 1997).

However, to date there are no specific recommendations for these various types of exercise; it can only be assumed that differing types of training may result in differing physiological adaptations. Due to the limited number of high quality studies, it is concluded that further research is needed to explore and improve the impact of specific training programmes in the SCI population (Valent *et al.*, 2007a).

2.3.3 The rise of Paralympic sport

The popularity of wheelchair sport has been growing steadily over the years, since its first introduction at the Stoke Mandeville games in 1952. This is evident by the increasing number of disciplines; the number of summer sports for which medals were awarded has increased from six in 1952 to twenty at the Beijing Paralympic games in 2008 (Gold & Gold, 2007). Likewise, the number of competing athletes has increased from 130 in 1952 to 4.237 (from 164 countries) in the London 2012 Paralympic games. Since the Paralympic games held in Seoul (Korea) in 1988 (3,053 athletes from 61 countries), they have been hosted by the same city, in the same year and at the same venues as the Olympic competitions, making efforts to incorporate the Paralympic games into the Olympic movement clear. The Paralympic games today sport 20 disciplines, including both team (i.e. basketball, rugby) and individual sports (i.e. athletics, fencing, shooting, swimming) (IPC, 2011).

Whilst in the early days an athlete with a disability was only able to perform sport on a recreational basis, the advancements in wheelchair design (Ardigo' *et al.*, 2005) combined with greater funding opportunities and sports professionalism have resulted in a better quality of the sports. This is supported by both the sports scientist's and coaching support staff's analysis of objective markers of physical performance, which, when investigating $\dot{V}O_{2peak}$ as an example, have increased around two-fold within 30 years (Goosey-Tolfrey, 2005; Hullemann *et al.*, 1975).

Three different types of sport will be presented in the following section, as the population studied in this thesis consists of athletes competing in these disciplines. Further to a simple description of the sports, the physical demands and the impacts on physical health will be discussed.

Wheelchair basketball. Wheelchair basketball is played on a standard basketball court and baskets placed on standard height (3.05 m). It is a team sport played in manual wheelchairs and a standard basketball; points are scored in the same way as in AB basketball. A game consists of four ten-minute quarters; the game clock is started when the ball goes into play and is stopped at each stoppage in play. Wheelchair basketball is designed for athletes who have a physical disability that prevents running, jumping and pivoting, such as paraplegia, amputations, or joint and musculoskeletal conditions. A classification system assesses the functional physical ability of each player, ranging from 1.0 (least able) to 4.5 (most able). Each team fields five players, and the total permitted classification point value of all players on the court is 14.0 per team. This requires teams to field a mix of players with different classification point values and ensures as much as possible that the balance of players on both teams will be fair (IWBF, 2012). Wheelchair basketball was introduced to the Paralympic programme in Rome in 1960 and is one of the most popular sports in the Paralympic Games (IPC, 2011).

Time-motion analyses provide insight into the intermittent nature of wheelchair basketball (Bloxham *et al.*, 2001; Coutts, 1992). About 28% of the active portion of a game is spent performing high intensity anaerobic work, such as sprinting and contesting for the ball, and 22% of a game is played at an intensity above the ventilatory threshold – on the other hand, about 48% of the time is spent resting (Bloxham *et al.*, 2001). Further to the muscle work used for the acceleration of the wheelchair, it is important to acknowledge the substantial part of braking activity (36%) during basketball games, which should be considered when designing training programmes (Coutts, 1992). Most importantly from a training and health perspective, playing wheelchair basketball increases energy expenditure (Abel *et al.*, 2008), and it is generally accepted that wheelchair athletes are significantly fitter when compared with the sedentary SCI population (Bhambhani, 2002; Colivicchi *et al.*, 2002). Depending on the activity level, it is concluded that the energy expenditure during wheelchair basketball is sufficient to maintain (Abel *et al.*, 2008) and improve fitness (Bernardi *et al.*, 2010), and to prevent the development of cardiovascular diseases (Abel *et al.*, 2008).

Wheelchair rugby. Wheelchair rugby is a team sport, which is played in manual wheelchairs and with a standard volleyball on a court measuring 15 by 28 m. The aim is to beat the opponent by scoring goals. The ball can be passed or carried, and players are allowed to tackle their opponents or hinder their movement by strategically blocking their

wheelchairs. A point is scored when a player carries the ball across the opposing team's goal line at the end of the court. A game consists of four eight-minute quarters; the game clock is started when the ball goes into play and is stopped at each stoppage in play. Wheelchair rugby was introduced as a demonstration sport at the Atlanta 1996 Paralympic Games and became a full medal sport at the Sydney 2000 Paralympics (IWRF, 2012).

Most wheelchair rugby athletes have spinal cord injuries at the level of their cervical vertebrae, multiple amputations, polio or neurological disorders such as cerebral palsy and some forms of muscular dystrophy, among other medical conditions. Similar to wheelchair basketball, players are assessed by a classification system. They are classified on a scale according to their functional physical ability, with a range from 0.5 (least able) to 3.5 (most able). Each team can field four players at one time. The total permitted classification point value of all players on the court is 8.0 per team, which again should ensure fairness about the overall disability level in both teams (IWRF, 2012).

Wheelchair rugby is a game designed by TETRA athletes for TETRA athletes and as such the influence of players on the development of the sport was significant (Goodwin *et al.*, 2009). It is a sport dominated by frequent, intermittent, short-term power demands superimposed on a background of aerobic activity (Goosey-Tolfrey *et al.*, 2006). As the aerobic capacity in TETRA individuals is reduced in comparison with PARA individuals (Haisma *et al.*, 2006; van Loan *et al.*, 1987), it is not surprising that the energy expenditure during wheelchair rugby is lower when compared with wheelchair basketball (Abel *et al.*, 2008). As a consequence, wheelchair rugby players do not reach the minimal energy expenditure needed to maintain fitness based on recommendations for AB individuals (Abel *et al.*, 2008). However, as no recommendations for athletes with a disability exist (Abel *et al.*, 2008), these results must be interpreted with caution. Investigating this problem from another perspective, it is known that wheelchair rugby players cover considerable distances (3500 – 5650 m) during the course of a game (Sarro *et al.*, 2010). This is in the range of the distances covered (~5000 m) by wheelchair basketball athletes during a game (Coutts, 1992). In summary, due to their higher level of disability, energy expenditure in wheelchair rugby players is clearly lower in comparison with wheelchair basketball players, but their physical fitness is higher when compared with an inactive population group with similar disabilities (Goosey-Tolfrey *et al.*, 2006).

Wheelchair tennis. Wheelchair tennis is played in the open class (athletes with a range of disabilities, such as amputations or lower limb deformations) and the quad division (athletes with a tetraplegia or upper extremity disabilities) (ITF, 2011). The aerobic capacity of elite tennis players is found to be at a considerable level, however slightly lower than what is found in wheelchair basketball players. Match analyses reveal that average HR is lower in tennis when compared with basketball, which is partly due to the higher number of breaks between game actions (Croft *et al.*, 2010). Therefore, this sport is considered to be slightly less physical than wheelchair basketball at an elite level: Work to rest ratio during a game is about 15 – 20%, whereas this value in wheelchair basketball is 50% (Croft *et al.*, 2010). Preliminary findings using distance logging devices (Sporner *et al.*, 2009) during match play indicate that distances covered during a game are in the range of 1338 ± 370 m (low ranked players) – 1922 ± 945 m (high ranked players) for athletes competing in the open class (Sindall *et al.*, 2011).

2.4 Physiological testing of wheelchair athletes

The performance of Paralympic court sport athletes is highly dependent upon athletes' aerobic fitness (Bernardi *et al.*, 2010). Performance markers, such as speed at $\dot{V}O_{2peak}$ or mechanical efficiency, correlate with wheelchair racing performance (Cooper, 1992), whereas the ventilatory threshold correlates with average work intensity in the field (Bernardi *et al.*, 2010). Likewise, anthropometric factors such as arm length or range of motion correlate with wheelchair basketball performance (Wang *et al.*, 2005). Still, the literature is not as extensive as in AB sport, where, for example, a considerable number of researchers have investigated the physiological and morphological characteristics that determine success in AB football players (Castagna *et al.*, 2010; Mujika *et al.*, 2009; Nevill *et al.*, 2009).

Table 2.2 Selected ergometers and test protocols to establish peak performance in wheelchair athletes.

Ergometer and protocol details (all tests performed to exhaustion)	**N**	**Participant activity level**	**Body mass [kg]**	**$\dot{V}O_{2max}$ (relative in mL·kg^{-1}·min^{-1}; absolute in L·min^{-1})**	**Reference**
ACE: increasing load (15 W·2min^{-1})	12 PARA	recreational and elite athletes	63.5 ± 11.5	relative 31.1 ± 6.1	Al-Rahamneh & Eston, 2011b
ACE: increasing load (5 W·min^{-1})	8 TETRA	WR, WT elite athletes	71.6 ± 10.8	relative 13.5 ± 2.6	Goosey-Tolfrey *et al.*, 2006
ACE: increasing load (10 W·2min^{-1})	13 (11 PARA)	not stated	not reported	relative 19.0 ± 5.2 absolute 1.23 ± 0.35	Arabi *et al.*, 1997
WERG: increasing speed (0.5 m·s^{-1}·min^{-1})	12 (7 PARA)	WB elite athletes	74.7 ± 14.4	absolute 2.83 ± 0.53	Goosey-Tolfrey, 2005
WERG: increasing speed (1 km·h^{-1}·2min^{-1})	13 (11 PARA)	not stated	not reported	relative 19.0 ± 5.2 absolute 1.23 ± 0.35	Arabi *et al.*, 1997
WERG: increasing strike frequency (8 strikes·min^{-1})	7 (2 PARA, 5 TETRA)	not stated	58.7 ± 8.2	relative 25.6 ± 12.6 absolute 1.50 ± 0.99	Bhambhani *et al.*, 1991
WERG: increasing speed, resulting in increasing power output (~10 W·min^{-1})	9 (6 PARA)	elite athletes	63.9 ± 12.6 (all) 69.3 ± 12.1 (PARA)	relative 46.6 ± 11.1 (all) 42.9 ± 9.8 (PARA)	Coutts, 1990
TM: constant speed, increasing gradient (0.5%·2min^{-1}).	11 (8 PARA)	WB athletes	72.7 ± 16.9	relative 35.1 ± 4.9	Knechtle & Kopfli, 2001
TM: constant gradient, increasing speed (0.25 m·s^{-1}·min^{-1})	11 (3 PARA)	elite athletes	64.0 ± 14.0	relative 38.0 ± 6.0	Tropp *et al.*, 1997
TM: constant gradient, increasing speed (1 km·h^{-1}·2min^{-1})	13 (11 PARA)	not stated	not reported	relative 19.0 ± 5.2 absolute 1.23 ± 0.35	Arabi *et al.*, 1997
3 TM protocols: constant speed, increasing gradient / increasing speed, constant gradient / increasing speed and gradient	7 (5 PARA)	recreational athletes	73.4 ± 20.0	relative 24.5 ± 6.9	Hartung *et al.*, 1993

ACE, arm crank ergometer; **WERG**, wheelchair ergometer; **TM**, treadmill; **WR**, wheelchair rugby; **WT**, wheelchair tennis; **WB**, wheelchair basketball; **AB**, able-bodied.

The use of a wheelchair for propulsion is a unique feature not found in AB sport, and therefore, a range of ergometers is commonly used to assess submaximal and peak exercise responses for upper body exercise in general and wheelchair propulsion specifically (Bhambhani, 2011; Malone *et al.*, 2011; Bernardi *et al.*, 2010; Goosey-Tolfrey, 2005) (Table 2.2). Some of the laboratory testing procedures have been adapted from AB sports, such as the use of 4-min stages for submaximal intensities to elicit steady state conditions or for blood lactate profiling (Dallmeijer *et al.*, 2004). Similarly, the design of peak testing protocols has been adapted from the AB literature. Due to the reduced active muscle mass and the reduced $\dot{V}O_{2max}$ during upper body exercise, lower work increments are employed in these peak testing protocols when compared with lower body exercise (Table 2.2).

Similar limitations and benefits regarding test protocols and modalities apply in wheelchair athlete exercise testing as in AB exercise testing. For example, whilst power output can be accurately monitored with an arm crank ergometer, the mode of exercise is distinctively different to wheelchair propulsion. Also, the wheelchair ergometer and the treadmill both have the advantage of being able to accommodate the participants' wheelchair for testing, but only allow for pushing in a straight line. On the other hand, field-based testing procedures may be most ecologically valid, but do not allow exercise intensity to be as closely controlled as when using laboratory equipment.

Surprisingly, despite peak variables being important (in some contexts the most important) parameters, there seems to be a scarcity of studies looking into the reliability of peak variables. Whilst a few studies assessed the reliability of field-based tests (Goosey-Tolfrey & Tolfrey, 2008; Vanderthommen *et al.*, 2002; Vanlandewijck *et al.*, 1999), to the author's knowledge there is only one published laboratory study investigating this problem (Bhambhani *et al.*, 1991), which has limitations in terms of subject heterogeneity and participant number. In contrast, more information, even though not excessive, is found in AB populations, where the reliability of peak physiological parameters has been investigated in patient (Bhambhani *et al.*, 2003) and athletic populations (Leicht *et al.*, 2009; Weltman *et al.*, 1990b). New studies are therefore needed to further the knowledge on laboratory testing in wheelchair athletes to enhance the current body of evidence. Likewise, the impact of exercise on mucosal immune function in wheelchair athletes has

only been scarcely explored to date, which is an issue addressed in the field of exercise immunology.

2.5 Exercise immunology

Exercise immunology is a field that has been growing rapidly over the last 3 decades, with 95% of all existing peer reviewed publications being published since 1989 (Walsh *et al.*, 2011). As the term implies, exercise immunology is concerned with the impacts of exercise on immune function. In order to comprehend immunological responses to exercise, a basic understanding of the structure of the immune system and the underlying mechanisms of immune enhancement and suppression are required.

The immune system is divided into two major parts, the innate and the adaptive immune system. Innate defence mechanisms rely on detecting pathogens by their different structure when compared with bodily cells (for example, the cell membrane of gram-negative bacteria is distinctively different to human cells) or chemical signals released from infected or damaged cells. The responses of the innate immune system do not strengthen upon repeated pathogen exposure – in other words, the speed of the elimination of the pathogen does not increase. The adaptive immune system, however, is able to remember specific parts of a pathogen (for example, proteins presented on the cell surface), and repeated exposure results in a quicker immune response, and ultimately, faster elimination of the pathogen (Walsh *et al.*, 2011). Markers include circulating number of neutrophils, macrophages or natural killer cells for the innate immune system, and T and B cells, or immunoglobulins for the adaptive immune system. They are used to draw conclusions about the functioning of the immune system, with depressions and increases implying a worse or better host defence, respectively. Most importantly, it is not only the number of cells that define protection, but also their function, as expressed by cytotoxicity or reaction to pathogen exposure (Walsh *et al.*, 2011). As this section will focus on mucosal immune function, the reader is referred to the excellent textbook by Delves *et al.* (2006) for more details in the broader field of immunology.

2.5.1 Mucosal immune function

Salivary secretory immunoglobulin A (sIgA) is the predominant immunoglobulin in saliva and other mucosal secretions. It plays an important role in mucosal immunity and has therefore been described as 'first line of defence' against pathogens and antigens presented at the mucosa, such as cold-causing viruses (Walsh *et al.*, 2011; Bishop & Gleeson, 2009). sIgA is excreted by plasma cells (differentiated B cells) beneath the mucous membrane and then transferred through the epithelial wall into the lumen, where it forms part of the salivary gland excretion (Lamm, 1998) (Figure 2.4). The antimicrobial barrier is further supported by other components of the adaptive immune system (for example, other types of immunoglobulins) and by the innate mucosal immune system, consisting of other antimicrobial proteins such as α-amylase, lactoferrin, and lysozyme (Bishop & Gleeson, 2009).

The importance of a well-functioning mucosal immune defence is documented by the increased susceptibility to illness when immune parameters are depressed. For example, it was shown that sIgA concentration is low in children prone to respiratory infections (Lehtonen *et al.*, 1987). Likewise, sIgA concentration in children (measured when they were not ill) was found to be negatively correlated with the number of infections occurring throughout the year (Isaacs *et al.*, 1984). However, it is important to note that the majority of individuals with IgA deficiency show no obvious signs of vulnerability to infection, one probable reason being that the production of another immunoglobulin subclass is increased as a result (Woof & Kerr, 2006). Even though these results are drawn from a study analysing plasma immunoglobulin A (IgA), not sIgA, they should emphasize the significance of the redundancy of the immune system. Redundancy may pose a problem in studies of the immune system (or in this thesis in particular), as the investigator is limited to a finite number of parameters which can be analysed. With no control over other, potentially compensatory factors, it is difficult to draw conclusions about immune function status as a whole. This problem can to a certain extent be overcome by selecting parameters that have been shown to correlate with health measures, as outlined above.

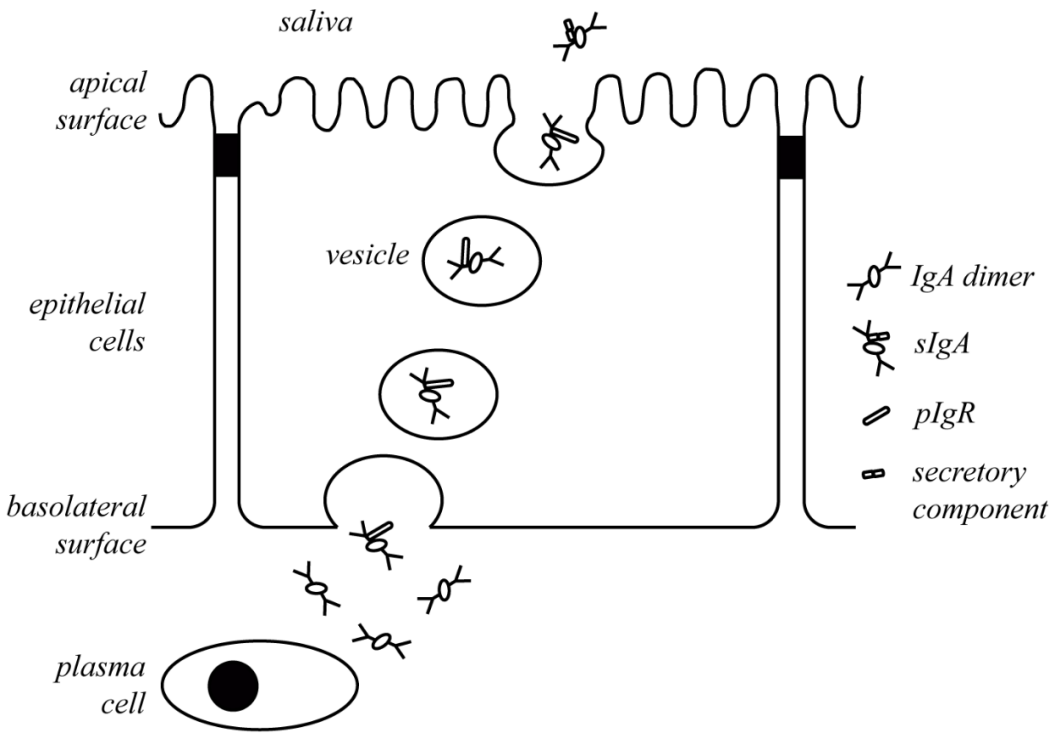

Figure 2.4 Epithelial transport of immunoglobulin A (IgA) into saliva.

IgA produced by local plasma cells binds to the polymeric immunoglobulin receptor (pIgR) at the basolateral membrane of the glandular epithelial cell After being transported through the cell, pIgR is cleaved, leaving its external domain, secretory component bound to IgA. This form of IgA is salivary secretory IgA (sIgA). Adapted from Bishop and Gleeson (2009).

2.5.2 The impact of exercise on mucosal immune function

Today, it is widely accepted that both acute and chronic exercise has the potential to alter markers of the immune system (Walsh *et al.*, 2011; Bishop & Gleeson, 2009). Likewise, hormonal changes occur in response to exercise, including increases in the plasma concentration of several hormones (e.g., adrenaline, cortisol or growth hormone) that are known to have immunomodulatory effects (Gleeson, 2007). Intensive exercise is associated with enhanced sympathetic nervous system activity, and it is known from laboratory studies in rodents that saliva composition and secretion of sIgA can be modified by both parasympathetic and sympathetic nerve stimulation (Proctor & Carpenter, 2007). Therefore, it seems logical to assume that physical activity could modify secretion of saliva

and its constituent proteins (Bishop & Gleeson, 2009). Indeed, decreased sIgA secretion rates were reported following bouts of strenuous exercise, whereas secretion rates of sIgA are generally unaffected following moderate intensity exercise (Table 2.3).

Table 2.3 Impact of acute exercise on sIgA secretion rate.

Type and intensity of exercise	**Duration of exercise**	**N**	**Participant activity level**	**sIgA secretion rate**	**Reference**
Repeated cycling bouts (each at limit of tolerance)	5 x 60 s	12	recreational	⇩52%	MacKinnon & Jenkins, 1993
Nordic skiing race, 20 and 50 km	not indicated	8	elite	⇩50%	Tomasi *et al.*, 1982
3 kayaking interval training sessions, rated as hard – very hard	~30 min	8	elite	⇩27 – 38%	Mackinnon *et al.*, 1993
Marathon race	~4.4 ± 0.6 h	98	recreational	⇩34%	Nieman *et al.*, 2002
70% $\dot{V}O_{2max}$, cycling	120 min	15	recreational	⇩20%	Walsh *et al.*, 2002
Continuous (10 $km \cdot h^{-1}$) and interval (6 – 21 $km \cdot h^{-1}$) soccer-specific running protocol	90 min	8	recreational	⇨	Sari-Sarraf *et al.*, 2006
20 1-min periods at 100% $\dot{V}O_{2max}$, cycling	60 min	8	recreational	⇨	Walsh *et al.*, 1999
60% $\dot{V}O_{2max}$, brisk walking	30 min	15	untrained	⇨	Nieman *et al.*, 2005
60% $\dot{V}O_{2max}$, cycling	120 min	8	recreational	⇨	Li & Gleeson, 2004
70% $\dot{V}O_{2max}$, cycling	90 min	11	endurance-trained	⇧24%, with caffeine ⇨ without caffeine	Bishop *et al.*, 2006
GXT, 50% $\dot{V}O_{2max}$, and 75% $\dot{V}O_{2max}$, cycling	22 min	10	recreational	⇧50% for GXT ⇨ for 50 and 75% $\dot{V}O_{2max}$	Allgrove *et al.*, 2008

Percentage changes compare post with pre exercise values. **sIgA**, salivary immunoglobulin A; **GXT,** graded exercise test to exhaustion. ⇧, increase; ⇨, no change; ⇩, decrease.

It is not fully understood whether immunosuppression as a result of hard exercise is caused by the reallocation of scarce metabolic components (O'Kennedy, 2000). It has been suggested that exercise-induced factors such as oxidative stress or the above mentioned changes in circulating hormones can worsen pathogen recognition by altering the expression of recognition molecules, altering haematopoieisis (the formation of blood cellular components), or affecting antigen processing and presentation (Walsh *et al.*, 2011). It is possible that the down-regulation of the immune system represents a mechanism to reduce the potential of an autoimmune response (O'Kennedy, 2000), but this remains speculative. In the field of mucosal immune function, exercise-induced depressions in pIgR messenger ribonucleic acid (mRNA) expression accompany the depressions of sIgA (Kimura *et al.*, 2008) This provides a mechanistic explanation for the reduced sIgA secretion rate following intense exercise, as reduced pIgR availability implies a reduced transcytosis of sIgA through epithelial cells (Figure 2.4). However, there seems to exist a threshold for changes to occur: When investigating mucosal immunity, both exercise intensity and duration seem to be critical in the downregulation of sIgA secretion rate (Table 2.3).

Whilst a number of studies did not find any relationships between upper respiratory symptoms (URS) and sIgA concentration (Cunniffe *et al.*, 2011; Gleeson *et al.*, 2000), longer lasting longitudinal studies were able to demonstrate that decreased resting sIgA concentration preceded the occurrence of URS (Neville *et al.*, 2008; Fahlman & Engels, 2005). Consequently, sIgA was suggested to be a clinical biomarker to predict incidence of URS (Fahlman & Engels, 2005). This is of practical relevance, as URS is one of the most common medical problems in elite AB athletes (Robinson & Milne, 2002), leading to missed training sessions and compromises in athletic performance. Further, according to some surveys, sore throats and flu-like symptoms are more common and colds may last longer in athletes than in the general population (Gleeson, 2007; Heath *et al.*, 1991; Nieman *et al.*, 1990). Positive relationships between URS incidence and high training loads were found in both athletic AB (Fahlman & Engels, 2005) and PARA (Furusawa *et al.*, 2007) populations. Furthermore, negative relationships between training load and sIgA concentration or training load and sIgA secretion rate were documented previously (Neville *et al.*, 2008; Fahlman & Engels, 2005).

2.5.3 Spinal cord injury and immune function

It is important to note that the field of exercise immunology is sparsely explored in the SCI population, and further to the study by Furusawa *et al.* (2007) discussed above there appear to be only a few more studies in SCI individuals: For example, it was found that arm-cranking exercise for 20 min at 60% $\dot{V}O_{2max}$ does not alter natural killer cell cytotoxic activity in TETRA individuals, whereas an increase was observed in the AB control group (Yamanaka *et al.*, 2010). On the other hand, natural killer cell number and cytotoxicity following 30 min of electrically stimulated exercise were increased in TETRA individuals, despite depressed resting levels (Nash, 1994).

Other published studies investigating immune function and spinal cord injury did not explore the influence of exercise on the responses: At rest, innate immunity (expressed as natural killer cell number) was shown to be depressed in the SCI population when compared with AB controls (Campagnolo *et al.*, 2008). The limited published work on IgA in SCI populations shows a similar increase in serum IgA following vaccinations when compared with AB controls (Lynch *et al.*, 2002). On the other hand, the production of IgA in the mucosa of the urinary tract was shown to be altered in SCI patients, with IgA being absent or largely depressed in more than half of the participants studied (Vaidyanathan *et al.*, 2000). Similarly, but using animal models, Proctor *et al.* (2000) showed that sympathetic decentralisation abolishes the increase in sIgA secretion normally found during parasympathetic stimulation.

As stated previously, the most common causes of death in SCI individuals are related to respiratory illnesses (Brown *et al.*, 2006), and individuals with a higher lesion level appear to be at a higher risk for pulmonary complications (Lucke, 1998). Underlying mechanisms include lesion-dependent losses of respiratory muscle innervation (Baydur *et al.*, 2001). This leads to impairments in respiratory muscle function, resulting in a decreased ability to cough and clear secretions, and as a consequence, various types of respiratory diseases such as pneumonia or respiratory failure (Brown *et al.*, 2006). Furthermore, uncoordinated autonomic control in TETRA individuals may be responsible for abnormal bronchial secretion, airway hypersensitivity, and other respiratory issues (Krassioukov, 2009). Therefore, furthering the knowledge of mucosal immune function and the impacts of exercise on immunity in the SCI population is of great practical relevance.

2.6 Conclusions

Physical activity and exercise training has a positive impact on SCI individuals, improving physiological factors, such as endurance and strength parameters (Buchholz *et al.*, 2009; Jacobs, 2009; Devillard *et al.*, 2007), and psychological factors, such as independence, self-esteem and confidence (Goodwin *et al.*, 2009; Kehn & Kroll, 2009; McVeigh *et al.*, 2009). However, there is evidence in the AB population that exercise can impact negatively on mucosal immune function, which can be depressed after strenuous exercise or long lasting periods of intensified training (Walsh *et al.*, 2011).

Given the rising profile of Paralympic sport (IPC, 2011) and the on-going development of recreational sport (Hettinga *et al.*, 2010) it is important that there is a sound scientific base to develop research in an exercise context for SCI individuals. This is even more important, as the majority of this population is inactive (Buchholz *et al.*, 2003; Tasiemski *et al.*, 2000; Dearwater *et al.*, 1985). Given the known benefits of physical activity, any impetus to initiate a training regimen would be favourable. On the other hand, training strategies which do not enhance or may even be detrimental to health must be investigated and defined. This is likely to be most relevant in athletes who have a high training load and/or engage in intense individual exercise sessions with potential negative consequences. Scientific evidence that can potentially inform annual training plans or individual exercise sessions to minimise any detrimental effects would help to maintain health and optimise performance in wheelchair athletes.

The SCI population is very specific due to functional (i.e. altered HR response and blood redistribution) and morphological (i.e. decreased heart mass) adaptations caused by the disability (Janssen & Hopman, 2005). As a result, findings related to exercise physiology and exercise immunology may be very specific for this population group – even more as within this population, the different lesion levels of a spinal cord injury can potentially affect physiological responses. Therefore, new data need to be generated and analysed because a simple adoption of principles based on findings in the AB population may be inadequate.

3

General methods

Several studies of this thesis required the use of similar methods (for example, oxygen consumption was measured in all laboratory studies, while sIgA was analysed in all studies investigating the mucosal immune function). To avoid repetition, methods used in more than one of the experimental chapters (Chapters 4 – 8) are described in this section and will only be described in short in the following experimental chapters.

3.1 Participant recruitment

The majority of participants were recruited through the support staff of British Wheelchair Basketball and the Great Britain Wheelchair Rugby Ltd. The remaining participants were recruited by approaching them individually. As a result, participants consisted of athletes only, competing at a minimum level of national competition.

3.2 Consent, preliminary questionnaires, and body mass

All studies were approved by Loughborough University's Ethics committee. Before testing, all participants provided written informed consent and completed separate health, training and disability characteristics questionnaires. Body mass was obtained to the nearest 0.1 kg using a wheelchair double-beam scale (300 series, Marsden, London, UK), in the clothing worn during exercise.

3.3 Treadmill testing

All laboratory exercise tests were performed on a motorised treadmill (HP Cosmos, Traunstein, Germany, Figure 3.1) in the participants' own individual sports wheelchairs. The wheelchairs were secured to a safety bar running alongside the treadmill, allowing the wheelchair to run freely back and forth, but preventing it from moving sideways. The back end of this safety bar was fitted with a spring to prevent the wheelchair from falling off the back. Touching this spring repeatedly further served as an indication of exhaustion, which will be described in detail in the studies investigating testing to exhaustion. Since tyre pressure can have an influence on rolling resistance (Sawatzky *et al.*, 2005), it was controlled before each test and set at levels participants would normally compete with (80 – 150 psi).

Figure 3.1 The treadmill used for laboratory testing.

3.4 Spirometric measurements

Spirometric data were recorded continuously using an online gas analysis system in breath to breath mode (MetaLyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany). Before each test, the system was calibrated according to the manufacturer's recommendations, gases using a 2-point calibration (O_2 = 17.0%, CO_2 = 5.0% against room air); volumes with a 3L-syringe at flow rates of 0.5 – 3.0 $L \cdot s^{-1}$. Data were recorded at a frequency of 1 Hz. Any $\dot{V}O_2$ data points lying outside the local 60 s rolling average were excluded, in line with methods employed earlier (Rossiter *et al.*, 2006; Day *et al.*, 2003) (Figure 3.2). For submaximal testing procedures where data was obtained in 4-min exercise blocks of a constant speed, data was averaged over the final minute. For peak testing, the peak value was defined as the highest 30 s average value achieved in the test.

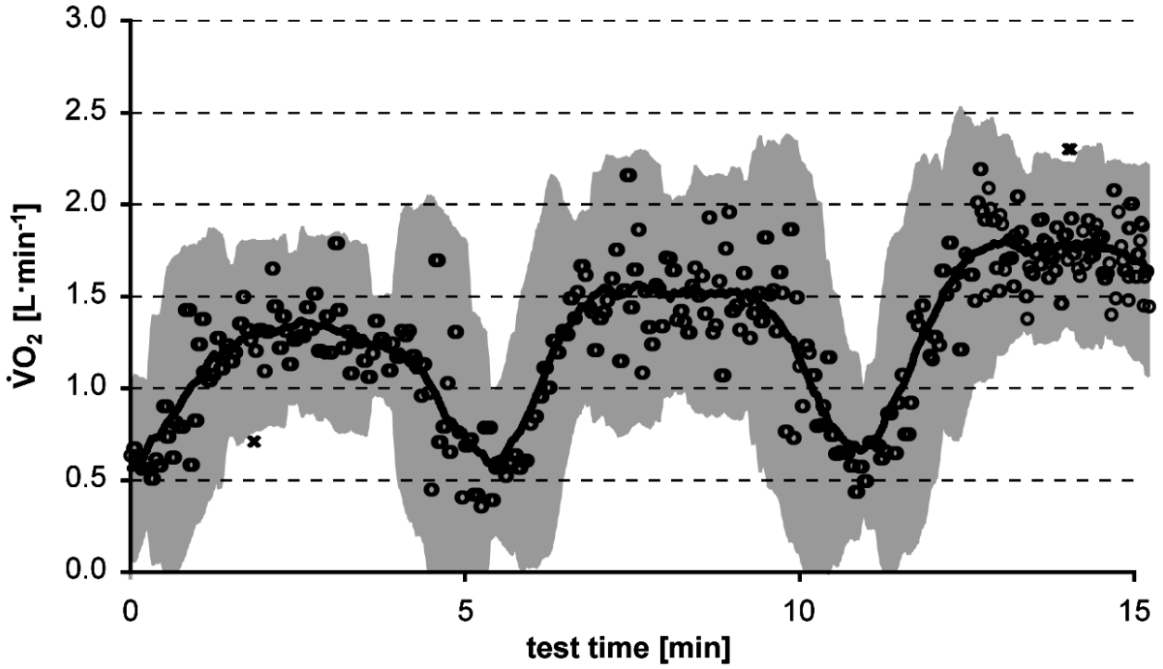

Figure 3.2 Oxygen uptake data processing.

Example data obtained in three submaximal 4-min exercise blocks. Raw data points and average line, the shaded area spans three standard deviations around the 60 s rolling average. **Circles**, data points included; **crosses**, data points excluded to calculate rolling average. **$\dot{V}O_2$**, oxygen uptake.

3.5 Blood lactate analysis

Blood samples for lactate analysis were taken from the earlobe, using a safety lancet (Sarstedt Ltd, Leicester, UK) and a heparinized capillary tube. In all laboratory studies, BLa was analysed using a lactate analyser (YSI 1500 SPORT, YSI Incorporated, Ohio, USA), which was calibrated before each test using a lactate standard solution of 5 mmol·L^{-1} provided by the manufacturer.

3.6 Heart rate data collection

HR was continuously recorded at 5-s intervals using Polar belts (Polar, Kempele, Finland). A Polar PE 4000 monitor watch was used for the laboratory studies, and a Polar team system for the field-based study, which allowed simultaneous collection of HR data from all participants without the participants wearing monitor watches.

3.7 Salivary parameters

Timed, unstimulated saliva samples were collected into sterile plastic containers. For this, participants rinsed their mouth with water and sat still with their head slightly tilted forward with minimal orofacial movement. After an adequate sample volume was obtained (~1.0 mL, usually collected within 2 – 5 min), participants were asked to collect all saliva remaining in the oral cavity and expectorate it in the containers provided. In the laboratory study, saliva samples were frozen and stored at -20 ºC immediately following collection. In the field-based studies, samples were stored on ice immediately after collection and stored at -20 °C within 6 h.

Analytical methods. Samples were defrosted and weighed to the nearest 10 mg. Saliva volume was estimated assuming saliva density to be 1.00 g·mL^{-1} (Cole & Eastoe, 1988) and saliva flow rate calculated from saliva volume and collection time. Samples were then spun for 2 min at 13,400 rpm. A sandwich ELISA was conducted using flat-bottomed microtitration plates (Nunc-Immunoplate, Thermo Fisher Scientific, Denmark). After coating the plates with rabbit anti human capture antibody (Dako UK, Ely, UK), they were

washed and blocked with a blocking protein solution (2.0% bovine serum albumin in phosphate buffered saline (PBS)). Depending on saliva flow rate, saliva samples were diluted in PBS by 1:375 – 1:2000 (samples with a lower flow rate were diluted by a higher factor). Purified secretory IgA from colostrum was used as a standard (Sigma-Aldrich, St. Louis, USA). Duplicate samples of 50 µl were applied to the plates and incubated overnight at 4 ºC. After washing the plates, detection antibody (Dako UK, Ely, UK) was applied and plates were incubated at 25 ºC for 90 min. After a final wash, a colouring substrate (OPD substrate, Dako UK, Ely, UK) was added, and the absorbance of the individual samples was determined spectrophotometrically at 490 nm (Opsys MR, Dynex Technologies Inc., Chantilly, USA). SIgA secretion rate was calculated by multiplying sIgA concentration with saliva flow rate.

Salivary α-amylase activity was measured using the same spectrophotometer and microtitration plates mentioned above. Briefly, 20 µl saliva diluted 1:100 in 1.0 mmol·L^{-1} $CaCl_2$ were mixed with 180 µl of amylase reagent (Infinity amylase, Thermo Electron, Melbourne, Australia). The plate was incubated at 25 °C and the increase in absorbance at 405 nm was recorded for minutes 1 and 3. The difference in absorbance per minute was multiplied by 1994, which is a reagent and temperature specific factor provided by the manufacturer of the amylase reagent.

All samples from the same participant were analysed in duplicate on one microplate, and the average was reported. For each study separately, coefficients of variation (CV) were calculated from these duplicate samples. CVs are presented in the respective chapters.

4

Study 1: The verification phase and reliability of physiological parameters in peak testing of elite wheelchair athletes

This chapter has been published in slightly modified form in the *European Journal of Applied Physiology*: Leicht C.A., Tolfrey K., Lenton J.P., Bishop N.C. & Goosey-Tolfrey V.L. The verification phase and reliability of physiological parameters in peak testing of elite wheelchair athletes. *Eur.J.Appl.Physiol.*, accepted June 02 2012.

4.1 Abstract

The aims of this study were 1) to examine the value of a verification phase (VER) in a peak testing protocol and 2) to assess the reliability of peak physiological variables in wheelchair athletes.

Methods: On two separate days, eight tetraplegic (TETRA), eight paraplegic (PARA) and eight non-spinal cord-injured (NON-SCI) athletes performed a graded exercise test to exhaustion (GXT) on a treadmill, followed by a VER. Peak oxygen uptake ($\dot{V}O_{2peak}$) was compared 1) between GXT and VER and 2) between test days.

Results: $\dot{V}O_{2peak}$ did not differ between GXT and VER (P = 0.27), and coefficients of variation (CVs) between GXT and VER were in the range of 2.9 and 6.4% for all subgroups. Coefficients of variation of $\dot{V}O_{2peak}$ between test days were 9.3% (TETRA), 4.5% (PARA) and 3.3% (NON-SCI).

Conclusions: Whilst a VER can be used for a more robust determination of $\dot{V}O_{2peak}$, a CV of up to ~6% between GXT and VER should be considered as acceptable. For between day analyses, relatively large changes in $\dot{V}O_{2peak}$ are required to confirm "true" differences, especially in TETRA athletes. This may be due to their lower aerobic capacity, which results in a larger relative variation compared with the other subgroups.

4.2 Introduction

$\dot{V}O_{2max}$ is defined as the highest rate at which oxygen can be taken up and utilised during high-intensity exercise and is one of the main variables in exercise physiology to describe physical capacity related to endurance performance (Levine, 2008; Bassett & Howley, 2000). In both cross-sectional and longitudinal scenarios, $\dot{V}O_{2max}$ helps to distinguish the aerobic capacity (as a measure of cardiorespiratory fitness or training status) between individuals (van der Woude *et al.*, 2002; Veeger *et al.*, 1991) or during the course of a training or rehabilitation period (Devillard *et al.*, 2007; Goosey-Tolfrey, 2005). Since $\dot{V}O_{2max}$ is such an important measure, it has been reviewed extensively (Levine, 2008; Bassett & Howley, 2000). Furthermore, the validity, reliability, and sensitivity of protocols to establish $\dot{V}O_{2max}$ have been studied in detail (Kirkeberg *et al.*, 2011; Currell & Jeukendrup, 2008; Bar-Or & Zwiren, 1975).

Historically, the primary criterion of a valid $\dot{V}O_{2max}$ has been a plateau in oxygen uptake with increasing workload (Howley *et al.*, 1995). However, it has been pointed out that this plateau is not evident in all individuals (Day *et al.*, 2003; Bassett & Howley, 2000), especially during upper-body exercise (Smith *et al.*, 2006). Consequently, the term $\dot{V}O_{2peak}$, rather than $\dot{V}O_{2max}$, is commonly used to describe the highest achieved $\dot{V}O_2$, which does not require a plateau phase by definition (Rossiter *et al.*, 2006). In the absence of a plateau, secondary criteria have been utilised, such as defined values of peak respiratory exchange ratio (RER_{peak}), peak blood lactate concentration (BLa_{peak}) or HR_{peak}. Again, upper-body exercise has the potential to evoke different responses in these parameters, which may further be affected in populations with a disability, where a reduced muscle mass and potentially disrupted autonomic innervation may have a profound impact on them (Campbell *et al.*, 2004; Bhambhani *et al.*, 1994).

Performing a verification phase (VER) following a GXT can be used to confirm the $\dot{V}O_{2peak}$ obtained in a GXT (Scharhag-Rosenberger *et al.*, 2011; Midgley & Carroll, 2009; Midgley *et al.*, 2006; Rossiter *et al.*, 2006). Including a VER into a test protocol therefore generates data to complement the "weaker" secondary criteria. This may be of special significance in populations with a disability where these secondary criteria are potentially

compromised. However, there is a paucity of research data investigating this issue in wheelchair ergometry.

Reliability and validity of peak variables have been investigated in wheelchair sport previously; however, most of this research has been conducted in field-based settings (Goosey-Tolfrey & Tolfrey, 2008; Vanlandewijck *et al.*, 1999). In contrast to the abundance of AB literature, there seems to be very limited and dated literature analysing the reliability of peak physiological responses in a more tightly controlled (i.e. laboratory) environment investigating upper-body exercise (Price & Campbell, 1997; Bar-Or & Zwiren, 1975) or wheelchair dependent populations (Bhambhani *et al.*, 1991). Given the advancement of wheelchair sport in recent years, it is somewhat surprising as $\dot{V}O_{2peak}$ is widely used in these populations both for between-group comparisons and also to demonstrate longitudinal intervention effects (Valent *et al.*, 2007a). Therefore, the purpose of this study was twofold: 1) To examine whether a VER can confirm the $\dot{V}O_{2peak}$ measured in a preceding GXT in wheelchair ergometry peak testing and; 2) To investigate the reliability of peak physiological variables obtained in a GXT, followed by a VER in three subgroups: TETRA, PARA and NON-SCI wheelchair athletes. It is hypothesised that $\dot{V}O_{2peak}$ obtained in a GXT can be confirmed by performing a VER and that $\dot{V}O_{2peak}$ can be reliably measured in these subgroups.

4.3 Methods

4.3.1 Participants

Twenty-four trained male wheelchair athletes volunteered to participate in this study. They were grouped into three subgroups of eight individuals; 1) TETRA (motor complete), 2) PARA (motor complete) and 3) NON-SCI (including disabilities such as amputations, club foot, brittle bones). All athletes performed their respective sports to a minimum standard of national level competition with 70% competing at an international level. A summary of their physical and physiological characteristics is presented in Table 4.1. To compare the body size-independent $\dot{V}O_{2peak}$ values, the mass exponent of 0.82 was adopted (i.e., $mL \cdot kg^{-0.82} \cdot min^{-1}$) as described by Goosey-Tolfrey *et al.* (2003).

Table 4.1 Participants' characteristics.

Parameter	TETRA	PARA	NON-SCI
Body mass [kg]	68.0 ± 6.7	67.6 ± 12.0	76.3 ± 11.6
Age [years]	28.1 ± 5.2	31.7 ± 8.7	24.0 ± 6.2
Lesion level/ disability types	C6 – C7	T4/5 – T12, Spina bifida, polio	Amputations, hip dysplasia, club foot, brittle bones
Wheelchair sport	Rugby (N = 8)	Basketball (N = 8)	Basketball (N = 7) Tennis (N = 1)
Wheelchair sport experience [years]	6.9 ± 5.2	12.3 ± 5.1	8.0 ± 5.8
Training volume [h·week^{-1}]	14 ± 5	12 ± 5	11 ± 6
$\dot{V}O_{2peak}$ [L·min^{-1}]	1.57 ± 0.35*†	2.47 ± 0.34	3.06 ± 0.63
$\dot{V}O_{2peak}$ [mL·kg^{-1}·min^{-1}]	23.1± 4.2*†	37.1 ± 5.8	39.9 ± 3.3
$\dot{V}O_{2peak}$ [mL·kg$^{-0.82}$·min^{-1}]	49.3 ± 9.3*†	78.9 ± 11.0	87.0 ± 8.6
GXT time to fatigue [s]	516 ± 148	640 ± 100	554 ± 40

With the exception of lesion level/disability types and sport, data are means ± standard deviation. All exercise data presented are from the first graded exercise test to exhaustion (GXT-1). *Significantly different from PARA; †significantly different from NON-SCI; at $P < 0.05$ level.

4.3.2 Experimental design

Athletes attended the laboratory on two separate days to perform identical procedures on both occasions at the same time of day. Visits were separated by 7.3 ± 2.9 days. A 24-h food diary was provided and athletes were asked to eat and drink the same types and amounts of food before both visits, to abstain from caffeine and to avoid any exercise 24 h before the tests. Questionnaires were completed and body mass recorded as explained in Chapter 3.2. All exercise tests were performed in the athletes' sports wheelchair on a motorised treadmill, with details described in Chapter 3.3.

Athletes completed a discontinuous sub-maximal test consisting of six to eight 4-min stages with increments of speed, covering a range of ~40 – 80% $\dot{V}O_{peak}$. This 30 – 40-min

habituation period also served as an active warm-up. Following a 15-min break, athletes completed a GXT at a constant speed, which was chosen according to the responses elicited during the discontinuous sub-maximal test. The gradient at the start of the GXT was 1.0% for all subgroups; this was then increased by 0.3% per min for the PARA and NON-SCI, and by 0.1% per 40 s for the TETRA athletes (Figure 4.1A). After the GXT, athletes sat quietly for 2 min, then performed an active recovery at a low exercise intensity (1.0 $m \cdot s^{-1}$ at 1.0% gradient) for 5 min. Following this active recovery period, they performed a VER, designed as a test to exhaustion at the same constant speed but at a gradient which was constant, but higher than the maximal gradient achieved during the GXT (+0.6% for PARA and NON-SCI; +0.3% for TETRA). The GXT and the VER were terminated when athletes were unable to maintain the speed of the treadmill, i.e., when athletes had touched the spring of the safety bar for the third time. Verbal encouragement was given throughout the tests.

Before, immediately after the GXT and immediately after the VER a capillary blood sample was obtained from the earlobe in order to measure BLa using a lactate analyser (YSI 1500 SPORT, YSI Incorporated, Yellow Springs, Ohio, USA). HR was recorded continuously using a HR monitor (Polar PE 4000, Polar, Kempele, Finland), whereas respiratory data were recorded continuously, at a sampling frequency of 1 Hz, using a calibrated online gas analysis system (MetaLyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany). Immediately after the GXT and the VER, athletes were asked to indicate their RPE using a scale ranging from 6 – 20 (Borg, 1982). Time to fatigue for both the GXT and the VER were noted to the nearest second.

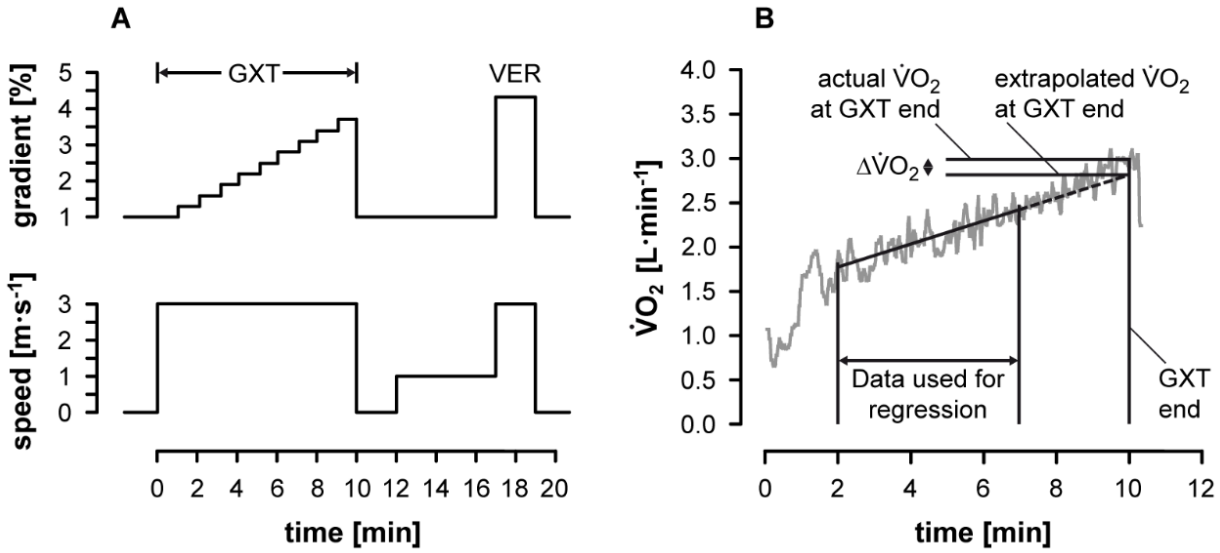

Figure 4.1 Test protocol and $\dot{V}O_2$ data analysis procedure.

A: Example for an athlete performing the exercise tests at 3 $m \cdot s^{-1}$, terminating the graded exercise test to exhaustion (GXT) at 10 min and the verification phase (VER) at 19 min. **B:** Analysis of $\dot{V}O_2$ raw data obtained in the GXT. $\Delta\dot{V}O_2$ is defined as difference between the extrapolated $\dot{V}O_2$ data at the point of exhaustion and $\dot{V}O_2$ measured at the end of the GXT.

4.3.3 Data processing and statistical analyses

Breath-by-breath data for each individual trial were edited as described in the general methods section (Chapter 3.4). A linear regression of the $\dot{V}O_2$-time data was then performed through the linear portion of the $\dot{V}O_2$-time data, i.e., after excluding the initial 2 min (to avoid the influence of $\dot{V}O_2$ kinetics on the early response) and the final 3 min (to avoid the influence of a possible $\dot{V}O_2$ plateau (Rossiter *et al.*, 2006) or excess $\dot{V}O_2$ (Smith *et al.*, 2006) at the end of the test). This linear fit was then extrapolated to the end of the GXT, and the difference to the 30 s $\dot{V}O_2$ average immediately before exhaustion was noted (Figure 4.1B). A negative deviation of < -0.1 $L \cdot min^{-1}$ was defined as plateau response, a positive deviation of > 0.1 $L \cdot min^{-1}$ as excess $\dot{V}O_2$ response, and any deviation within 0.1 $L \cdot min^{-1}$ as linear response (Day *et al.*, 2003).

For determination of peak data, $\dot{V}O_2$ and the respiratory exchange ratio (RER) were computed as rolling 30 s averages throughout the tests, and HR as 5 s averages. Four peak

values were obtained for each of these parameters: One each for the data obtained in the GXT measured on each respective day (GXT-1 and GXT-2), and in analogy, one each for VER-1 and VER-2. Furthermore, for every parameter the peak value of a particular day was defined as the higher value obtained in either the GXT or the VER for each day separately.

The SPSS 19 statistical package (SPSS Inc., Chicago IL, USA) was used for all statistical analyses. Means and SD were computed for all variables. Normality was checked with the Shapiro Wilk and homogeneity with Levene's statistic. Participants' characteristics were examined using a one-way analysis of variance (ANOVA). A logarithmic transformation was applied to the BLa data, as these violated normality and homogeneity assumptions required for parametric testing. A two-way (group x test) repeated measures ANOVA was then applied to all peak data obtained in GXT-1, VER-1, GXT-2 and VER-2. This was with the exception of RER and GXT time to fatigue data, where, due to violations of assumptions required for parametric testing, multiple Bonferroni-corrected Wilcoxon signed rank tests were used. Significant test effects of the ANOVAs were analysed in more detail using two-way (group x test) Bonferroni-corrected ANOVAs, focusing on dual comparisons of the four peak values. Intraclass correlation coefficients (ICC) and coefficients of variation (CV) were computed for peak variables. After applying a logarithmic transformation to account for heteroscedasticity, Bland-Altman plots were created for the VER – GXT comparisons of $\dot{V}O_{2peak}$ data. Limits of agreement (LoA) were therefore presented as ratios rather than ranges (Bland & Altman, 1999), a ratio >1.0 indicating a higher value in the VER. The spread of the residuals in these plots were compared between days using a two-way (group x day) ANOVA. Finally, all data were pooled, and the correlation of VER time to fatigue vs. VER-$\dot{V}O_{2peak}$ – GXT-$\dot{V}O_{2peak}$ ratio was examined. Statistical significance for all analyses was accepted at $P < 0.05$.

4.4 Results

Whilst $\dot{V}O_{2peak}$ did not differ between tests in all subgroups (P = 0.27, Figure 4.2), the CV comparing $\dot{V}O_{2peak}$ between days was two- to threefold higher in the TETRA subgroup when compared with the other subgroups (Table 4.2). Furthermore, VER time to fatigue was significantly greater in TETRA athletes when compared with the other subgroups (P < 0.01, Figure 4.3D). Interestingly, despite no difference in $\dot{V}O_{2peak}$, time to fatigue was significantly increased in GXT-2 when compared with GXT-1, with an increase of 46 ± 69 s in all subgroups (P < 0.01). Athletes of all subgroups showed all types (plateau, linear relationship, and excess $\dot{V}O_2$) of $\dot{V}O_2$-kinetics in the GXT (Table 4.3). Reliability analysis further revealed 95% LoA ratios of 1.00 ×/÷ 1.16 and 0.98 ×/÷ 1.11 when comparing $\dot{V}O_{2peak}$ measured in the VER and the GXT for days 1 and 2, respectively (Figure 4.3A/B), heteroscedasticity not being apparent (R = 0.25, P = 0.24 and R = -0.06, P = 0.77 for days 1 and 2, respectively). The spread of the residuals was not significantly different between test days (P = 0.09), indicating no improvement of the variation of the LoA ratio between day 1 and day 2.

Athletes with a high aerobic capacity tended to exhibit a lower $\dot{V}O_{2peak}$ in the VER when compared with the $\dot{V}O_{2peak}$ in the GXT (Figure 4.3A/B). This observation is confirmed by the significant proportional systematic bias of the pooled data between the LoA ratio (VER-$\dot{V}O_{2peak}$ vs. GXT-$\dot{V}O_{2peak}$) and GXT-$\dot{V}O_{2peak}$ (R = -0.45, P = 0.03 and R = -0.43, P = 0.04 for days 1 and 2, respectively). However, it must be noted that short VER time to fatigue resulted in a reduced VER-$\dot{V}O_{2peak}$ when compared with the GXT-$\dot{V}O_{2peak}$ (Figure 4.2D). The correlation between VER-$\dot{V}O_{2peak}$ – GXT-$\dot{V}O_{2peak}$ ratio and VER test time was significant when including all data (R = 0.51, P < 0.001); however, excluding VER time to fatigue below 90 s (N = 14) and 100 s (N = 21) reduced the significance of this relationship. This means VER time to fatigue did not have a systematic influence on the VER-$\dot{V}O_{2peak}$ – GXT-$\dot{V}O_{2peak}$ ratio in athletes who were able to exercise for 100 s or longer.

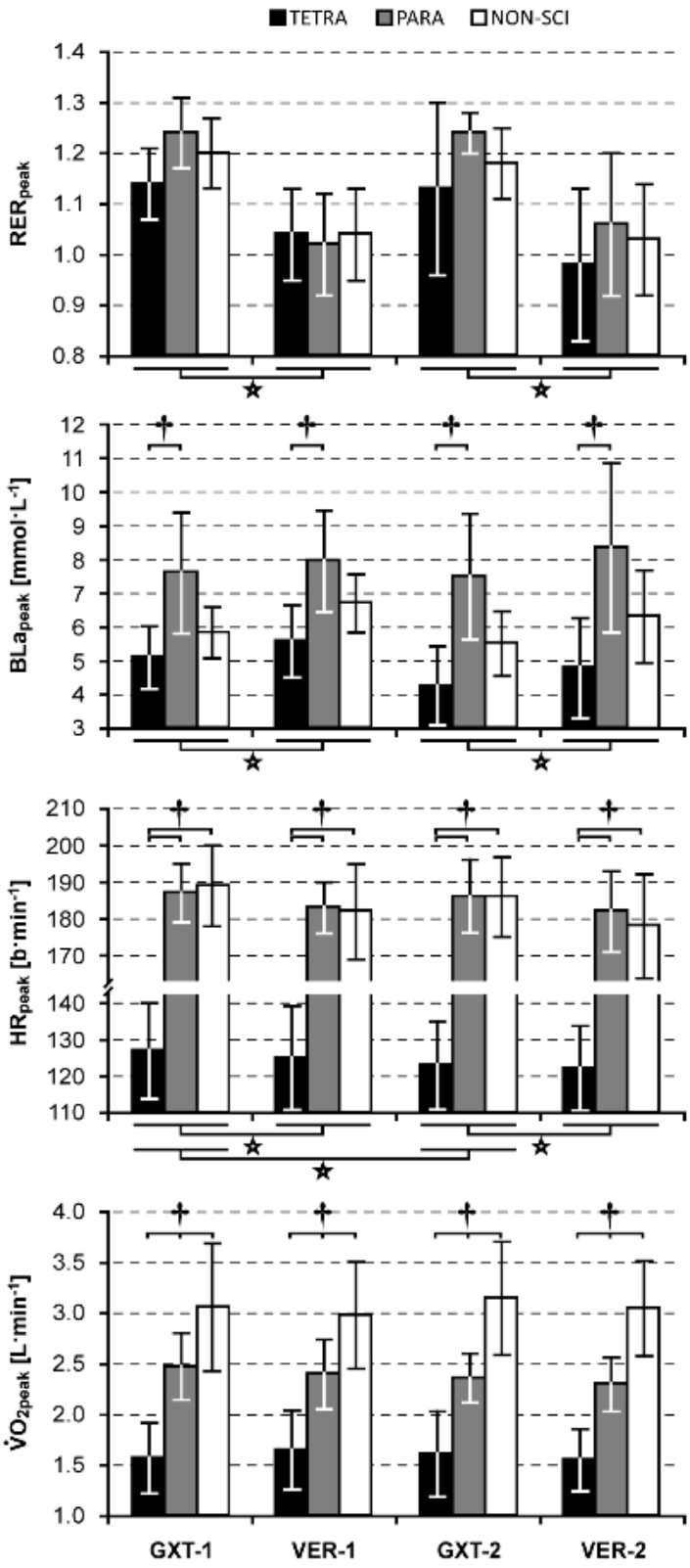

Figure 4.2 Main parameters derived in peak exercise testing.

Values are means ± SD. **VER**, verification phase; **RER**, respiratory exchange ratio; **BLa**, blood lactate concentration; **HR**, heart rate. Significantly different between ★tests and †subgroups; at $P < 0.05$ level.

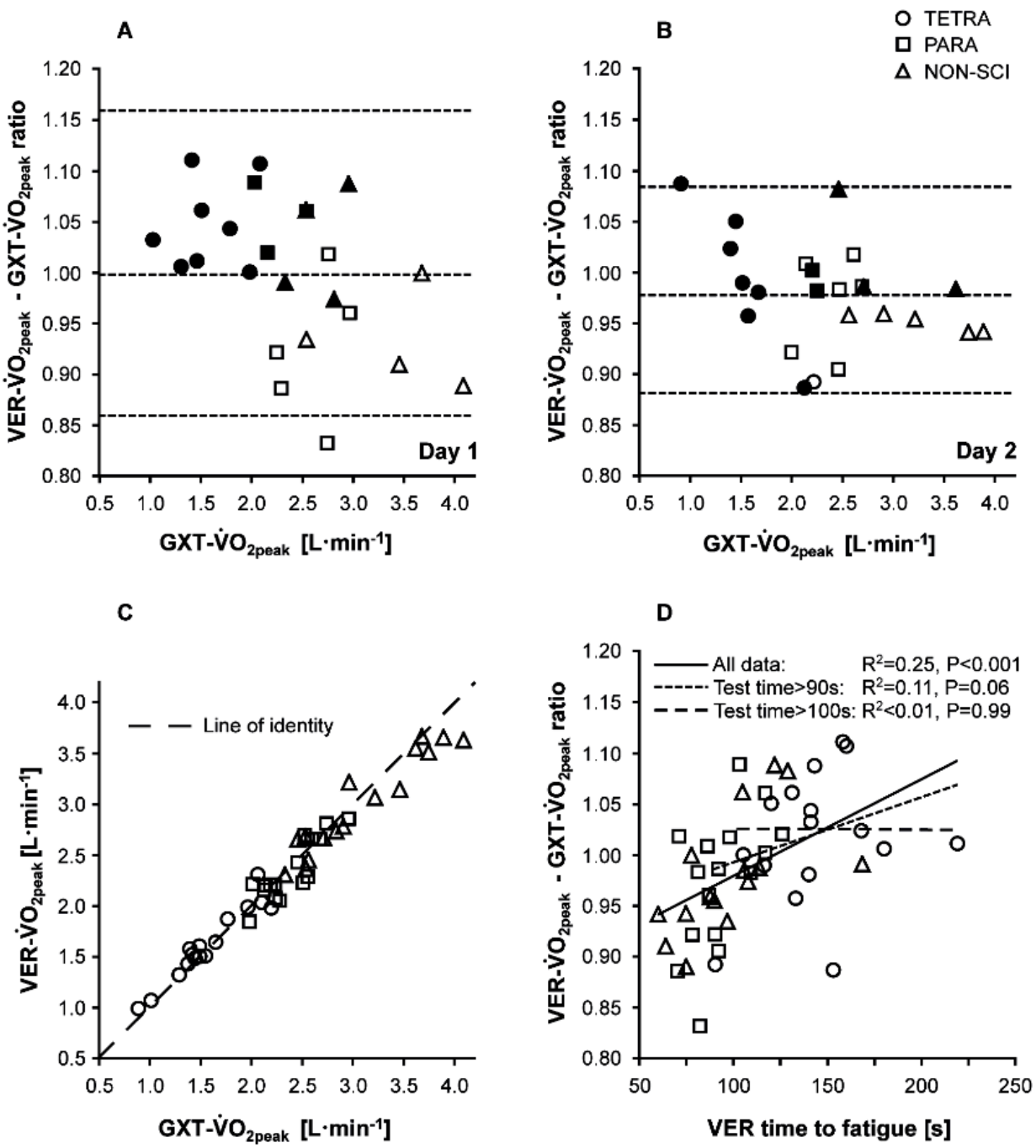

Figure 4.3 Reliability analysis.

Bland-Altman plots of the comparison of $\dot{V}O_{2peak}$ between the GXT and VER for the first (**A**) and the second (**B**) day. Dashed lines, mean and limits of agreement; solid symbols, VER test time >100s; open symbols, VER test time <100s. **C:** Comparison of $\dot{V}O_{2peak}$ between the GXT and the VER for all tests (n = 48). **D:** Influence of VER test time on the GXT-$\dot{V}O_{2peak}$ – VER-$\dot{V}O_{2peak}$ ratio for all tests (n = 48), data points and corresponding regression lines.

Table 4.2 Reliability measures for key physiological variables in peak testing.

Measure	Parameter	Subgroup	GXT-1 vs. GXT-2	GXT-1 vs. VER-1	GXT-2 vs. VER-2	Day 1 vs. Day 2
Intraclass correlation coefficient	$\dot{V}O_{2peak}$	TETRA	0.86	0.99	0.96	0.90
		PARA	0.82	0.84	0.96	0.90
		NON-SCI	0.98	0.95	0.98	0.98
	RER_{peak}	TETRA	0.16	0.51	0.90	0.34
		PARA	0.30	0.29	0.34	0.30
		NON-SCI	0.80	-0.14	0.62	0.80
	BLa_{peak}	TETRA	0.28	0.85	0.85	0.67
		PARA	0.81	0.86	0.79	0.34
		NON-SCI	0.41	0.14	0.85	-0.34
	HR_{peak}	TETRA	0.95	0.96	0.97	0.96
		PARA	0.96	0.95	0.95	0.96
		NON-SCI	0.95	0.97	0.95	0.95
Coefficient of variation [%]	$\dot{V}O_{2peak}$	TETRA	10.7	3.5	5.7	9.3
		PARA	6.0	6.4	2.9	4.5
		NON-SCI	3.1	5.5	3.1	3.3
	RER_{peak}	TETRA	10.6	5.4	5.8	9.5
		PARA	4.0	6.8	7.4	4.0
		NON-SCI	2.9	7.3	5.6	2.9
	BLa_{peak}	TETRA	20.2	8.6	13.7	15.8
		PARA	12.2	9.4	14.9	21.1
		NON-SCI	12.3	12.2	9.1	19.6
	HR_{peak}	TETRA	2.6	2.6	2.1	2.4
		PARA	1.3	1.2	1.6	1.2
		NON-SCI	1.6	1.5	1.9	1.6

Day 1 and 2 are defined as the highest score of the respective measure achieved on the day. **RER**, respiratory exchange ratio.

Reliability analysis of secondary criteria revealed low ICCs in RER_{peak} and high CVs in BLa_{peak}, respectively. On the other hand, HR_{peak} was highly correlated between days and trials and exhibited a low CV in all subgroups (Table 4.2). However, the achievement of critical thresholds in RER_{peak}, BLa_{peak} and HR_{peak} obtained during the GXT varied considerably between individuals (Table 4.3). RER_{peak} and HR_{peak} were significantly lower,

and BLa_{peak} significantly higher in the VER when compared with the GXT in all subgroups ($P < 0.05$, Figure 4.2). Median RPE values did not vary between subgroups, and were in the range of 19 – 20 for all GXTs and VERs.

Table 4.3 Ratio of tests fulfilling GXT peak criteria based on a range of parameters.

Parameter	Criterion	TETRA	PARA	NON-SCI
$\dot{V}O_{2peak}$	Plateau response	5/16	6/16	8/16
	Linear response	6/16	5/16	7/16
	Excess $\dot{V}O_2$ response	5/16	5/16	1/16
RER_{peak}	higher than 1.05	14/16	All	All
	higher than 1.10	13/16	All	15/16
	higher than 1.15	12/16	All	12/16
	higher than 1.20	6/16	15/16	7/16
BLa_{peak}	higher than 4.0 mmol·L^{-1}	15/16	All	All
	higher than 5.0 mmol·L^{-1}	7/16	15/16	15/16
	higher than 6.0 mmol·L^{-1}	3/16	14/16	10/16
HR_{peak}	higher than 85% APM	N/A	All	All
	higher than 90% APM	N/A	15/16	14/16
	higher than 95% APM	N/A	14/16	7/16
	higher than 100% APM	N/A	8/16	4/16

APM, age-predicted maximum.

4.5 Discussion

The main finding of this study was that inclusion of a VER can confirm the $\dot{V}O_{2peak}$ derived in a preceding GXT. The lack of significant differences of $\dot{V}O_{2peak}$ across repeat measurements further confirms that $\dot{V}O_{2peak}$ can be measured reliably on different days in the subgroups investigated; however, the TETRA subgroup exhibited a larger CV in the between day analysis.

4.5.1 Verification phase

Although $\dot{V}O_{2peak}$ is not the only measure of performance, it clearly is one of the major characteristics that determine performance in endurance sport (Levine, 2008), and it contributes to performance in patient, recreationally trained and endurance trained athletic populations. Therefore, the need for appropriate testing procedures which most accurately determine this variable is warranted. In AB sport, due to the limitations of secondary parameters like RER_{peak}, BLa_{peak} and HR_{peak} (Midgley *et al.*, 2009; Poole *et al.*, 2008), a VER can be used to accurately confirm the $\dot{V}O_{2peak}$ measured in a GXT (Midgley *et al.*, 2009; Midgley *et al.*, 2006). This finding can be confirmed with the protocol and athlete subgroups used in this study, with the LoA of $\dot{V}O_{2peak}$ (VER vs. GXT) found to be comparable to previous results, where LoAs spanning a range of 16 – 20% have been reported (Midgley *et al.*, 2007; Midgley *et al.*, 2006).

Physiological responses of secondary criteria related to the VER are also comparable with the AB population, with HR_{peak} being lower (by 1 – 2 $b \cdot min^{-1}$) during the VER when compared with the GXT (Midgley *et al.*, 2006). The same observation was made for $RER_{peak,}$ which was found to be 0.04 – 0.06 units lower for the VER when compared with the GXT. A possible reason could be that VER time to fatigue was not long enough to achieve physiological steady-state conditions for these parameters. Likewise, BLa_{peak} was consistently higher following the VER, which is likely to be due to the fact that BLa at the start of the VER was still elevated from performing the GXT, owing to the BLa half-life of around 15 min (Leicht & Perret, 2008) and the short break between GXT and VER. In summary, it cannot be recommended to use any VER-derived variables other than $\dot{V}O_{2peak}$ to verify results derived in a preceding GXT.

4.5.2 Reliability analysis

Whilst the literature review conducted for this thesis did not reveal any laboratory-based reliability studies examining peak testing in wheelchair sports, peak responses in only seven non-athletic participants with a spinal cord injury were found to be highly reliable (Bhambhani *et al.*, 1991). However, the spread of peak variables in the aforementioned investigation ($\dot{V}O_{2peak}$ = 0.54 – 3.00 $L \cdot min^{-1}$) due to within-group related disability

differences was substantial, and this heterogeneity may have affected the correlation coefficients reported in this study (Hopkins, 2000). By creating subgroups and recruiting athletes from an equally trained pool in the present study, the danger of inflated reliability parameters (in particular ICC) caused by within-group differences should have been largely reduced. Nonetheless, it must be noted that even with careful selection of athletes of a similar training and disability level, larger discrepancies within subgroups were found in $\dot{V}O_{2peak}$ when compared with the discrepancies found in equally trained pools of AB athletes (Midgley *et al.*, 2009; Poole *et al.*, 2008; Midgley *et al.*, 2006).

For PARA and NON-SCI athletes, the day to day CVs of $\dot{V}O_{2peak}$ found in this study compare well to the AB literature, where CVs of 3.5 – 5.6% (Midgley *et al.*, 2007; Shephard *et al.*, 2004; Katch *et al.*, 1982) have been reported for lower body exercise, whilst a CV of 8.9% was reported for arm-crank exercise in not specifically trained participants (Price & Campbell, 1997). A larger day to day CV of $\dot{V}O_{2peak}$ was noted for the TETRA athletes, which may partly be due to their lower aerobic capacity, where small absolute changes translate into larger relative changes of $\dot{V}O_{2peak}$. Further, it may be that device-dependent measurement errors have a greater impact when the variable under discussion is small in magnitude – for example, the manufacturer reports the accuracy of the gas sensors of the Cortex MetaLyzer 3B to be 0.1 Vol. %, independent of the absolute gas concentration. Finally, it cannot be excluded that the larger variation may be disability related, and it may therefore be that TETRA individuals have larger variations in their performance on a day to day basis. However, this is highly speculative and warrants further investigation.

It is striking that GXT time to fatigue was increased on the second occasion in all subgroups. Even though athletes were blinded to time to fatigue, they may have pushed themselves harder on their second day to beat their own score, a phenomenon that has been observed previously (Midgley *et al.*, 2006; Price & Campbell, 1997; Bhambhani *et al.*, 1991). Another explanation for this observation could be a learning effect, which cannot be completely ruled out, even though it can be assumed that due to the elite nature of the participants, they were able to perform to exhaustion consistently. However, as the differences in time to fatigue did not result in significant differences in $\dot{V}O_{2peak}$ between day 1 and day 2, which is a further indication that the "true" $\dot{V}O_{2max}$ has been found.

4.5.3 Secondary parameters

It has been suggested previously that a RER_{peak} above defined values (typically, 1.00 – 1.15), a HR_{peak} within a range of the age-predicted maximum (typically, ~90 – 100%) or BLa_{peak} above a certain threshold (typically, 8 – 10 $mmol \cdot L^{-1}$) may serve as secondary criteria to help determine whether peak performance has been achieved (Midgley *et al.*, 2007; Howley *et al.*, 1995). However, firstly, these criteria have been developed using specific exercise modalities, test protocols and participants; and it has been proposed that applying these criteria directly to exercise tests using different methodologies are unlikely to be valid (Midgley *et al.*, 2006). For wheelchair exercise, it was therefore to be expected that these defined values would potentially need reconsideration. Secondly, the validity of currently used $\dot{V}O_{2max}$ criteria (RER_{peak}, BLa_{peak} and HR_{peak}) has recently been questioned, as the threshold values typically used for these criteria could be attained at exercise intensities as low as 73% $\dot{V}O_{2max}$ (Poole *et al.*, 2008). It has, therefore, been suggested to reject these secondary criteria as a means of validating $\dot{V}O_{2max}$ measured in protocols of an incremental nature (Midgley *et al.*, 2009; Poole *et al.*, 2008). In light of the present data, this appears to be a reasonable approach, considering the big variation of athletes reaching respective levels of these parameters in the GXT. It seems that the use of these secondary parameters is not to help decide whether peak performance has been achieved, but rather to help decide whether peak performance has *not* been achieved; i.e. for an $RER_{peak} < 1.05$, $BLa_{peak} < 4.0$ $mmol \cdot L^{-1}$ (TETRA) or $RER_{peak} < 1.10$, $BLa_{peak} < 5.0$ $mmol \cdot L^{-1}$ or $HR_{peak} < 90\%$ age-predicted maximum (PARA and NON-SCI). Similarly, it appears plausible that the occurrence of a $\dot{V}O_2$ plateau indicates that the "true" $\dot{V}O_{2max}$ has been attained, but the absence of a plateau does not indicate that it has *not* been attained, as a plateau only occurred in 19 out of 48 tests. During upper-body exercise, it is possible that the phenomenon of excess $\dot{V}O_2$ (a non-linear rise in $\dot{V}O_2$ during the latter stages of an arm ergometry incremental test (Smith *et al.*, 2006)), may counteract the occurrence of a plateau in some individuals, resulting in a linear relationship of the $\dot{V}O_2$-workload response throughout the GXT. In some individuals, the mechanisms responsible for excess $\dot{V}O_2$ may override the plateau phenomenon, whilst in others they are overridden by it, resulting in the mixed $\dot{V}O_2$ responses observed in the present study. However, it must be kept in mind that even in lower body exercise, the $\dot{V}O_2$ plateau does not occur in every GXT, which leads to the conclusion that the absence of a plateau does not necessarily indicate that a maximum

effort has not been given or that a "true" $\dot{V}O_{2max}$ has not been elicited (Midgley & Carroll, 2009; Day *et al.*, 2003; Bassett & Howley, 2000).

4.5.4 Practical applications

With regard to secondary criteria, the analysis of $\dot{V}O_2$ data obtained in a VER can be recommended as the only valid option to confirm whether a "true" $\dot{V}O_{2max}$ has been attained in a GXT. Given the relative simplicity of including a VER into a routine GXT protocol (around 10 minutes of added time), this seems to be a practical solution to obtain a confirmation for $\dot{V}O_{2peak}$ data. With regard to the present study, the LoA between GXT and VER may be narrowed further by excluding tests with VER time to fatigue < 100 s, suggesting that the VER protocol used could be improved. As it appears that a minimum VER time to fatigue is required so that the "true" $\dot{V}O_{2max}$ can be reached, the treadmill gradient of the VER should be reduced for PARA and NON-SCI athletes, as a significant number of the athletes of these subgroups were not able to exercise for longer than 100 s. Possibly, the workload increment for some of the PARA and NON-SCI athletes was too large, and this may have induced rapid accumulation of intramuscular hydrogen ions, causing volitional exhaustion before $\dot{V}O_{2max}$ in the VER was attained (Midgley *et al.*, 2006). As the absolute workload increment between GXT and VER was smaller in the TETRA subgroup, it may have enabled them to exercise for longer than the other subgroups. Hence, for future investigations using a protocol based on the present study it is suggested to reduce the gradient difference between the final stage of the GXT and the VER for PARA and NON-SCI athletes to 0.3%. An alternative approach would be allowing for a longer recovery period between GXT and VER.

Statistical fundamentals postulate that a minimum difference between measurements of 0.5·LoA (≈√2·CV) is required to determine a real individual change with an 84% certainty (Hopkins, 2000). Based on the day to day variation of $\dot{V}O_{2peak}$ determined in a test consisting of a GXT and a VER, this translates to a 13.2%, 6.4%, and 4.7% change required to document an improvement or a reduction in TETRA, PARA and NON-SCI athletes respectively. This is in line with the able-bodied literature, where this figure has been shown to be 5% (Day *et al.*, 2003). Training programmes usually elicit changes in

$\dot{V}O_{2peak}$ of at least this magnitude (Devillard *et al.*, 2007), which supports the continued use of this variable to document the efficacy of training interventions. However, when investigating athletic populations, it must be appreciated that these are comparably large figures. It is therefore questionable whether $\dot{V}O_{2peak}$ should be used to track athletes, where much smaller differences in performance can differentiate between victory and defeat. Alternative, potentially more sensitive laboratory parameters to serve this purpose may include peak power output, or power output at lactate or ventilatory thresholds.

4.5.5 Limitations

Due to the elite nature of the participants, it was not possible to conduct a familiarisation trial of the procedures. This would have resulted in a total of three visits, taking six days out of the participants' training schedule (three testing days, and three days avoiding exercise before the tests). However, the majority of participants were familiar with the procedures as this was an integral part of their yearly training monitoring. Still, if feasible in future studies, familiarisation trials should be incorporated.

The time used to determine the linear part of the $\dot{V}O_2$-time relationship differed to what was used in the original study where the method for plateau determination was adapted from (Day *et al.*, 2003). As the GXT times in the present study were shorter when compared with the original study, it was necessary to reduce the data to be excluded at the start of the test from four to two minutes, in order to be able to include a sufficient amount of data into the regression analysis. However, visual inspection of the $\dot{V}O_2$-time plots showed that data remained linearly related, underpinning the validity of this adaptation to the methods.

4.6 Conclusions

Inclusion of a VER confirms the $\dot{V}O_{2peak}$ obtained in a GXT, however, a CV of up to ~6% between GXT and VER should be considered as acceptable. With regard to between day analyses, relatively large changes in $\dot{V}O_{2peak}$ are needed to confirm a "true" effect, especially in TETRA athletes. This may be due to their low aerobic capacity, where small

absolute changes result in larger relative changes when compared with the other subgroups. In summary, a VER provides a tool for a more robust determination of $\dot{V}O_{2peak}$ during wheelchair treadmill exercise. However, using defined values of secondary parameters to establish whether peak performance has been achieved is not recommended. The results of this study show the benefits of using a VER in peak testing protocols, and this method will hence be used in all following chapters determining laboratory peak exercise responses.

5

Submaximal exercise responses in tetraplegic, paraplegic and non-spinal cord-injured elite wheelchair athletes

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Study 1 was concerned with the investigation of peak exercise responses and has provided evidence that $\dot{V}O_{2peak}$ can be measured reliably on separate days in all disability subgroups. Study 2 seeks to investigate submaximal exercise responses and compare them between the same disability subgroups.

5.1 Abstract

The aim of this study was to compare submaximal physiological exercise responses between subgroups of wheelchair athletes with different disabilities.

Methods: Twenty-five wheelchair athletes, divided into three subgroups (8 tetraplegic (TETRA), 9 paraplegic (PARA), and 8 non-spinal cord-injured (NON-SCI)), performed an exercise test consisting of incremental submaximal stages, covering a range from 40 – 80% peak oxygen uptake ($\dot{V}O_{2peak}$). Oxygen uptake ($\dot{V}O_2$), heart rate (HR), blood lactate concentration (BLa) and rating of perceived exertion (RPE) were obtained for each stage.

Results: Expressed as a function of BLa, no differences were found between subgroups with respect to $\%\dot{V}O_{2peak}$ (group mean ± SD: 1.0 $mmol \cdot L^{-1}$: 53.9 ± 9.9%; 2.0 $mmol \cdot L^{-1}$: 70.7 ± 7.5%; 3.0 $mmol \cdot L^{-1}$: 78.5 ± 7.7%) and RPE (group median (lower and upper quartile): 1.0 $mmol \cdot L^{-1}$: 10.8 (9.9, 12.2); 2.0 $mmol \cdot L^{-1}$: 13.6 (12.7, 14.3); 3.0 $mmol \cdot L^{-1}$: 14.9 (13.7, 16.5)). Furthermore, no differences were found in the coefficient of determination (R^2) of the HR – $\dot{V}O_2$ relationship in any of the subgroups (TETRA: 0.90 ± 0.12; PARA: 0.97 ± 0.02; NON-SCI: 0.96 ± 0.04).

Conclusions: The results suggest that exercise prescription using measurements of $\%\dot{V}O_{2peak}$, BLa or RPE can be based on the same recommendations in all studied subgroups. This finding has added value for TETRA athletes, as it offers alternatives to HR monitoring.

5.2 Introduction

The ultimate goals of exercise prescription include maintaining health, controlling weight, or maintaining/developing aerobic and/or anaerobic capacity (American College of Sports Medicine, 1998). To achieve this successfully, physiological (Reilly *et al.*, 2009; Weltman *et al.*, 1990a) and psychophysiological (Kang *et al.*, 2003; Dishman, 1994; Dunbar *et al.*, 1992; Glass *et al.*, 1992; Eston & Williams, 1988) exercise responses serve as supporting markers. Depending on the goals and the resources available, common parameters to control and monitor exercise intensity are given percentages of maximum oxygen uptake (%$\dot{V}O_{2max}$), BLa, HR and RPE (Goosey-Tolfrey *et al.*, 2010b; Tolfrey *et al.*, 2001; American College of Sports Medicine, 1998; Dishman, 1994; Weltman *et al.*, 1990a).

However, it has been pointed out that metabolic responses for exercise at a given %$\dot{V}O_{2max}$ (Scharhag-Rosenberger *et al.*, 2010) or at the anaerobic threshold (Meyer *et al.*, 1999) may vary considerably between individuals. One important variable explaining this variation appears to be aerobic capacity (Held & Marti, 1999), which is a term often used to reflect fitness and training status, as it can be improved following training programmes (Rimaud *et al.*, 2005; American College of Sports Medicine, 1998; Magel *et al.*, 1978). Therefore, it seems important to account for differences in aerobic capacity (and hence, cardiorespiratory fitness) between individuals when prescribing exercise based on metabolic responses.

Wheelchair athletes are a heterogeneous pool of individuals. Depending on the lesion level of a spinal cord injury (SCI), large variations in active muscle mass and autonomic innervation between individuals lead to large variations in aerobic capacity, with high level TETRA individuals having the lowest and low level PARA individuals the highest aerobic capacity (Campbell *et al.*, 2004; Coutts *et al.*, 1983). Given the importance of aerobic capacity on the consistency of submaximal exercise responses (Held & Marti, 1999), the differences between disability types in wheelchair athletes may suggest that submaximal metabolic responses at any given %$\dot{V}O_{2max}$ have the potential to vary between subgroups of wheelchair athletes, even when comparing individuals from an equally trained pool.

Data in the literature analysing submaximal exercise responses are scarce, especially for TETRA individuals (Lewis *et al.*, 2007; Valent *et al.*, 2007b; Campbell *et al.*, 2004; McLean *et al.*, 1995; Coutts *et al.*, 1983). Furthermore, data are contradictory and, given the advances in disability sport (Hettinga *et al.*, 2010), potentially outdated at times. For example, the ventilatory threshold in TETRA athletes has been found to be at a higher $\%\dot{V}O_{2max}$ when compared with PARA athletes (Coutts & McKenzie, 1995); on the other hand, TETRA athletes have been found to race at a lower $\%\dot{V}O_{2max}$ when compared with PARA athletes (Bhambhani *et al.*, 1994).

A further step to understand the physiology of wheelchair athletes with different disabilities is therefore to examine how various subgroups of wheelchair athletes respond to exercise. Particularly, it is worth knowing whether there are ways to make them comparable to each other with respect to physiological and psychophysiological exercise responses. Only if this issue can be solved, one can consider using similar exercise response based prescriptions to gauge and monitor exercise intensity in different subgroups of wheelchair athletes.

Therefore, the aim of this study was to analyse submaximal exercise responses in three subgroups (TETRA, PARA and NON-SCI) of wheelchair athletes and to explore whether physiological and psychophysiological parameters can be used to express exercise intensity in a comparable way in all subgroups. Due to the low aerobic capacity in TETRA individuals, it is hypothesised that their $\%\dot{V}O_{2max}$ is higher for the same perceived intensities when compared with the other subgroups. Hence, their submaximal exercise responses (expressed as BLa and RPE) for any given $\%\dot{V}O_{2max}$ are expected to be lower when compared with the other subgroups.

5.3 Methods

5.3.1 Participants

Twenty-five male wheelchair athletes volunteered to participate in this study. Participants were eight motor complete TETRA, nine motor complete PARA and eight NON-SCI

individuals. All participants performed their sport on at least a national level; a summary of their physical, physiological and sport characteristics is presented in Table 5.1.

Table 5.1 Participants' characteristics.

Parameter	**TETRA**	**PARA**	**NON-SCI**
Body mass [kg]	67.9 ± 6.7	71.9 ± 12.6	79.6 ± 11.4
Age [years]	29.2 ± 3.8	30.6 ± 9.0	24.0 ± 6.2
Lesion level/ disability types	C6 – C7	T5 – T12, Spina bifida, polio	Amputations, club foot, brittle bones
Wheelchair sport	Rugby (N = 8)	Basketball (N = 9)	Basketball (N = 7) Tennis (N = 1)
Wheelchair sport experience [years]	7.8 ± 4.6	12.2 ± 5.3	6.2 ± 5.2
Training volume [h·week^{-1}]	14 ± 6	12 ± 4	13 ± 6
$\dot{V}O_{2peak}$ [L·min^{-1}]	1.67 ± 0.38*†	2.47 ± 0.33†	3.35 ± 0.57
$\dot{V}O_{2peak}$ [mL·kg^{-1}·min^{-1}]	24.5 ± 4.9*†	34.9 ± 5.1†	42.0 ± 2.8
$\dot{V}O_{2peak}$ [mL·kg$^{-0.82}$·min^{-1}]	52.4 ± 10.6*†	75.0 ± 9.7†	92.2 ± 7.2
RER_{peak}	1.13 ± 0.07*	1.27 ± 0.06	1.22 ± 0.08
Peak heart rate [beats·min^{-1}]	129 ± 12*†	184 ± 10	186 ± 11
Peak blood lactate concentration [mmol·L^{-1}]	4.95 ± 1.28*	8.47 ± 2.75	6.56 ± 1.36

Apart from lesion level/disability types and sport, data are means ± standard deviation. *Significantly different from PARA; †significantly different from NON-SCI; at $P < 0.05$ level.

5.3.2 Experimental design

Participants visited the laboratory on two occasions and were asked to abstain from caffeine and not to perform any exercise 24 h prior to the tests. Questionnaires were completed and body mass recorded as explained in Chapter 3.2. All exercise tests were

performed in the athletes' sports wheelchair on a motorised treadmill, with details described in Chapter 3.3.

5.3.3 Familiarisation trial

The familiarisation trial preceded the main trial and included constant load 4-min exercise blocks at 1.0% gradient at a minimum of five submaximal intensities, followed by a GXT described below. $\dot{V}O_2$, BLa and RPE were obtained in order to familiarise participants with the procedures performed in the main trial.

5.3.4 Main trial

In analogy to study 1, participants performed six to eight submaximal constant load 4-min exercise blocks at ascending speeds at a fixed gradient of 1.0%, in order to elicit physiological responses covering a range of ~40 – 80% $\dot{V}O_{peak}$ (Figure 5.1), according to Goosey-Tolfrey (2008). This was followed by a 15-min passive recovery. A GXT was then performed at a constant speed, which was chosen according to the responses elicited during the submaximal exercise blocks. The gradient at the start of the GXT was 1.0% for all subgroups; the gradient was then increased by 0.3% every minute for PARA and NON-SCI, and by 0.1% every 40 s for TETRA, in order to achieve total GXT test times between 8 and 14 min. After the GXT, participants sat quietly for 2 min and recovered actively at a low intensity (1.0 $m \cdot s^{-1}$ at 1.0% gradient) for 5 min. In order to confirm the $\dot{V}O_{2peak}$ attained in the GXT, they then performed a VER, designed as a test to exhaustion at the same constant speed but at a gradient which was higher than the maximal gradient achieved during the GXT (+0.6% for PARA and NON-SCI; +0.3% for TETRA). The GXT and the VER were terminated when participants were unable to maintain the speed of the treadmill, and verbal encouragement was given throughout the test.

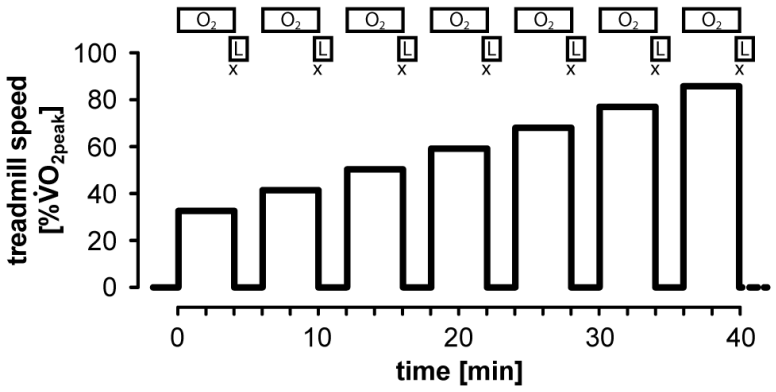

Figure 5.1 Test protocol for main trials.

O_2, spirometric measurements for determination of oxygen uptake; **L**, blood sample for lactate analysis; **x**, rating of perceived exertion.

5.3.5 Data collection

Spirometric, BLa and HR data were collected as described in Chapter 3. Following each submaximal exercise block, immediately after the GXT and immediately after the VER a small capillary blood sample was obtained from the earlobe in order to measure BLa. At the same time points, participants were asked to indicate RPE using a scale ranging from 6 – 20 (Borg, 1982). $\dot{V}O_2$ of each submaximal exercise block was averaged over the final minute. $\dot{V}O_{2peak}$ and RER_{peak} were defined as highest average value over 30s, whereas the higher of the two values obtained in the GXT and the VER was reported and used for further analysis. The same procedure was applied to determine HR_{peak} and peak BLa.

5.3.6 Data processing

The PASW 18.0 statistical package (SPSS Inc., Chicago IL, USA) was used for all data processing and statistical analyses. In a first step, submaximal data were processed, using the linear velocity - $\dot{V}O_2$ relationship to match each BLa value obtained at a discrete speed to a $\dot{V}O_2$ value. Regression analyses were then performed to model the nonlinear $\dot{V}O_2$ - BLa relationship using the formula below, discussed in more detail by Hughson *et al.* (1987):

$$BLa = a \cdot e^{b \cdot \dot{V}O_2} + C$$

From the resulting regression slope, $\dot{V}O_2$ at discrete BLa values was calculated at 1.0, 1.5, 2.0, 2.5, and 3.0 mmol·L^{-1} (Figure 5.2). A linear interpolation was applied to RPE data to obtain exact RPE scores at the same BLa reference points. In a second step, a linear interpolation was applied to RPE data to obtain exact RPE scores at discrete $\%\dot{V}O_{2peak}$ values (40, 50, 60, 70, and 80 $\%\dot{V}O_{2peak}$). Furthermore, R^2 was computed for the $\dot{V}O_2$ - HR relationship, according to methods described earlier (Tolfrey *et al.*, 2001). To compare the body size-independent $\dot{V}O_{2peak}$ values, the mass exponent of 0.82 was adopted (i.e., mL·kg$^{-0.82}$·min^{-1}) as described by Goosey-Tolfrey *et al.* (2003).

Finally, the HR reserve and $\dot{V}O_2$ reserve were calculated, using peak and lowest submaximal exercise values as boundaries. The ratio between HR reserve and $\dot{V}O_2$ reserve was further calculated (HR/$\dot{V}O_2$ reserve ratio).

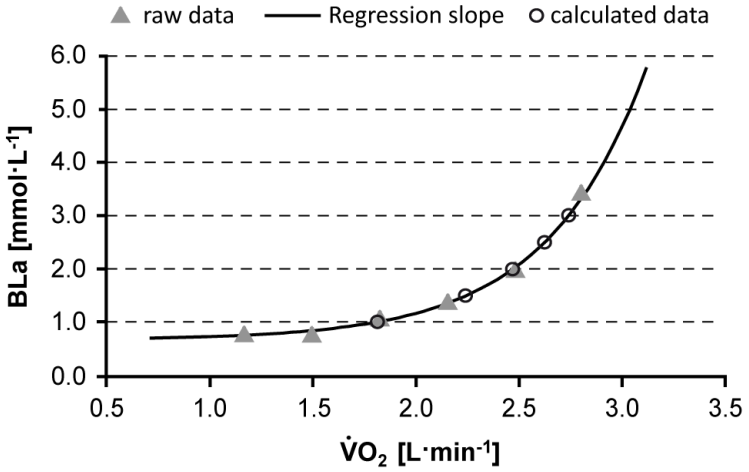

Figure 5.2 Regression analysis of the blood lactate concentration – $\dot{V}O_2$ relationship.
Data from an example participant. For all analyses, $\dot{V}O_2$ values were calculated for BLa concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0 mmol·L^{-1}.

5.3.7 Statistical analyses

Means and SD were computed for all variables. Normality was checked with the Shapiro Wilk, homogeneity with Levene's statistic. A one-way ANOVA was applied to explore participants' characteristics, HR and $\dot{V}O_2$ reserve, and the HR/$\dot{V}O_2$ reserve ratio. A

two-way (group x BLa) repeated measures ANOVA was performed to explore $\%\dot{V}O_{2peak}$ at the calculated BLas of 1.0, 1.5, 2.0, 2.5 and 3.0 $mmol \cdot L^{-1}$. Multiple Kruskall-Wallis tests were applied to explore absolute $\dot{V}O_2$ data at discrete BLa levels, as these violated the assumptions required for parametric testing. Likewise, RPE vs. $\%\dot{V}O_{2peak}$ and RPE vs. BLa was analysed using multiple Kruskall-Wallis tests. Bonferroni corrections were applied for all multiple comparisons. For all comparisons where the assumption of sphericity was violated, a Greenhouse Geisser correction was applied. Statistical significance for all analyses was accepted at $P < 0.05$.

5.4 Results

5.4.1 Submaximal exercise responses

Absolute $\dot{V}O_2$ at the BLa reference points 1.5, 2.0, 2.5, and 3.0 $mmol \cdot L^{-1}$ differed significantly between TETRA and PARA and between TETRA and NON-SCI ($P < 0.05$, Figure 5.3). However, when $\dot{V}O_2$ was expressed as $\%\dot{V}O_{2peak}$, no differences were found between subgroups at these BLa reference points ($P > 0.05$). The mean R^2 for the nonlinear $\dot{V}O_2$ - BLa regression was 0.98 for TETRA, and 0.99 for PARA and NON-SCI athletes. Furthermore, no differences ($P > 0.05$) were found when RPE was expressed as a function of BLa (Figure 5.4) and $\%\dot{V}O_{2peak}$ (Figure 5.5). It is worth noting that these differences between subgroups were also non-significant before applying Bonferroni corrections.

The HR reserve was significantly reduced in TETRA when compared to the other subgroups; however, the $HR/\dot{V}O_2$ reserve ratio did not differ between groups. Furthermore, the variables $\dot{V}O_2$ - HR were highly correlated and R^2 did not differ between subgroups (Table 5.2). The RPE range covering these reserve responses did not differ between subgroups (TETRA 7 (6, 8) – 19 (18, 19); PARA 7 (6, 7) – 19 (19, 20): NON-SCI 6 (6, 7) – 19 (18, 20)).

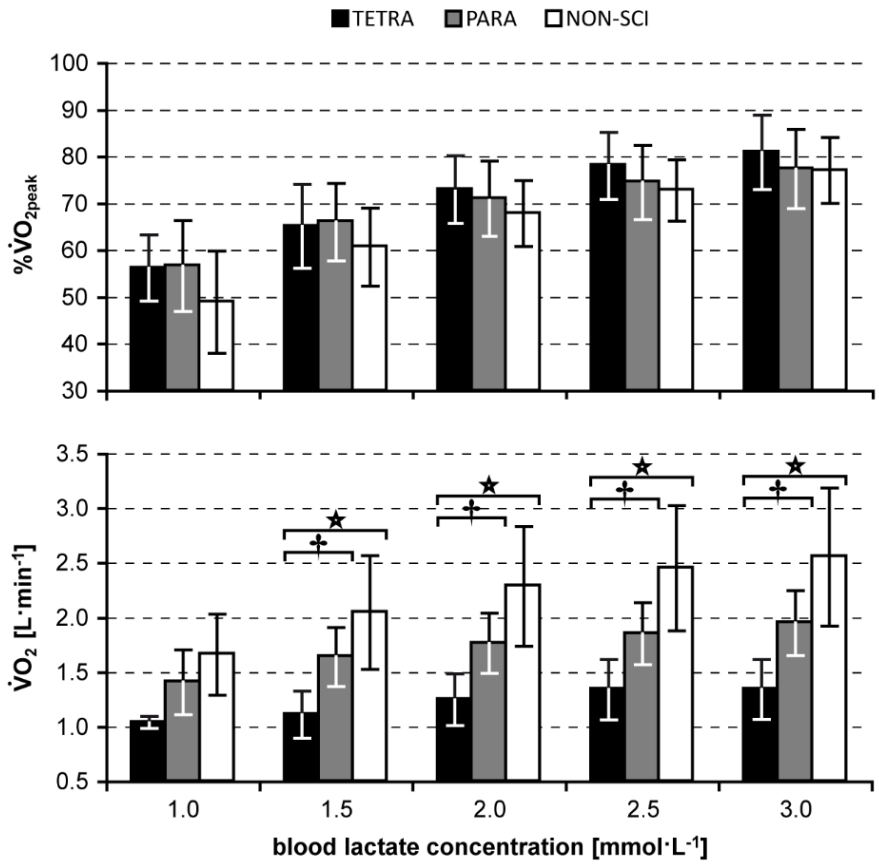

Figure 5.3 Oxygen uptake vs. blood lactate concentration during submaximal exercise.

Data are means ± standard deviation. †Significant difference, TETRA *vs.* PARA; ⋆significant difference, TETRA *vs.* NON-SCI; at $P < 0.05$ level.

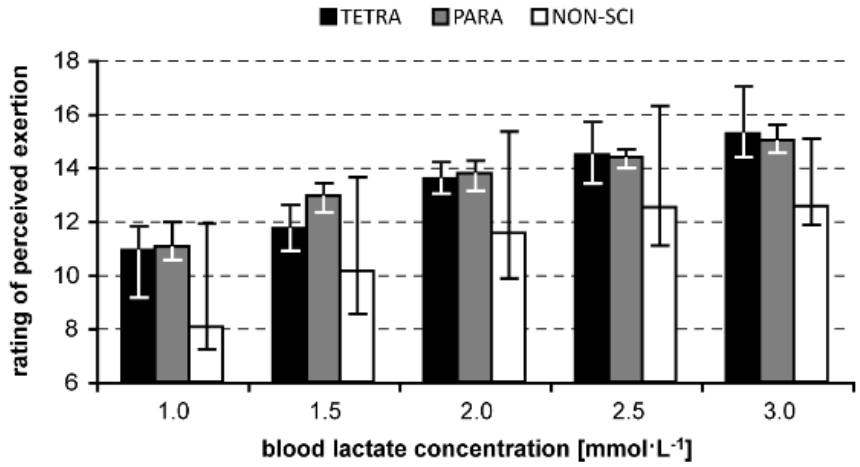

Figure 5.4 Rating of perceived exertion vs. blood lactate concentration during submaximal exercise.

Data are medians and interquartile range.

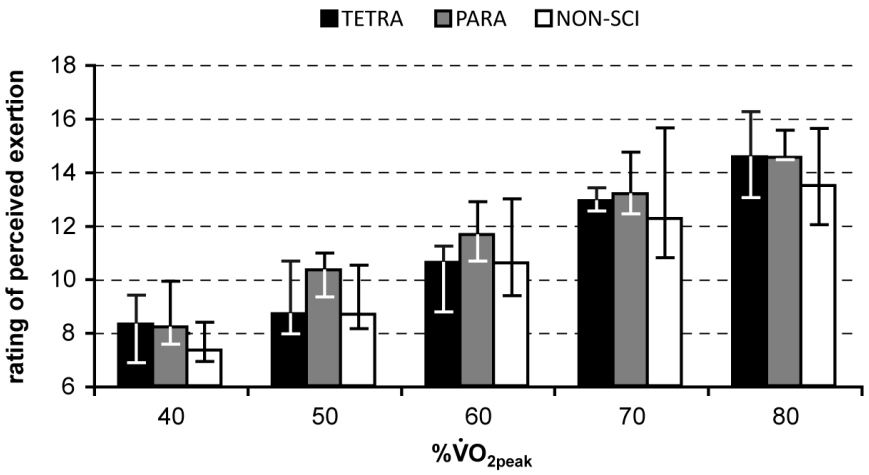

Figure 5.5 Rating of perceived exertion vs. $\%\dot{V}O_{2peak}$ during submaximal exercise.
Data are medians and interquartile range.

Table 5.2 Heart rate reserve and oxygen uptake reserve responses.

Parameter	TETRA	PARA	NON-SCI
HR reserve [$b \cdot min^{-1}$]	48 ± 14*†	96 ± 12	91 ± 11
$\dot{V}O_2$ reserve [$L \cdot min^{-1}$]	0.99 ± 0.31*†	1.71 ± 0.28†	2.30 ± 0.48
HR/$\dot{V}O_2$ reserve ratio	53 ± 22	57 ± 11	41 ± 9
$\dot{V}O_2$ – HR correlation [R^2]	0.90 ± 0.12	0.97 ± 0.02	0.96 ± 0.04

Data are means ± standard deviation. *Significantly different from PARA; †significantly different from NON-SCI; at $P < 0.05$ level.

5.4.2 Peak exercise responses

Significant differences between TETRA individuals and the subgroups PARA and NON-SCI were found in absolute, relative, and body-mass scaled $\dot{V}O_{2peak}$ and HR_{peak}. Furthermore, significant differences between PARA and NON-SCI individuals were found in absolute and relative $\dot{V}O_{2peak}$. Peak BLa and RER_{peak} were significantly higher in PARA when compared with TETRA individuals (Table 5.1). Finally, no differences in $\dot{V}O_{2peak}$ in a comparison of the GXT and the VER were found ($P > 0.05$).

5.5 Discussion

5.5.1 Main findings

In contradiction to the hypothesis, the main finding of this study is that TETRA, PARA and NON-SCI individuals do not differ in the exercise responses of their individual %$\dot{V}O_{2peak}$ and RPE when related to a range of submaximal BLa reference points. Further, when expressed as a function of %$\dot{V}O_{2peak}$, RPE does not differ between subgroups. Therefore, it appears to be possible to prescribe exercise in a comparable way for all subgroups by expressing exercise intensity as a function of %$\dot{V}O_{2peak}$, BLa or RPE. Finally, all subgroups exhibit a similar R^2 in the $\dot{V}O_2$ - HR relationship.

It must be noted that comparative wheelchair exercise data of disability subgroups are scarce. However, existing data confirm the present findings: No differences in RPE responses have previously been found in disability subgroups across intensities from resting to peak work rate (PARA vs. TETRA), and RPE has been reported to increase as a function of exercise intensity (Lewis *et al.*, 2007). Furthermore, significant correlations of the parameters $\dot{V}O_2$ - HR have been documented in TETRA and PARA individuals, even though it must be noted that these were clearly weaker when compared with the present findings (Coutts *et al.*, 1985). It has also been observed that both TETRA and PARA can maintain velocities equivalent to 75%$\dot{V}O_{2peak}$ for prolonged periods (Campbell *et al.*, 2004), which, in light of the data of this study, underpins that a similar relative performance in these subgroups can be achieved when the demand is related to the individual $\dot{V}O_{2peak}$. Finally, when compared with AB athletes, Schmid *et al.* (1998a) found no differences in work rate and HR at a fixed BLa level in PARA athletes. However, it must be said that whilst previous studies examined the relationship of %$\dot{V}O_{2peak}$, HR, RPE and (in some cases) BLa in wheelchair users, they were not designed optimally for data to be used to compare a range of disabilities with respect to submaximal exercise responses. Either very low numbers of a particular disability subgroup (mostly an under-represented TETRA subgroup) were investigated (Campbell *et al.*, 2004; Campbell *et al.*, 1997), or submaximal data was analysed in non-steady state conditions (Coutts & McKenzie, 1995), and very few data points were obtained for the TETRA subgroup (Coutts *et al.*, 1985). In

the majority of investigations, BLa was not analysed (Lewis *et al.*, 2007; Coutts & McKenzie, 1995; Coutts *et al.*, 1985). There appears to be no previous study on submaximal exercise responses in wheelchair athletes that based its findings on BLa modelling, which therefore is a novel approach in this context.

5.5.2 Blood lactate monitoring in wheelchair athletes

Controlling and monitoring exercise intensity by BLa measurements is well established in AB sports (American College of Sports Medicine, 1998). In addition to using BLa as a tool to analyse performance, it has the additional advantage that its modelling has been examined previously (Hughson *et al.*, 1987). Therefore, BLa modelling can be used to express BLa as a function of $\dot{V}O_2$, allowing more extensive data analyses. Especially for TETRA individuals, where exercise prescription on the basis of physiological parameters other than BLa has been found to be limited (McLean *et al.*, 1995), inclusion of BLa as an additional marker to prescribe and control exercise intensity may be of particular benefit. Adding to the paucity of BLa based findings in wheelchair populations, the present data showing similar BLa responses between subgroups complement the work of Leicht & Perret (2008), who found no differences in BLa elimination following exhaustive exercise between PARA and AB athletes. It must be noted that the formulas describing the BLa - $\dot{V}O_2$ relationship in the present study were derived from lower body exercise (Hughson *et al.*, 1987), and that they have yet to be validated in upper body and wheelchair exercise. However, the high coefficients of determination suggest that this model reflects the BLa - $\dot{V}O_2$ relationship in wheelchair propulsion accurately.

5.5.3 Submaximal exercise: Disability-related discrepancies

Even though subgroups do not differ with respect to $\%\dot{V}O_{2peak}$, BLa, and RPE, closer inspection of NON-SCI data reveals a larger variation in RPE when related to discrete BLa levels. It is well known that practice improves the accuracy of RPE based estimates of physiological variables (Eston *et al.*, 2008; Eston *et al.*, 2005). Therefore, it may be possible that the perception of effort is experienced more homogenously in a population that uses a wheelchair on a daily bases rather than only for a given sporting activity.

Whereas TETRA and PARA individuals depend on a wheelchair for daily ambulation, this is not the case for the majority of the NON-SCI subgroup (only one NON-SCI participant of this study used a wheelchair for daily ambulation). Furthermore, the disabilities within the NON-SCI subgroup are much more varied (club foot, brittle bones, or amputations) when compared with the TETRA and PARA subgroups, which all have a SCI. These facts may explain some of the additional RPE variation observed in NON-SCI athletes. On a practical note, this finding suggests that if non-individualised between-subject exercise prescription and monitoring should be based on RPE in individuals with a disability, it must be ensured that these individuals have similar disabilities requiring similar modes of (daily) ambulation. However, future studies for wheelchair athletes are needed to confirm earlier findings on RPE guided training (Lewis *et al.*, 2007; Grange *et al.*, 2002; McLean *et al.*, 1995), as only little data exist for athletic populations (Goosey-Tolfrey *et al.*, 2010b).

5.5.4 Considerations for tetraplegic athletes

The disrupted autonomic innervation of the heart leads to an altered HR response in TETRA individuals. In line with the findings of the present study, decreased HR_{peak} in TETRA individuals have been observed earlier (Goosey-Tolfrey *et al.*, 2006; Haisma *et al.*, 2006). However, it has also been shown that the linear $\dot{V}O_2$ - HR relationship is lacking or markedly reduced in some TETRA individuals, and it has therefore been suggested that HR is an inappropriate tool to control exercise intensity in this subgroup of SCI individuals (Valent *et al.*, 2007b; McLean *et al.*, 1995). However, it should be noted that lower level TETRA individuals tend to be the group where the majority of individuals exhibit linear $\dot{V}O_2$ - HR relationships (Valent *et al.*, 2007b; McLean *et al.*, 1995). This type of TETRA individuals was studied in the present study (lesion levels between C6 and C7 – as opposed to higher level TETRA individuals with lesion levels above C6). Further to this, a highly trained TETRA participant group may exhibit slightly elevated HR_{peak}, as HR_{peak} data from less trained TETRA individuals found in the literature tend to be lower than the HR_{peak} data observed in the present study (Valent *et al.*, 2007b; Haisma *et al.*, 2006). Assuming a constant resting HR, an increased HR_{peak} results in an increased HR reserve. Since it appears that TETRA individuals with the largest HR reserves exhibit the best $\dot{V}O_2$ - HR correlations (Valent *et al.*, 2007b), a higher HR_{peak} may therefore favour higher $\dot{V}O_2$ - HR correlations. Whilst at this moment, a connection between increased HR_{peak}, a more linearly

controlled $\dot{V}O_2$ - HR relationship and fitness level can only be guessed, future studies may elucidate this issue.

During constant load exercise, HR and $\dot{V}O_2$ can be reliably measured up to 30 min of wheelchair propulsion (Keyser *et al.*, 2001). However, using HR as a tool to monitor exercise intensity in TETRA individuals remains controversial. The decreased HR_{peak} in TETRA individuals results in a pronounced reduction of the HR reserve, which again results in a markedly reduced HR range exercise could be prescribed or monitored in. Given the biological variation in submaximal exercise parameters (Bagger *et al.*, 2003), any prescribed HR may therefore result in larger fluctuations of $\%\dot{V}O_{2peak}$ in TETRA individuals when compared with individuals with a normal HR reserve. On the other hand, the HR-$\dot{V}O_2$ reserve ratio between subgroups in the present study was comparable, implying that for a given increase in $\dot{V}O_2$, HR increased by the same amount in all subgroups. This may encourage the use of HR monitors as training tools in all subgroups. Nonetheless, it must be acknowledged that due to the wider spread of the R^2 in the TETRA subgroup, some individuals exhibited a noticeably weaker $\dot{V}O_2$ - HR relationship than the group mean. This stresses the fact that exercise prescription based on HR may not be suitable for all TETRA athletes. For some individuals, the likelihood of exercising at suboptimal or wrong intensities would be largely increased when compared with populations with intact autonomic heart innervation, and hence, normal HR reserve.

5.5.5 Limitations

Caution is advised when interpreting data not showing any significant differences between groups. Large inter-individual differences may mask "true" differences between subgroups, hence, more participants would be required in order to detect the "signal" of a given mean difference (Batterham & Atkinson, 2005). Comparing the data of this study with the literature, the few laboratory-controlled studies with more participants than the present study (Lewis *et al.*, 2007) confirm the limits of recruiting a homogenous pool of wheelchair dependent populations outside a patient environment. Therefore, follow-up research or the conduction of meta-analyses to strengthen the significance of published results in the area of wheelchair sport in the long term are encouraged.

5.6 Conclusions

The similar relationships of BLa, %$\dot{V}O_{2peak}$ and RPE between disability subgroups suggest that exercise prescription and monitoring relating to these physiological and psychophysiological measurements can be based on the same recommendations in all three subgroups of wheelchair athletes. Given that most literature on wheelchair exercise focuses on PARA individuals, it is suggested that %$\dot{V}O_{2peak}$, BLa or RPE based prescriptions for PARA individuals can be adopted by TETRA and NON-SCI individuals. The present study also encourages further research to investigate HR as a training tool for TETRA athletes, since data show that HR – $\dot{V}O_2$ correlations do not differ between subgroups.

6

Study 3: Mucosal immune responses to treadmill exercise in elite wheelchair athletes

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Studies 1 and 2 provided evidence that a protocol based on a percentage of $\dot{V}O_{2peak}$ should result in similar exercise responses in all disability subgroups. Therefore, in this study mucosal immune responses were investigated using a laboratory protocol based on $\%\dot{V}O_{2peak}$.

6.1 Abstract

The aim of this study was to examine salivary secretory immunoglobulin A (sIgA) responses and α-amylase activity following constant load and intermittent exercise in elite wheelchair athletes.

Methods: Twenty-three wheelchair athletes divided into three subgroups (8 tetraplegic (TETRA), 7 paraplegic (PARA), and 8 non-spinal cord-injured (NON-SCI)) performed two randomised and counterbalanced 60-min sessions on a treadmill. These consisted of a constant load (60% peak oxygen uptake ($\dot{V}O_{2peak}$)) and an intermittent (80% and 40% $\dot{V}O_{2peak}$) exercise block. Timed, unstimulated saliva samples were obtained pre, mid, post, and 30 min post exercise and analysed for sIgA and α-amylase. Furthermore, blood lactate concentration and rating of perceived exertion (RPE) were measured during both sessions.

Results: sIgA secretion rate, sIgA concentration and α-amylase activity were increased during exercise in all subgroups ($P < 0.05$). However, the increase of sIgA secretion rate during exercise was greater in TETRA individuals (post exercise average data for both trials in comparison with pre: TETRA + 60 ± 31%, PARA + 30 ± 35%, NON-SCI + 11 ± 25%, $P < 0.05$). Yet, subgroups were comparable with respect to blood lactate concentration and RPE for both exercise sessions.

Conclusions: Despite the disruption of autonomic salivary gland innervation in TETRA athletes, their ability to increase sIgA secretion rate seems comparable to wheelchair athletes with intact autonomic salivary gland innervation. The similar responses between subgroups may stem from sympathetic reflex activity during exercise or a predominant contribution of parasympathetic activity, which are still intact systems in the TETRA population. The results of this study support the positive role of acute exercise on oral immune function in wheelchair athletes independent of disability type.

6.2 Introduction

The production of sIgA is the major function of the mucosal immune system, and sIgA is the predominant immunoglobulin in saliva and other mucosal secretions. It has been described as 'the first line of defence' against pathogens and antigens presented at the mucosa, such as cold-causing viruses (Bishop & Gleeson, 2009; Woof & Kerr, 2006). Specifically, decreased levels of sIgA concentration have been associated with subsequent episodes of upper respiratory tract infection (Neville *et al.*, 2008; Fahlman & Engels, 2005) and sIgA has therefore been suggested to be the most useful clinical biomarker to predict the incidence of this infection (Fahlman & Engels, 2005).

Saliva composition and secretion of sIgA can be modified by both parasympathetic and sympathetic nerve stimulation (Proctor & Carpenter, 2007). Since intensive exercise is associated with enhanced sympathetic nervous system activity, it seems logical to assume that physical activity could modify secretion of saliva and its constituent proteins (Bishop & Gleeson, 2009). Indeed, decreased sIgA secretion rates have been reported following bouts of strenuous exercise (Nieman *et al.*, 2002; Walsh *et al.*, 2002; MacKinnon & Jenkins, 1993; Mackinnon *et al.*, 1993). In contrast, following moderate intensity exercise (typically below 70% maximal oxygen uptake), secretion rates of sIgA are generally unaffected (Allgrove *et al.*, 2008; Nieman *et al.*, 2005; Li & Gleeson, 2004). However, it should be noted that data in the literature are conflicting, and a number of studies have shown no decrease in sIgA secretion rate following strenuous exercise (Bishop & Gleeson, 2009; Allgrove *et al.*, 2008; Bishop *et al.*, 2006; Walsh *et al.*, 1999).

The impact of controlled bouts of exercise on mucosal immune function has yet to be analysed in SCI individuals. An understanding of this is of interest for a number of reasons: Firstly, certain parameters of innate immunity are depressed in the SCI population in resting conditions (Campagnolo *et al.*, 2008). This may potentially lead to differences when comparing mucosal immune function following exercise between PARA and NON-SCI individuals. Furthermore, it has been suggested that differences in exercise protocols may have an impact on sIgA concentration and secretion rate (Walsh *et al.*, 2002), thus it is of interest to examine wheelchair propulsion and how this may influence exercise-induced immune responses. Finally, given the above-mentioned effects of sympathetic activation on

sIgA in the AB population, the decreased sympathetic outflow in TETRA individuals (Schmid *et al.*, 1998c; Stjernberg *et al.*, 1986) may affect their immune response following exercise.

Analysing the mucosal immune function in SCI individuals is of practical relevance. The most common causes of death in SCI individuals are related to respiratory illnesses (Brown *et al.*, 2006), and individuals with a higher lesion level appear to be at a higher risk for pulmonary complications (Lucke, 1998). Underlying mechanisms include lesion-dependent losses of respiratory muscle innervation, which lead to impairments in respiratory muscle function (Baydur *et al.*, 2001). While low level PARA individuals are comparable with AB individuals with respect to respiratory and upper body strength, respiratory muscle strength in tetraplegia is markedly reduced (Haisma *et al.*, 2006). This results in a decreased ability to cough and clear secretions, and as a consequence, various types of respiratory diseases such as pneumonia or respiratory failure occur in SCI individuals (Brown *et al.*, 2006). Furthermore, uncoordinated autonomic control in TETRA individuals may be responsible for abnormal bronchial secretion, airway hypersensitivity, and other respiratory issues (Krassioukov, 2009). On the other hand, SCI persons are still capable to fortify their systemic immune system despite depressed resting levels, as shown following electrically stimulated exercise in TETRA individuals (Nash, 1994). However, data about impacts of exercise on mucosal immune function in this population are scarce.

Knowledge about the adaptations of the mucosal immune function following exercise in wheelchair athletes could serve as a base of health promotion and monitoring in this specific population, using sIgA as a biomarker, which also has a practical advantage as saliva is easy to collect. Salivary α-amylase may support the protective function of sIgA, as it can bind to some oral bacteria (Scannapieco *et al.*, 1994). Given that α-amylase has been proposed as a non-invasive marker of the sympathetic nervous system (Nater & Rohleder, 2009), the impact of exercise on this enzyme is of particular interest, especially in individuals with a disrupted sympathetic nervous system, i.e., TETRA individuals. Therefore, the purpose of this investigation was to explore the impacts of a 60-min constant load (moderate) and intermittent (strenuous) bout of laboratory-controlled exercise on the sIgA and α-amylase response in a group of wheelchair athletes. Due to the reduced sympathetic outflow in TETRA individuals, a less pronounced sIgA decrease and a less

pronounced α-amylase increase induced by strenuous exercise in this population was hypothesised.

6.3 Methods

6.3.1 Participants

Twenty-three male wheelchair athletes volunteered to participate in this study. Participants consisted of eight motor complete TETRA, seven motor complete PARA, and eight NON-SCI individuals, competing in wheelchair basketball, rugby and tennis. All participants performed their sport on a national level at least; a summary of their physical, physiological and sport characteristics is presented in Table 6.1.

6.3.2 Experimental design

Participants visited the laboratory on two occasions, separated by a minimum of 4 days. After reporting to the laboratory between 09:30 and 11:00, participants completed questionnaires and body mass was recorded as explained in Chapter 3.2. All exercise tests were performed in the athletes' sports wheelchair on a motorised treadmill, with details described in Chapter 3.3. A food diary was provided and participants were asked to eat and drink the same types and amounts of food before both visits, to abstain from caffeine and not to perform any exercise 24 h prior to the tests. To minimise the risk of autonomic dysreflexia, participants emptied their bladder immediately before each exercise session. In a preliminary testing phase, which was performed in both visits, exercise intensities for the main trial were obtained. The same standardised lunch was then provided for both visits; participants were not allowed any other food intake apart from lunch during the laboratory tests, and drink intake was limited to water *ad libitum*. The main trial was conducted 1 h following lunch (13:00 – 15:30 for all participants). For one visit, this consisted of a constant load (CL), for the other visit, of an intermittent (IM) exercise block. The order of visits was randomised and counterbalanced for all participant subgroups.

Table 6.1 Participants' characteristics.

Parameter	TETRA	PARA	NON-SCI
Body mass [kg]	67.9 ± 6.7	71.2 ± 11.0	76.3 ± 11.6
Body height [m]	1.80 ± 0.09	1.71 ± 0.12	1.82 ± 0.14
Age [years]	29.2 ± 3.8	28.0 ± 5.1	24.0 ± 6.2
Lesion level/ disability types	C6 – C7	T6 – T12, spina bifida	Amputations, club feet, brittle bones
Time since onset of disability [years]	9.3 ± 4.8*†	17.8 ± 9.2	21.4 ± 2.8
Wheelchair sport	Rugby (N = 8)	Basketball (N = 7)	Basketball (N = 7) Tennis (N = 1)
Training volume [h·week^{-1}]	14 ± 6	13 ± 3	11 ± 6
Wheelchair sport experience [years]	7.8 ± 4.6	11.6 ± 5.6	8.0 ± 5.8
$\dot{V}O_{2peak}$ [L·min^{-1}]	1.77 ± 0.43*†	2.60 ± 0.31	3.08 ± 0.60
$\dot{V}O_{2peak}$ [ml·kg^{-1}·min^{-1}]	26.0 ± 5.4*†	37.2 ± 6.5	40.3 ± 4.1
Peak heart rate [beats·min^{-1}]	136 ± 21*†	190 ± 6	189 ± 11

Apart from lesion level/disability types and sport, values are means ± standard deviations. *Significant difference, TETRA *vs.* PARA; †significant difference, TETRA *vs.* NON-SCI, at $P < 0.05$ level.

6.3.3 Preliminary testing

Following the protocols of studies 1 and 2, participants performed six to eight submaximal constant load 4-min exercise blocks at 1.0% gradient and ascending speeds, in order to elicit physiological responses covering a range of ~40 – 80% $\dot{V}O_{peak}$. This was followed by a 15-min passive recovery. A GXT was then performed at a constant speed, which was chosen according to the responses elicited during the submaximal exercise blocks. The gradient at the start of the GXT was 1.0% for all subgroups; the gradient was then increased by 0.3% every minute for PARA and NON-SCI, and by 0.1% every 40 s for TETRA, in order to achieve total GXT test times between 8 and 14 min. After the GXT, participants sat quietly for 2 min and then recovered actively at a low intensity (1.0 m·s^{-1} at

1.0% gradient) for 5 min. In order to confirm the $\dot{V}O_{2peak}$ attained in the GXT, they then performed a VER, designed as a test to exhaustion at the same constant speed but at a gradient which was higher than the maximal gradient achieved during the GXT (+0.6% for PARA and NON-SCI; +0.3% for TETRA). The GXT and the VER were terminated when participants were unable to maintain the speed of the treadmill, and verbal encouragement was given throughout the test. Spirometric data were recorded continuously with an online gas analysis system (MetaLyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany), and $\dot{V}O_2$ of each submaximal exercise block was averaged over the final minute, whereas $\dot{V}O_{2peak}$ was defined as highest average value over 30 s for both the GXT and the VER. From the linear workload - $\dot{V}O_2$ relationship and from the higher of the two $\dot{V}O_{2peak}$ readings attained in the GXT and the VER, speeds at 1.0% gradient for 40, 60, and 80% $\dot{V}O_{2peak}$ were then calculated for each individual from the preliminary testing of the first visit and used for both main trials. Saliva samples during the preliminary testing were obtained before participants started to exercise and immediately after the VER, which represented the final bout of exercise; saliva sampling procedures are outlined in Chapter 3.7. The results of the preliminary testing of the second visit were ignored, but all procedures were carried out to ensure an identical preload before the main trials.

6.3.4 Main trial

Participants performed 60 min of exercise at 1.0% gradient, which was divided by a 5-min break after 30 min, allowing data collection for mid exercise responses. In the CL trial, the speed was set constantly at 60% $\dot{V}O_{2peak}$ for the whole duration of exercise. In the IM trial, participants completed twenty 2-min periods at 80% $\dot{V}O_{2peak}$, each separated by a 1-min recovery at 40% $\dot{V}O_{2peak}$ (Figure 6.1).

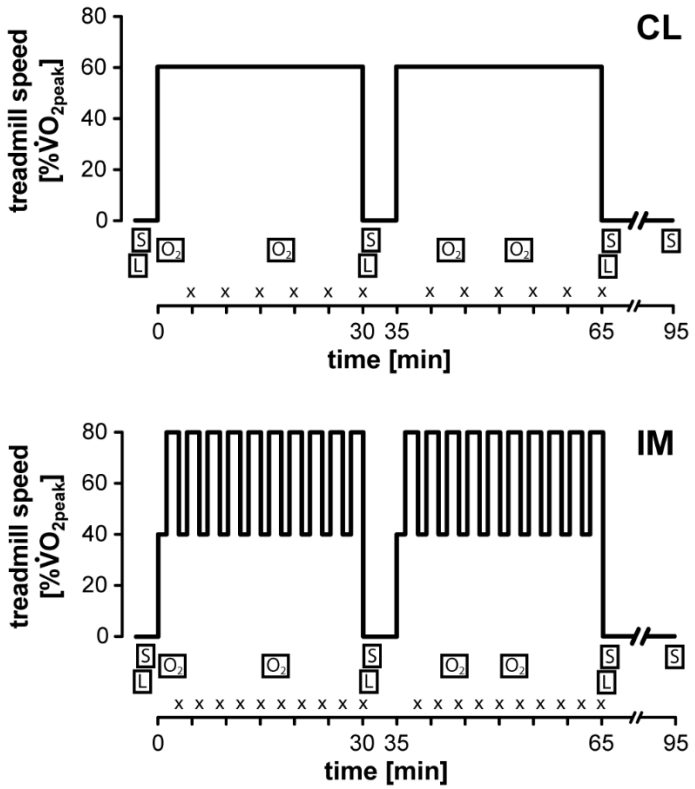

Figure 6.1 Test protocol for main trials.

CL, constant load trial; **IM**, intermittent trial. **S**, saliva sample; **L**, blood sample for lactate analysis; **O_2**, spirometric measurements for determination of $\dot{V}O_2$; **x**, rating of perceived exertion.

6.3.5 Data collection main trial

Timed, unstimulated saliva samples were collected into sterile plastic containers pre, mid, post, and 30 min post exercise. Participants were allowed to consume water *ad libitum* apart from 6 min prior to each collection. At pre, mid, and post exercise, small capillary blood samples were obtained from the earlobe in order to measure BLa. For participants who terminated the main trial prior to 60 min due to exhaustion, a saliva and capillary blood sample was obtained at the time of exhaustion and treated as post exercise data. Further, participants were asked to indicate their RPE using a scale ranging from 6 – 20 (Borg, 1982) every 5 min during the CL trial and following each period at 80%$\dot{V}O_{2peak}$ in the IM trial. HR was continuously recorded, whereas spirometric data were recorded during four 4-min intervals (for details, see Figure 6.1) using a calibrated online gas analysis system as described in Chapter 3.4.

6.3.6 Analytical methods

For the analysis of sIgA and salivary α-amylase the procedures as outlined in Chapter 3.7 were followed. The CV of the methods based on analyses of duplicate samples were 2.1 ± 2.0% for sIgA and 1.7 ± 1.5% for α-amylase.

6.3.7 Data processing and statistical analyses

The SPSS 16.0 statistical package (SPSS Inc., Chicago IL, USA) was used for all statistical analyses. The sample size calculation was based on previous unpublished pilot data of relative increases in sIgA secretion rates (22 ± 16%) immediately after an intensive training session compared to resting levels in elite PARA athletes from the same population as the present study. Using GPower 3.1.2, it was calculated that 7 participants in each subgroup would be required to detect a similar change in sIgA secretion rate, with an effect size of 1.38, 90% power and an α of 5%.

Means and SD were computed for all variables. In order to achieve normality and homogeneity of data, a logarithmic transformation was applied to sIgA and amylase data of the main trial, and a square root transformation to amylase data and a logarithmic transformation to saliva flow rate data of the preliminary testing. Normality was checked with the Shapiro Wilk, homogeneity with Levene's statistic. A three-way (group x time x exercise type) repeated measures ANOVA was then applied to these data. To compare the observed interaction effects of the analysis of sIgA data of the main trial, PARA and NON-SCI data were collapsed into one group, and multiple two-way (group x time) repeated measures ANOVAs were conducted to compare different time points, applying a Bonferroni correction to take into account these multiple comparisons. For all comparisons where the assumption of sphericity was violated, a Greenhouse Geisser correction was applied.

$\dot{V}O_2$ data was averaged over 1 min at minutes 3, 18, 44, and 53. RPE and HR data were averaged over each 30-min exercise block separately. $\dot{V}O_2$ data were compared between trials using Bonferroni corrected paired *t*-tests. Saliva flow rate, RPE, HR, BLa data violated the normality and/or homogeneity of variances assumption required for parametric

testing. Therefore, multiple Wilcoxon signed rank tests were used to analyse RPE, HR, and BLa data between trials, and multiple Kruskall-Wallis tests for between-groups analyses. Saliva flow rate data were analysed with multiple Wilcoxon signed rank tests, comparing pre exercise data to mid, post, and 30 min post data. For all multiple comparisons, Bonferroni corrections were applied. Statistical significance for all analyses was accepted at $P < 0.05$.

6.4 Results

6.4.1 Preliminary testing

Submaximal exercise, followed by a GXT and a VER resulted in significant elevations of sIgA concentration, sIgA secretion rate, and α-amylase activity, whilst saliva flow rate significantly decreased in all three participant subgroups when compared with resting values. No difference between subgroups was apparent (Figure 6.2, Table 6.2).

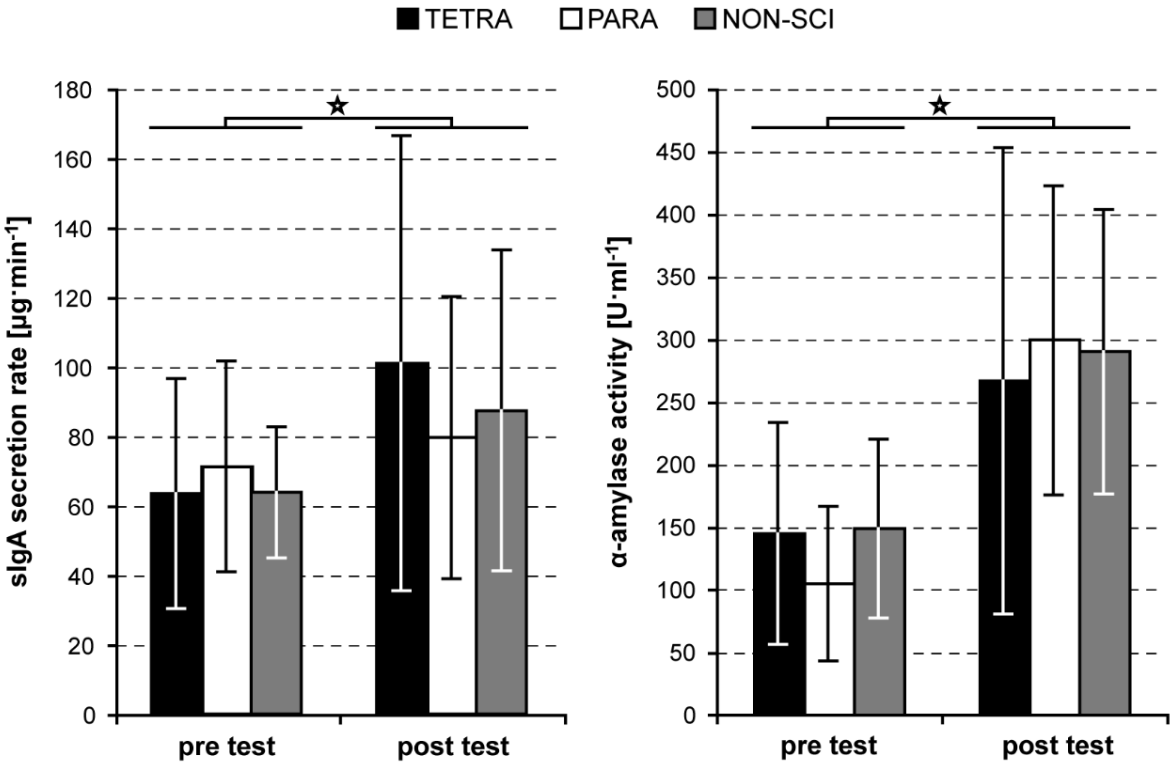

Figure 6.2 Effect of exhaustive exercise on sIgA secretion rate and α-amylase activity.

*Significantly different between tests, at $P < 0.05$ level.

Table 6.2 Salivary responses to exhaustive exercise.

Parameter	TETRA	PARA	NON-SCI
sIgA concentration pre test [mg·L^{-1}]	166 ± 117	191 ± 121	132 ± 50
sIgA concentration post test [mg·L^{-1}]*	337 ± 167	294 ± 170	222 ± 127
Saliva flow rate pre test [mL·min^{-1}]	0.56 ± 0.41	0.44 ± 0.18	0.55 ± 0.26
Saliva flow rate post test [mL·min^{-1}]*	0.36 ± 0.22	0.36 ± 0.26	0.47 ± 0.33

Values are means ± standard deviation. *Significantly different from pre test, at $P < 0.05$ level.

6.4.2 Main trial

All participants completed the CL trial, while three participants terminated the IM trial early (1 TETRA at 44 min; 2 NON-SCI at 30 and 38 min). The IM trial resulted in significant elevations of $\dot{V}O_2$, BLa and RPE when compared with the CL trial in all three participant subgroups. However, no differences between subgroups were found in $\%\dot{V}O_{2peak}$ and BLa at any time ($P > 0.05$, Table 6.3). HR was consistently lower in the TETRA subgroup when compared with the other subgroups ($P < 0.05$), whereas RPE did not differ between subgroups ($P > 0.05$, Table 6.3). Finally, water consumption during exercise did not differ between subgroups ($P > 0.05$).

In line with the preliminary findings, the impact of exercise resulted in an elevated sIgA concentration ($P < 0.001$) and secretion rate ($P < 0.05$, Figure 6.3), which remained elevated 30 min post exercise, whereas saliva flow rate was unaffected by exercise ($P > 0.05$, Table 6.4). Both sIgA concentration and secretion rate did not differ between participant subgroups at any time point, and the responses evoked by exercise did not differ between the CL and the IM trials ($P > 0.05$). A group x time interaction ($P < 0.05$) indicated a different development of sIgA secretion rate (but not IgA concentration), and following visual inspection of data, group x time interactions between TETRA and a collapsed group of PARA and NON-SCI showed a further increase of sIgA secretion rate for TETRA following mid exercise, whereas sIgA secretion rates for PARA and NON-SCI decreased ($P < 0.05$, Figure 6.4). As a consequence, 60 min of exercise resulted in a more

pronounced overall increase of sIgA secretion rate in TETRA when compared with the other subgroups ($P < 0.05$).

Table 6.3 Physiological and psychophysiological responses to 60 min of exercise.

Parameter	Subgroup	pre CL	mid CL	end CL	pre IM	mid IM	end IM
$\%\dot{V}O_{2peak}$	TETRA		61.5 ± 4.8*	62.7 ± 5.2†		78.5 ± 9.1	84.2 ± 8.1
	PARA		58.9 ± 3.7*	59.6 ± 3.6†		78.5 ± 3.9	80.4 ± 6.2
	NON-SCI		58.2 ± 4.7*	59.8 ± 4.4†		77.6 ± 5.1	77.7 ± 7.1
Blood lactate concentration [mmol·L^{-1}]	TETRA	1.19 ± 0.31	1.07 ± 0.20*	0.96 ± 0.21†	1.33 ± 0.46	3.26 ± 1.77	2.20 ± 0.66
	PARA	1.37 ± 0.39	1.11 ± 0.24*	0.87 ± 0.15†	1.36 ± 0.27	2.91 ± 1.18	2.48 ± 1.06
	NON-SCI	1.30 ± 0.55	0.92 ± 0.33*	0.80 ± 0.31†	1.16 ± 0.39	2.80 ± 1.21	2.14 ± 1.04
Heart rate [beats·min^{-1}]	TETRA		101 ± 11*$^{\Lambda}$	100 ± 11$^{\dagger\Lambda}$		109 ± 13$^{\Lambda}$	108 ± 11$^{\Lambda}$
	PARA		140 ± 20*	134 ± 19†		156 ± 10	154 ± 10
	NON-SCI		134 ± 13*	130 ± 15†		151 ± 11	145 ± 16
RPE	TETRA		12.0* (11.0, 13.0)	12.0† (11.5, 13.0)		15.5 (14.5, 17.0)	17.0 (16.5, 18.5)
	PARA		13.0* (13.0, 14.5)	15.0† (13.0, 15.0)		15.0 (13.0, 15.5)	17.0 (15.5, 17.5)
	NON-SCI		13.5* (13.0, 15.0)	13.0† (12.5, 14)		14.0 (14.0, 16.5)	16.0 (16.0, 18.0)

Values are means ± standard deviation, apart from RPE values, which are medians (interquartile range). **CL**, constant load trial; **IM**, intermittent trial; **RPE**, rating of perceived exertion. *Significant difference, mid CL *vs.* mid IM; †significant difference, end CL *vs.* end IM; $^{\Lambda}$significant difference, TETRA *vs.* PARA and NON-SCI; at $P < 0.05$ level.

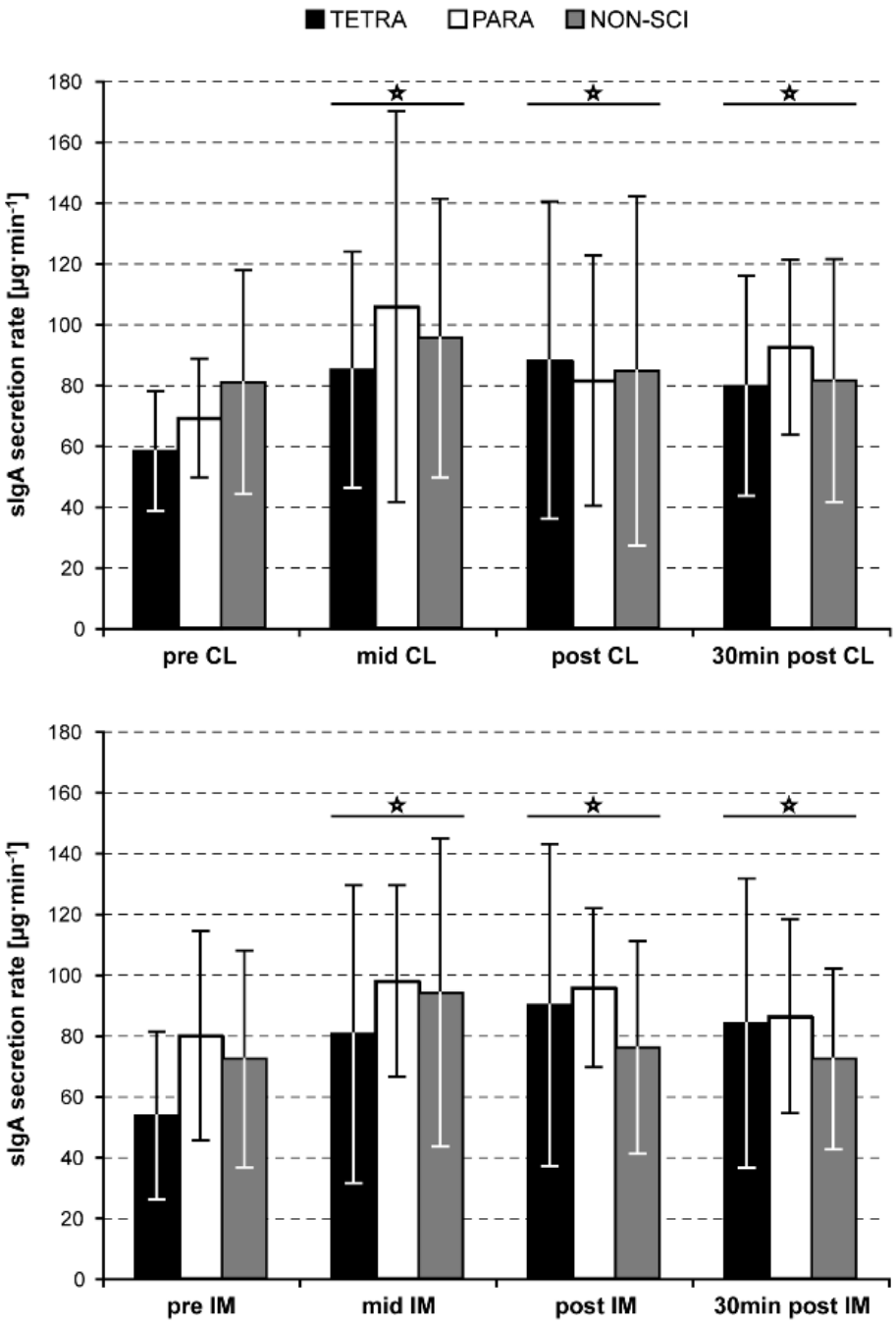

Figure 6.3 Effect of a 60-min exercise trial on sIgA secretion rate.

CL, constant load trial; **IM**, intermittent trial. ⋆Significantly different from pre, at $P < 0.05$ level.

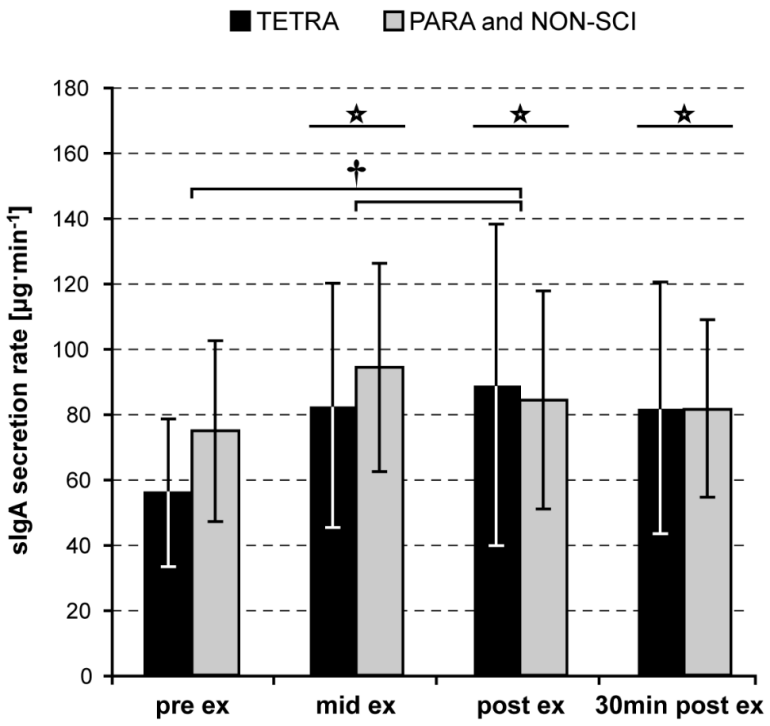

Figure 6.4 Effect of a 60-min exercise trial on sIgA secretion rate.

ex, exercise (pooled data of constant load and intermittent trial). *Significantly different from pre, at $P < 0.05$ level; †significant group x time interaction, at $P < 0.05$ level.

The impact of exercise resulted in an elevated α-amylase activity ($P < 0.001$), which returned to resting levels 30 minutes post exercise. In analogy to the sIgA concentration data, α-amylase activity did not differ between participant subgroups at any time point, and the responses evoked by exercise did not differ between the CL and the IM trials (Table 6.4).

Table 6.4 Salivary responses to 60 min of exercise.

Parameter	TETRA CL	PARA CL	NON-SCI CL	TETRA IM	PARA IM	NON-SCI IM
sIgA concentration pre ex [mg·L^{-1}]	103 ± 63	118 ± 39	103 ± 34	107 ± 64	133 ± 54	104 ± 37
sIgA concentration mid ex [mg·L^{-1}]	167 ± 67*	146 ± 57*	151 ± 56*	171 ± 108*	186 ± 109*	158 ± 45*
sIgA concentration post ex [mg·L^{-1}]	184 ± 104*	164 ± 76*	148 ± 59*	183 ± 104*	187 ± 104*	142 ± 36*
sIgA concentration 30 min post ex [mg·L^{-1}]	210 ± 195*	147 ± 36*	136 ± 160*	189 ± 144*	186 ± 138*	114 ± 49*
α-amylase activity pre ex [U·mL^{-1}]	160 ± 156	153 ± 99	227 ± 124	176 ± 166	169 ± 106	310 ± 203
α-amylase activity mid ex [U·mL^{-1}]	279 ± 161†	220 ± 100†	307 ± 121†	239 ± 172†	346 ± 191†	336 ± 121†
α-amylase activity post ex [U·mL^{-1}]	294 ± 150†	246 ± 85†	299 ± 131†	251 ± 132†	347 ± 207†	303 ± 88†
α-amylase activity 30 min post ex [U·mL^{-1}]	187 ± 152	140 ± 68	208 ± 115	190 ± 146	184 ± 145	207 ± 135
Saliva flow rate pre ex [mL·min^{-1}]	0.68 ± 0.34	0.58 ± 0.19	0.83 ± 0.35	0.57 ± 0.29	0.63 ± 0.22	0.77 ± 0.43
Saliva flow rate mid ex [mL·min^{-1}]	0.55 ± 0.25	0.63 ± 0.18	0.65 ± 0.20	0.57 ± 0.33	0.66 ± 0.23	0.71 ± 0.48
Saliva flow rate post ex [mL·min^{-1}]	0.52 ± 0.23	0.54 ± 0.13	0.57 ± 0.21	0.61 ± 0.47	0.60 ± 0.25	0.58 ± 0.36
Saliva flow rate 30 min post ex [mL·min^{-1}]	0.50 ± 0.22	0.61 ± 0.17	0.66 ± 0.35	0.57 ± 0.45	0.60 ± 0.22	0.70 ± 0.29

Values are means ± standard deviation. **CL**, constant load trial; **IM**, intermittent trial; **ex**, exercise. *Significantly different from pre ex, †significantly different from pre ex and 30 min post ex, at $P < 0.001$ level.

6.5 Discussion

6.5.1 Main findings

The main finding of this study is that resting levels and the main trends in sIgA secretion rate following exercise do not differ when comparing TETRA, PARA and NON-SCI participants and result in an increase of this parameter during 60 min of both CL and IM exercise. However, TETRA participants exhibit a different pattern in the evolution of sIgA secretion rate, resulting in a greater magnitude of increase from pre to post levels. Furthermore, α-amylase activity was shown to increase during exercise and return to resting levels 30 min following exercise, irrespective of the type of exercise and the participant subgroup. The preliminary testing results show the same pattern, with sIgA secretion rate and α-amylase activity increasing in all participant subgroups as a result of exhaustive exercise.

It should be noted that the participants of this study comprised of a highly trained group, with $\dot{V}O_{2peak}$ values similar to or exceeding existing literature of international level wheelchair athletes (Goosey-Tolfrey *et al.*, 2006; Goosey-Tolfrey *et al.*, 2003). This means, with respect to training status, they were comparable to participants of a number of previous studies investigating sIgA and/or α-amylase responses to exercise (Sari-Sarraf *et al.*, 2007; Bishop *et al.*, 2006; Sari-Sarraf *et al.*, 2006; Li & Gleeson, 2004; Walsh *et al.*, 1999; Mackinnon *et al.*, 1993).

The sIgA responses evoked by exercise in wheelchair athletes are in line with findings in AB athletes using a similar (though soccer-specific) protocol to the present study, where no differences in sIgA concentration and secretion rate have been found between CL and IM exercise, despite higher RPE scores in the IM trial (Sari-Sarraf *et al.*, 2006). The same research group also found increased sIgA secretion rates following IM exercise (Sari-Sarraf *et al.*, 2007), which again is in line with the present findings but is challenged by Walsh *et al.* (1999) who found no changes in sIgA secretion rate following IM exercise of a similar protocol. Likewise, similar α-amylase responses have been observed following laboratory

controlled bouts of moderate (Allgrove *et al.*, 2008; Li & Gleeson, 2004) and intense (Allgrove *et al.*, 2008; Walsh *et al.*, 1999) exercise.

It is commonly accepted that the sympathetic nervous system may at least partly be responsible for the changes in salivary markers, such as sIgA and α-amylase (Bishop & Gleeson, 2009; Allgrove *et al.*, 2008; Sari-Sarraf *et al.*, 2007; Bishop *et al.*, 2006; Chicharro *et al.*, 1998). Hence, it may appear surprising that no distinct differences in the mucosal immune responses between TETRA, and the PARA and NON-SCI group were found. TETRA individuals represent a model with no centrally mediated sympathetic nervous control (Krassioukov, 2009), as centrally mediated sympathetic stimuli do not activate the decentralised part below the level of lesion (Corbett *et al.*, 1971). The disrupted autonomic innervation of the heart therefore leads to the observed decreased HR, which is a common observation in TETRA individuals (Goosey-Tolfrey *et al.*, 2006; Haisma *et al.*, 2006; Schmid *et al.*, 2001). Most importantly in the context of the present study, the innervation of the salivary glands in TETRA individuals is disrupted as well, as it originates from the upper thoracic segments, although it remains unclear precisely where in this region (Proctor & Carpenter, 2007). In rats, it has been shown that sympathectomy results in a decreased sIgA secretion (Proctor *et al.*, 2000). Furthermore, both parasympathetic and sympathetic stimulation of rat salivary glands evokes changes in both saliva flow rate and sIgA secretion (Proctor & Carpenter, 2007). As the literature review did not reveal any scientific study investigating sIgA in human TETRA individuals during exercise, it seems possible that the differences to the responses observed in denervated animal models may be species-related, or may stem from other, exercise related factors, such as circulating metabolites (Victor *et al.*, 1988), which may alter the autonomic neural output.

During exercise, TETRA individuals may compensate the lack of a centrally mediated neural drive with a spinal reflex. The observed increase in adrenaline and noradrenaline plasma concentrations in TETRA following bladder stimulation (Karlsson *et al.*, 1998) or electrically stimulated cycle exercise (Bloomfield *et al.*, 1994) both support the theory of a remaining, but qualitatively altered, sympathetic function due to reflex activity. Moreover, a hyper-responsiveness of α-adrenoreceptors in TETRA individuals (Arnold *et al.*, 1995) may further compensate for some of the lack of the centrally mediated neural drive. With

respect to physical exercise, reflex activity may also be driven by afferent signals from mechanoreceptors (Vissing *et al.*, 1991), and it is possible that a reflex increase in sympathetic outflow due to muscle acidosis (Victor *et al.*, 1988) is responsible for the observed increase in sIgA secretion rate and α-amylase activity during exercise. No differences in BLa between participant subgroups were found, suggesting that any reflex activity stemming from muscle acidosis may result in similar responses in all participant subgroups. With respect to mucosal immune function, this would underline the value of exercise for a population which is disadvantaged with regard to immune function (Yamanaka *et al.*, 2010; Campagnolo *et al.*, 2008). However, it must be appreciated that BLa is not a direct measure of muscle acidosis and that therefore muscle acidosis may be different between disability subgroups, even more as disability-dependent differences in active muscle mass and blood volume (Houtman *et al.*, 2000) potentially impact on BLa.

Even though the main trends in sIgA secretion rate are similar between subgroups and show an exercise-induced increase, the lack of centrally mediated sympathetic control may manifest itself in the fine-tuning of the mucosal immune response. It appears that sIgA secretion rate is down-regulated following 30 min of exercise in PARA and NON-SCI, whereas this is not the case in TETRA individuals. Therefore, it is possible that centrally mediated sympathetic signals may have a greater impact in the down-regulation, whereas the above mentioned peripheral signals contribute to an up-regulation of sIgA secretion rate. A further interesting observation is that sIgA secretion rate before exercise in TETRA individuals tends to be slightly lower. However, following 60 min of exercise, this is increased to average values found in the other subgroups.

Salivary α-amylase is increasingly being used as a biomarker for autonomic nervous system activation, even though it has been pointed out that the correlation of α-amylase activity and sympathetic markers, such as noradrenaline or adrenaline plasma concentrations, are relatively small (Nater & Rohleder, 2009). In the present study, no differences in α-amylase between subgroups were found. However, it should again be noted that compensatory mechanisms, such as sympathetic reflex activity, may enable individuals, who lack central sympathetic drive, to access parts of their sympathetic system. This suggestion is supported by the reduced, but still existent increase in adrenaline and noradrenaline plasma concentrations following exercise in TETRA individuals (Schmid *et*

al., 1998b). Therefore, the observed increase in α-amylase in TETRA individuals is not necessarily proof that this biomarker should not be used to deduce sympathetic overall drive. However, it should clearly not be used as a biomarker for sympathetic central drive.

6.5.2 Limitations

Autonomic dysreflexia is a condition sometimes found in high-level SCI individuals and includes a high outflow of catecholamines (Schmid *et al.*, 2001). It must be noted that no self-reported episodes of autonomic dysreflexia and no episodes of acute bradycardia, indicating autonomic dysreflexia, were noted during any of the exercise tests. Furthermore, only two of the TETRA participants had a history of exercise-related autonomic dysreflexia. However, to control autonomic dysreflexia more closely, data collection relating to objective symptoms of autonomic dysreflexia (such as blood pressure, skin color or sweating) in future exercise studies with TETRA individuals is encouraged. This is of particular importance as sympathetic nervous system hyperactivity and the concomitant increased outflow of adrenaline and noradrenaline may affect sIgA and α-amylase secretion.

Further, it should be noted that the statistical power of this study was limited, owing to the high variability of salivary data. If feasible, more participants should be recruited for future studies; even though it is appreciated that it is very difficult to recruit large participant numbers in homogenous SCI elite athlete cohorts.

6.6 Conclusions

It is concluded that TETRA, PARA, and NON-SCI wheelchair athletes likewise experience the positive acute effects of exercise on markers of the mucosal immune function. However, the impaired autonomic nervous system in TETRA seems to influence the fine-tuning of their sIgA response when compared with PARA and NON-SCI. This results in a greater increase of sIgA secretion rate in TETRA, which may potentially result in more pronounced immuno-protective effects in this subgroup. Furthermore, the results of this study question the use of α-amylase as a marker of centrally mediated autonomic nervous system activity.

7

Study 4: Mucosal immune responses during court training in elite tetraplegic athletes

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Study 3 has provided first insights into the mucosal immune responses to exercise in wheelchair athletes and a slightly altered response has been found in tetraplegic athletes when compared with the other subgroups. Study 4 will extend the laboratory finding into a field-based setting, investigating tetraplegic athletes.

7.1 Abstract

The aims of this study were to examine salivary secretory immunoglobulin A (sIgA) responses and α-amylase activity during court training in highly trained tetraplegic athletes.
Methods: Seven highly trained wheelchair rugby athletes with tetraplegia performed two separate wheelchair rugby court training sessions, lasting 23 and 41.5 min, respectively, with either an aerobic or an interval focus. Timed, unstimulated saliva samples were obtained pre, post, and 30 min post exercise and analysed for sIgA and α-amylase. Furthermore, blood lactate concentration and rating of perceived exertion (RPE) immediately after training were measured.
Results: sIgA secretion rate and α-amylase were unaffected by exercise during both sessions. However, the increases of sIgA concentration (30 min post exercise: +67 ± 29%) during the aerobic session were accompanied by decreases in saliva flow rate (-35 ± 22%). Athletes' physiological responses to exercise document the highly strenuous nature of the sessions, with blood lactate concentrations reaching 8.1 ± 1.0 and 8.7 ± 1.6 $mmol \cdot L^{-1}$ and RPE reaching 18 (17, 18) and 16 (15, 17) for the aerobic and the interval session, respectively.
Conclusion: Acute bouts of highly strenuous exercise do not have negative impacts on mucosal immune responses in tetraplegic athletes, nor do they influence the production of α-amylase, a marker of sympathetic nervous activity. This contrasts with responses previously observed in able-bodied athletes. The disruption of the sympathetic nervous system may prevent the down-regulation of sIgA secretion rate following intense exercise, which is a response previously observed in able-bodied athletes.

7.2 Introduction

In analogy to study 3, study 4 is concerned with the analysis of the mucosal immune function, and sIgA specifically. This field has been introduced extensively in the previous chapter, therefore, only the most important facts are given in short.

sIgA is the predominant immunoglobulin in saliva and other mucosal secretions and has been described as 'the first line of defence' against pathogens and antigens presented at the mucosa, such as viruses responsible for the common cold (Walsh *et al.*, 2011; Bishop & Gleeson, 2009). sIgA has been suggested to be a useful clinical biomarker to predict the incidence of upper respiratory tract infection (Fahlman & Engels, 2005), as decreased levels of sIgA concentration have been associated with subsequent episodes of this ailment (Neville *et al.*, 2008; Fahlman & Engels, 2005). Regarding exercise, decreased sIgA secretion rates have been reported following strenuous exercise in AB individuals (Nieman *et al.*, 2002; Walsh *et al.*, 2002; Mackinnon *et al.*, 1993), which may be due to the association of intensive exercise and enhanced sympathetic nervous system activity (Meyer *et al.*, 1988).

TETRA individuals represent a model with no centrally mediated sympathetic nervous control (Krassioukov, 2009), as centrally mediated sympathetic stimuli do not activate the decentralised part below the level of lesion (Corbett *et al.*, 1971). It must further be noted that the innervation of the salivary glands in TETRA individuals is also disrupted, as it originates from the upper thoracic segments (Proctor & Carpenter, 2007). Some of these changes affect the immune response of this population and ultimately, its health, as a number of comorbidities have been associated with the impaired autonomic (Krassioukov, 2009), immune (Riegger *et al.*, 2009) and/or respiratory function (Brown *et al.*, 2006). Given the above-mentioned effects of sympathetic activation on sIgA, the decreased sympathetic outflow in TETRA individuals (Schmid *et al.*, 2001; Stjernberg *et al.*, 1986) can result in an altered immune response following intense exercise when compared with populations with intact sympathetic innervation (Chapter 6). Extending the insights of this laboratory-based study into a more applied, field-based environment is likely to increase the practical value for the exercising individual. The data obtained could hence serve as a base of health promotion and monitoring in this specific athletic population. Looking

beyond the research context, the use of salivary markers, with the practical advantage of easy and non-invasive data collection, may help providing feedback for athletes and coaches. Therefore, the purpose of this investigation was to explore the impacts of highly strenuous court training sessions on mucosal immune responses in TETRA athletes. Due to the nature of their disability with a disruption of autonomic nerve pathways, it is hypothesised that sIgA will not be down-regulated as a result of exercise-induced changes of sympathetic activity.

7.3 Methods

7.3.1 Participants

Seven male highly trained TETRA wheelchair rugby athletes with motor and sensory complete lesions volunteered to participate in this study. All participants regularly attended structured training sessions with the Great Britain national team under the supervision of appointed strength and conditioning coaches. Participant C performed the sport at a national level, all other participants at an international level. A weekly training volume of at least 10 h was required for inclusion in this study. Prior to the study, all participants were classified for wheelchair rugby functional class according to the International Wheelchair Rugby Federation Classification system (IWRF, 2012); a summary of their physical and sport characteristics is presented in Table 7.1.

7.3.2 Experimental design

Participants were monitored during two regular court training sessions, which were designed and supervised by the same coach and separated by 7 – 14 days for all athletes (Figure 7.1). As the sessions were part of the participants' yearly training schedule, the investigators could not influence the order of the sessions (five athletes completed the aerobic training session first). Both sessions took place at the same indoor location and started at 11:00, and participants were advised to consume the same breakfast on both days of testing. Before the sessions, questionnaires were completed and body mass recorded as explained in Chapter 3.2. Training sessions were performed in the participants' competition

wheelchair. Both sessions were preceded by a 20-min warm-up period, which included easy pushing, agility practice, short sprints and stretching.

Table 7.1 Participants' characteristics.

Participant	Age [years]	Lesion level	Body mass [kg]	Wheelchair sport experience [years]	Rugby classi-fication	Training volume [h·week^{-1}]
A	28	C6	56.2	10	0.5	13.5
B	39	C5/6	64.4	11	1.0	10.0
C	20	C7	65.4	1	2.0	16.0
D	32	C6/7	64.2	13	2.0	13.5
E	30	C6/7	69.8	10	2.5	13.5
F	32	C6/7	94.8	12	2.5	15.0
G	30	C6/7	68.9	5	2.5	19.0
Mean	30	N/A	69.1	8.9	-	14.4
SD	6	N/A	12.2	4.3	-	2.8

SD, standard deviation. Rugby classification according to the international wheelchair rugby federation (IWRF, 2012).

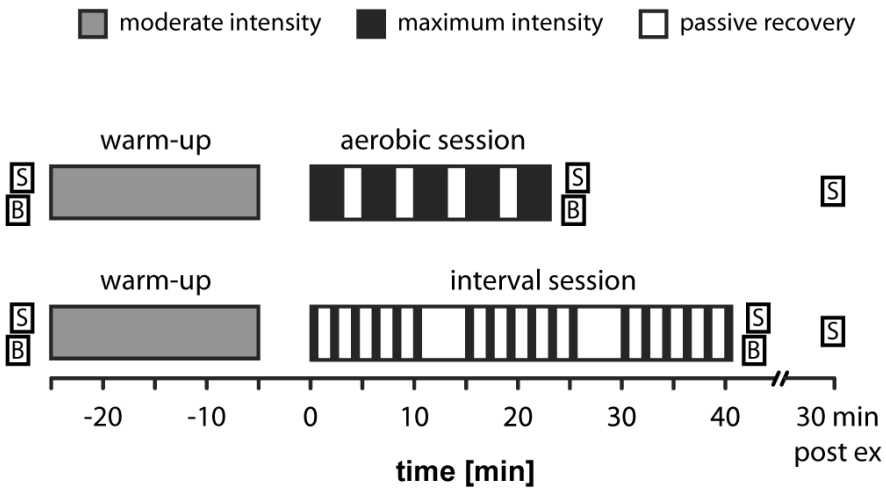

Figure 7.1 Study design.

B, blood sample for lactate analysis; **S**, saliva sample for analysis of sIgA and α-amylase; **ex**, exercise.

Session 1 ("aerobic" session) consisted of five blocks, each separated by 2 min of passive recovery. Each block consisted of 3 min of forward pushing on a lap, including a downhill and an uphill ramp, straight sections and slalom around cones. Athletes were required to cover as much distance as possible during each block. The whole training session lasted 23 min, whereas the active part of the session accounted for 15 min.

Session 2 ("interval" session) consisted of three blocks, each separated by 5 min of passive recovery. Each block consisted of six bouts of 30 s, where athletes were required to perform forward and backward pushing between lines 2 – 3 m apart for the first 15 s, followed by a sprint during the last 15 s, all at maximum effort. Bouts were repeated after 90 s of rest, meaning that the whole training session lasted 41.5 min, whereas the active part of the session accounted for 9 min.

7.3.3 Data collection

Timed, unstimulated saliva samples were collected into sterile plastic containers before, immediately after, and 30 min post exercise, as described in Chapter 3.7. Participants were

allowed to consume drinks *ad libitum* apart from 5 min prior to each collection. Before and immediately after the training session, small capillary blood samples were obtained from the earlobe in order to measure BLa using a lactate analyser (Lactate Pro, Arkray, Kyoto, Japan), which was calibrated before each session according to the manufacturer's guidelines.

Further, participants were asked to indicate their overall RPE using a scale ranging from 6 – 20 (Borg, 1982) at the end of the sessions. HR was continuously recorded using a HR monitor (Polar Team System, Polar, Kempele, Finland), and the highest average 5 s interval achieved during each session was defined as HR_{peak}. Finally, a data logger (Sporner *et al.*, 2009) was fitted to the wheelchairs in order to collect distance data during each court training session.

7.3.4 Analytical methods

Saliva samples were stored on ice immediately following collection and stored at -20 ºC upon return to the laboratory. sIgA concentration and salivary α-amylase activity were then determined as described in Chapter 3.7. All samples from the same participant were analysed in duplicate on one microplate. The CV of the methods based on analyses of these duplicate samples was 1.7 ± 1.4% for sIgA and 1.6 ± 1.2% for α-amylase.
Saliva osmolality was determined using a calibrated cryoscopic osmometer (Osmomat 030, Genotec, Berlin, Germany). Samples were analysed in duplicate, the CV was 1.1 ± 1.3%.

7.3.5 Data processing and statistical analyses

The SPSS 19 statistical package (SPSS Inc., Chicago IL, USA) was used for all statistical analyses. As in study 3, sample size calculation was based on previous unpublished pilot data of relative increases in sIgA secretion rates (22 ± 16%) comparing pre and post values of an intensive training session in elite PARA athletes. In analogy to study 3, GPower 3.1.2 was used to determine that 7 participants would be required to detect a similar change in sIgA secretion rate, with an effect size of 1.38, 90% power and an α of 5%.

All salivary responses were expressed as absolute values, and, to account for differences of pre exercise values, post and 30 min post exercise data were expressed as a percentage of the pre exercise value (from here on referred to as "percentage data"). sIgA responses were expressed as concentration, secretion rate, and sIgA concentration ratio to saliva osmolality (sIgA:osmolality).

Normality was checked with the Shapiro Wilk test. Means and SD were computed for normally distributed variables, medians and quartiles for all other variables. In order to normalise data, a logarithmic transformation was applied to absolute and percentage saliva flow rate data, and a square root transformation was applied to HR and percentage sIgA data. A two-way (time x exercise type) ANOVA was applied to normally distributed salivary percentage data, Friedman and Wilcoxon tests for non-normally distributed percentage and RPE data. Paired t-tests were performed to compare absolute salivary pre exercise values, distance data, HR_{peak} and BLa_{peak} between the two court training sessions. To examine the effects of saliva flow rate on sIgA concentration, a two-way ANOVA (time x parameter) was performed on log-transformed percentage post and 30 min post data of the aerobic session. For all comparisons where the assumption of sphericity was violated, a Greenhouse Geisser correction was applied. Statistical significance for all analyses was accepted at $P < 0.05$.

7.4 Results

As a result of the court training sessions, a time x exercise interaction in sIgA concentration was found, indicating a greater increase in sIgA concentration post and 30 min post exercise in the aerobic session (Figure 7.2, $P = 0.03$). However, the increases of sIgA concentration during the aerobic session were accompanied by decreases in saliva flow rate ($P < 0.001$). Therefore, sIgA secretion rate and sIgA:osmolality were unaffected by exercise ($P = 0.13$ and 0.48, respectively) and time of measurement ($P = 0.98$ and 0.57, respectively). α-amylase activity was not affected by any exercise type ($P > 0.87$, Table 7.2). Furthermore, saliva did not differ in osmolality between training sessions ($P = 0.42$) and time points ($P = 0.16$; aerobic - pre: 45 ± 9, post: 54 ± 21, 30 min post: 56 ± 11; interval - pre: 53 ± 11, post: 62 ± 16, 30 min post: 52 ± 7 mosmol·kg^{-1}).

Table 7.2 Salivary responses during wheelchair rugby court training.

	Aerobic session			Interval session		
Parameter	**pre ex**	**post ex**	**30 min post ex**	**pre ex**	**post ex**	**30 min post ex**
sIgA concentration [µg·mL^{-1}]	84±23*	118±56	137±34	142±58	127±65	123±46
sIgA secretion rate [µg·min^{-1}]	53±26	57±35	53±24	68±37	47±16	50±16
α-amylase activity [U·mL^{-1}]	305 (274,318)	310 (141,333)	258 (209,438)	364 (327,438)	363 (149,490)	248 (207,614)
Saliva flow rate [mL·min^{-1}]	0.65* (0.48,0.71)	0.42 (0.39,0.59)	0.36 (0.33,0.44)	0.46 (0.27,0.66)	0.32 (0.26,0.62)	0.40 (0.34,0.64)

sIgA data are presented as mean ± standard deviation, α-amylase activity and saliva flow rate data as median (quartiles). *Significant difference between aerobic and interval session, at $P < 0.05$ level.

Table 7.3 Physiological data during court training in wheelchair rugby.

	Aerobic session			Interval session		
Participant	**HR_{peak} [beats·min^{-1}]**	**BLa_{peak} [mmol·L^{-1}]**	**RPE**	**HR_{peak} [beats·min^{-1}]**	**BLa_{peak} [mmol·L^{-1}]**	**RPE**
A	113	7.2	17	126	7.7	13
B	135	8.4	18	150	11.2	16
C	125	8.9	20	128	6.9	15
D	no data	8.0	18	139	14.3	16
E	174	9.2	17	174	7.8	17
F	117	7.9	18	145	9.1	15
G	151	6.7	18	138	9.6	18
Mean / Median	136	8.1	18*	144	8.7	16
SD / Quartiles	23	1.0	17,18	18	1.6	15,17

Heart rate (HR) and blood lactate concentration (BLa) data are presented as mean and SD, rating of perceived exertion (RPE) data as median and quartiles. *Significant difference between sessions, at $P < 0.05$ level.

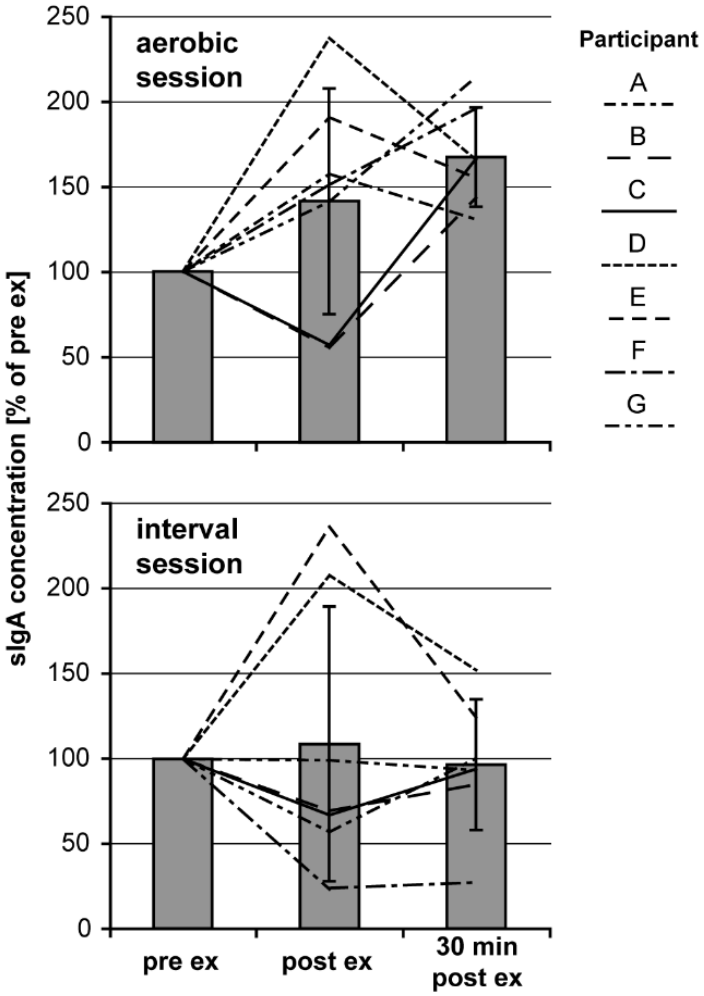

Figure 7.2 Changes in sIgA concentration as a percentage from pre ex during court training in wheelchair rugby.

Time x exercise interaction (P = 0.03).

Both court training sessions resulted in substantial elevations of markers associated with physical exertion in all athletes (Table 7.3). Athletes covered 4155 ± 483 m and 3754 ± 331 m in the aerobic and the interval session, respectively (P = 0.02). Finally, HR_{peak} did not differ between court training sessions (P = 0.23, Table 7.3).

7.5 Discussion

7.5.1 Main findings

This is the first study to demonstrate the effects of field-based training sessions on the mucosal immune function in a cohort of highly trained TETRA wheelchair athletes. In line with the hypothesis, the main findings are that acute bouts of highly strenuous exercise do not have negative impacts on the sIgA concentration and secretion rate in TETRA athletes, nor do they influence the production of α-amylase, a marker of sympathetic nervous activity. Further, despite the nature of a tetraplegia, which results in profound active muscle mass loss, remarkably high BLa_{peak} were measured during both training sessions.

It is commonly accepted that the sympathetic nervous system may at least partly be responsible for the changes in salivary markers, such as sIgA and α-amylase (Bishop & Gleeson, 2009; Chicharro *et al.*, 1998). In AB individuals, intense exercise typically results in a rise of catecholamines, which correlates with a rise in α-amylase (Chatterton *et al.*, 1996). Furthermore, depressions in sIgA secretion rate (Nieman *et al.*, 2002; Mackinnon *et al.*, 1993) and saliva flow rate (Walsh *et al.*, 1999) have been observed following this type of exercise. Therefore, the absence of significant alterations in sIgA and α-amylase in TETRA athletes underpins that a functional sympathetic drive has an influence on governing these responses. In line with this, it has been suggested previously that sympathoadrenal activity is responsible for a normal natural killer cell response to exercise, which is blunted in TETRA athletes as well (Klokker *et al.*, 1998).

However, it must be noted that saliva flow rate was decreased (with a concomitant increase in sIgA concentration) in the aerobic session of the present study. Whilst it has been suggested that increases in sIgA concentration can be caused by changing hydration status (Oliver *et al.*, 2007), this is not likely to be the case in the present study: Saliva osmolality, a marker of hydration status (Walsh *et al.*, 2004), did not change throughout the sessions. It seems that athletes maintained their hydration status by consuming drinks *ad libitum* (which is common procedure during training). Therefore, the decreases in saliva flow rate may be attributed to changes in sympathetic activation (Gatti & De Palo, 2010; MacKinnon

& Jenkins, 1993) rather than dehydration, suggesting the potential of exercise-induced activation of the sympathetic nervous system despite a central lesion. This gains further support as the participants perceived the aerobic session as more strenuous, suggesting a higher potential of sympathetic activity when compared with the interval session. It is important to note that the decrease in saliva flow rate is in line with the results presented in Chapter 6.4.1, where a test to exhaustion resulted in depressions in this parameter, potentially through this same suggested mechanism, sympathetic activation.

Due to the nature of their disability, abolishing central sympathetic signals to reach effector organs, a mechanism that could still be functional in TETRA individuals is sympathetic reflex activity (see also Chapter 6.5.1), which may be the mechanism potentially being responsible for decreasing saliva flow rate. The suggestion of a remaining, but qualitatively altered sympathetic function gain further support by research observing increased adrenaline and noradrenaline plasma concentrations in TETRA individuals as a result of bladder stimulation (Karlsson *et al.*, 1998) or electrically stimulated cycle exercise (Bloomfield *et al.*, 1994). It has therefore been proposed earlier that sympathetic reflex activity, driven by afferent signals from mechanoreceptors (Vissing *et al.*, 1991) or muscle acidosis (Victor *et al.*, 1988), is a potential mechanism still functional in TETRA individuals. Moreover, a hyper-responsiveness of α-adrenoreceptors in TETRA individuals (Arnold *et al.*, 1995) may further compensate for some of the lack of the centrally mediated neural drive. However, it must be appreciated that withdrawal of parasympathetic activity can also cause decreases in saliva flow rate, since parasympathetic stimulation results in marked increases of saliva flow rate (Carpenter *et al.*, 1998). Therefore, no definite conclusions about the effect of sympathetic activation on saliva flow rate can be made, as more than one parameter (sympathetic and parasympathetic activity) has an effect on the outcome measure (saliva flow rate).

The results of this study do not contradict the suggestion of α-amylase being used as a marker of sympathetic activation (Nater & Rohleder, 2009). Since α-amylase is an enzyme fulfilling functions like breaking down starch or supporting the immune system by neutralising pathogens (Nater & Rohleder, 2009; Scannapieco *et al.*, 1994), it is not to be expected that impaired sympathetic function would result in a complete suppression of this enzyme. Consequently, a baseline level of α-amylase activity could be measured

throughout the experiments, however, any exercise-related increases were not observed, being in line with the theory of an altered sympathetic function in the participants of this study.

Whilst the work described in Chapter 6 investigated the effects of strenuous exercise on immune function in TETRA athletes, one potential limitation of this previous investigation was that exercise intensity was chosen as a percentage of peak oxygen uptake, which may have caused differing, and not necessarily maximal strain in all individuals. In the present study, all individuals were instructed to exercise at the maximum of their abilities, which, in AB individuals, would cause a high degree of sympathetic activation, as sympathetic activation seems to increase as a function of muscle cell pH and exercise intensity (Victor *et al.*, 1988).

With regard to the present study, average HR_{peak} was higher when compared with the literature investigating TETRA individuals previously (Bhambhani *et al.*, 1994; Coutts *et al.*, 1983). In contrast to these previous investigations, the participants of this study comprised of a highly trained group with a high weekly training volume and a professional training structure. Even though speculative, it is therefore possible that long term elite training results in physiological adaptations that enable accessing larger parts of the sympathetic nervous system. This may result in greater sympathetic activity, which may in turn increase HR_{peak}. This theory is supported by a previous investigation in TETRA elite athletes, where a similar average HR_{peak} to the present study was observed (Goosey-Tolfrey *et al.*, 2006). However, it must be noted that different protocols were used to determine HR_{peak} in previous investigations (Bhambhani *et al.*, 1994; Coutts *et al.*, 1983), which may have influenced physiological responses.

A further fact underpinning the importance of transferring laboratory-based findings into the field is the elevated BLa_{peak} measured during the court training sessions when compared with laboratory data in populations of the same elite nature as the one in the present study, where an average BLa_{peak} of 4 – 6 $mmol \cdot L^{-1}$ was measured (Chapter 4; Abel *et al.*, 2003). It is appreciated that different BLa analysers are used for this comparison; however, the large differences in BLa_{peak} suggest these were most likely "real" differences, even more as the lactate analyser used in the present study compares well to other laboratory-based systems

(Pyne *et al.*, 2000a). Even though all BLa measurements were taken immediately after a high degree of exertion and from elite athletes (Chapter 4), the protocols used in the laboratory previously (notably peak tests to exhaustion) may not have been optimal to exhaust the potential of the anaerobic system in TETRA athletes. In contrast, exercising in their normal sporting environment provided optimal conditions, stressing the anaerobic system to a higher degree. It can only be assumed that similar principles apply to other physiological processes, such as the government of sympathetic activity. Therefore, a field environment may be more suitable to generate the greatest stress on the sympathetic system. It is worth noting that the magnitude of BLa_{peak} measured following court training sessions compares well to existing literature (Bhambhani *et al.*, 1994). Interestingly, despite the small active muscle mass in TETRA athletes, BLa_{peak} values even compare with measurements derived after highly strenuous cycling exercise (Walsh *et al.*, 1999). The elite athlete nature of the participants of this study and a potentially decreased total blood volume (Houtman *et al.*, 2000) are probably the most likely causes for these considerably high values.

On a final note, the distance covered during both training sessions compares well to distances measured during actual game play in wheelchair rugby (Sarro *et al.*, 2010). Measurement of this type of data can be used to confirm the ecological validity, and may further be used to help quantifying the exercise load of a training session. For future studies, collecting velocity and/or acceleration data to gain further insight into the dynamics and strain of wheelchair rugby training sessions and game play would increase the value of the data.

7.5.2 Limitations

Because of the yearly planning of the wheelchair athletes tested, it was not possible to conduct standard laboratory tests for the determination of peak oxygen uptake. However, if feasible in future studies, measuring objective data of aerobic capacity would facilitate comparison of the fitness status of the participants with existing literature.

It must further be appreciated that no functional tests were carried out to assess whether participants had a functional complete lesion of the sympathetic nervous system, even

though this is highly likely, as the spinal cord injury of every athlete was motor and sensory complete. Assuming complete lesions of the sympathetic nervous system, this opens an interesting discussion, as the results suggest that individuals with a spinal cord injury can compensate lost centrally mediated function by peripheral adaptations. This would be another area to be followed up on in future investigations. For example, it would be of interest to know the critical amount of exercise required to cause such improvements.

Finally, it should be noted that some data on sIgA secretion in the literature are conflicting in AB populations, and a number of studies have shown no decrease or even increases in sIgA secretion rate following strenuous exercise (Bishop & Gleeson, 2009; Allgrove *et al.*, 2008; Bishop *et al.*, 2006; Walsh *et al.*, 1999). However, all-out exercise protocols tend to give more consistent results, i.e. depression of the mucosal immune function (Nieman *et al.*, 2002; Walsh *et al.*, 2002; Mackinnon *et al.*, 1993). Still, future studies should acknowledge the influence of exercise modalities on mucosal immune responses, such as intensity, duration, or resting state of the participant.

7.6 Conclusions

Acute bouts of highly strenuous exercise do not have negative impacts on mucosal immune responses in TETRA athletes, nor do they influence the production of α-amylase, a marker of sympathetic nervous activity. This contrasts responses previously observed in AB athletes. The disruption of the sympathetic nervous system may prevent the down-regulation of sIgA secretion rate following intense exercise. However, the observed decreases in saliva flow rate and the relatively high HR observed during exercise may be attributed to the contribution of changing sympathetic activity, which may be qualitatively altered, but in parts still be functioning.

8

Study 5: Salivary immunoglobulin A and upper respiratory symptoms during five months of training in elite tetraplegic athletes

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Studies 3 and 4 revealed a positive impact of exercise on acute mucosal immune responses in all studied disability subgroups. Study 5 investigated chronic mucosal exercise responses in tetraplegic athletes.

8.1 Abstract

The aim of this study was to examine resting sIgA responses as a function of training load and episodes of upper respiratory symptoms (URS) in elite tetraplegic (TETRA) athletes.

Methods: Resting saliva samples were obtained from fourteen TETRA athletes at twelve pre-defined time points over five months and analysed for sIgA. Occurrence of self-reported URS and training load were recorded throughout the study duration. Regression analyses were performed to investigate the relationship between sIgA responses and training load. Furthermore, the relationships between sIgA responses and URS occurrence were examined.

Results: sIgA secretion rate was negatively correlated with training load ($P = 0.04$). However, training load accounted for only 8% of the variance. No significant relationships were found between sIgA responses and subsequent URS occurrence. Finally, sIgA responses did not differ between athletes with or without a recorded URS during the study period.

Conclusions: In line with findings in able-bodied athletes, negative relationships between sIgA secretion rate and training load were found in TETRA athletes. This may explain some of the higher infection-risk in wheelchair athletes with a high training load, which has been previously observed in paraplegic athletes. However, the non-significant relationship between sIgA responses and URS occurrence questions the use of sIgA as a prognostic tool for the early detection of URS episodes in the studied population.

8.2 Introduction

In analogy to studies 3 and 4, study 5 is concerned with the analysis of the mucosal immune function, and sIgA specifically. This field has been introduced extensively in the previous chapters, therefore, only the most important facts are given in short.

sIgA is the predominant immunoglobulin in saliva and other mucosal secretions. It plays an important role in mucosal immunity and has therefore been described as 'first line of defence' against pathogens and antigens presented at the mucosa, such as cold-causing viruses (Walsh *et al.*, 2011; Bishop & Gleeson, 2009). Whilst a number of studies have found no relationships between URS and sIgA concentration (Cunniffe *et al.*, 2011; Gleeson *et al.*, 2000), longer lasting longitudinal studies have been able to associate preceding decreased resting levels of sIgA concentration with URS (Neville *et al.*, 2008; Fahlman & Engels, 2005). Consequently, sIgA has been suggested to be a clinical biomarker to predict incidence of URS (Fahlman & Engels, 2005). This is of practical relevance, as URS is one of the most common medical problems in elite AB athletes (Robinson & Milne, 2002). URS may lead to missed training sessions and compromises in athletic performance. Further, according to some surveys, sore throats and flu-like symptoms are more common in athletes than in the general population, and, once infected, colds may last longer in athletes (Gleeson, 2007; Heath *et al.*, 1991; Nieman *et al.*, 1990).

Positive relationships between URS incidence and high training loads have been found in both athletic AB (Fahlman & Engels, 2005) and PARA (Furusawa *et al.*, 2007) populations. Furthermore, negative relationships between training load and sIgA concentration or training load and sIgA secretion rate have been documented previously (Neville *et al.*, 2008; Fahlman & Engels, 2005). Importantly, during periods of heavy training the sympathovagal balance at rest seems to be altered, with a rise in sympathetic and suppression of parasympathetic drive (Pichot *et al.*, 2000). Both parasympathetic and sympathetic nerve stimulation can modify saliva composition and secretion of sIgA (Proctor & Carpenter, 2007). This may lead to the conclusion that high training loads, altered sympathovagal balance, depressions in sIgA concentration or sIgA secretion rate and a higher incidence of URS are causally connected.

Since the sympathetic outflow in TETRA individuals is decreased (Schmid *et al.*, 2001), any stress-related sIgA response at rest has the potential to be altered when compared with populations with intact autonomic innervation. However, this issue has not been investigated until present. This would be of particular relevance, as SCI individuals are prone to respiratory diseases, such as pneumonia or respiratory failure (Brown *et al.*, 2006). In a sporting context, it should be noted that in line with findings in AB athletes (Robinson & Milne, 2002), anecdotal evidence from the national governing sports bodies' training log records shows a high proportion of URS-related drop-outs in athletic TETRA populations during heavy training periods and competition.

Knowledge of oral-respiratory mucosal immune responses to exercise in TETRA athletes could serve as a base for health promotion and monitoring in this specific population. Indeed, a compensatory effect on sIgA responses to acute exercise in elite TETRA athletes has recently been found (Chapter 6). Therefore, the purpose of this study was to examine resting sIgA responses as a function of training load and episodes of URS during a five-month training period in TETRA athletes.

8.3 Methods

8.3.1 Participants

Fourteen TETRA wheelchair rugby players (referred to as participants A – N throughout this chapter) volunteered to participate in this study. The group consisted of thirteen males and one female (participant I) and were all members of the Great Britain wheelchair rugby squad. A summary of their physical, physiological and sport characteristics is presented in Table 8.1.

8.3.2 Data collection

Data were collected on twelve pre-defined time points over five months during the squad's training building up to the 2010 World Championships in September (Figure 8.1). Timed, unstimulated saliva samples were collected between two and five minutes (depending on

each individuals' saliva flow rate) into sterile plastic containers. All samples were collected before 11:00 in a fasted state and prior to any physical activity.

Table 8.1 Participants' characteristics.

Parameter	Value
Body mass [kg]	70.4 ± 13.2
Body height [m]	1.81 ± 0.12
Age [years]	33 ± 5
Lesion level	C6 – C7
Motor completeness of injury	yes (N = 13) no (N = 1)
Time since onset of disability [years]	12 ± 5
Wheelchair rugby classification	0.5 – 2.5
Training volume [h·week^{-1}]	14 ± 3

Apart from lesion level and wheelchair rugby classification (range), numbers are average ± standard deviation.

On each data collection day, participants filled out exercise questionnaires in retrospect, a sample questionnaire was provided for guidance. For each day individually, average intensity for any given exercise (such as individual work-outs or game-play) was rated on a five-point scale (1 - very low, 2 - low, 3 - medium, 4 - hard, 5 - very hard), and exercise duration was noted. Training load was then determined by multiplying intensity with duration (Impellizzeri *et al.*, 2004), and a rolling average over 7 days was computed for the entire duration of the study (Figure 8.1). The 100% training load for each individual was defined as the highest 7-day average attained during the five-month study period. Finally, the location of the athletes and any sport-related international travels were recorded.

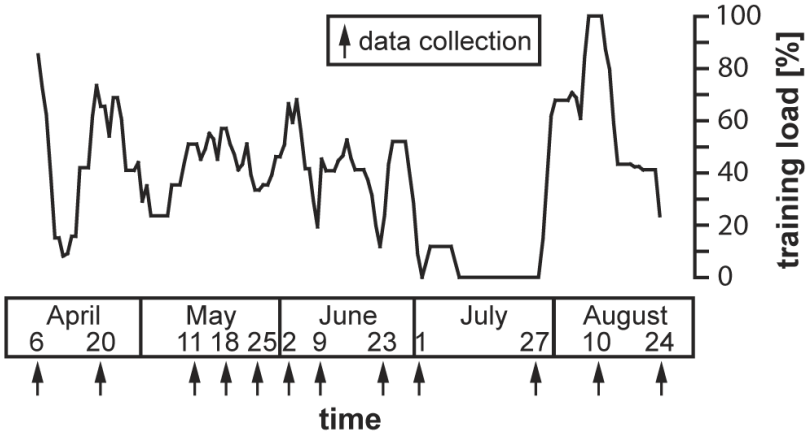

Figure 8.1 Study design.

Training load is given for an example participant. At data collection times (indicated by arrows in the diagram), a saliva sample and the exercise and illness questionnaires were obtained.

Illness questionnaires were also filled out in retrospect. The criteria for definition of an episode of URS have been published elsewhere (Gleeson *et al.*, 2011a). In short, the self-reported occurrence of symptoms like sore/scratchy throat, runny/plugged nose, cough or fever, were noted and their intensity was rated on a three-point scale (1 - light, 2 - moderate, 3 - severe). Multiplying the intensity-score with the number of symptoms and the number of days suffered, a minimum score of 12 was taken to indicate an URS was present. Finally, according to the methods employed by Neville *et al.* (2008), participants used a three-point scale to indicate their resting status on the day of data collection (worse than, same as, or better than normal).

With the exception of four dates (April 20th, May 11th, June 23rd, August 10th), data were collected on scheduled training days at the training venue. When participants were unable to attend these sessions and on the dates mentioned before, saliva samples were collected individually by the participants. A detailed written instruction was provided and it was ensured that procedures for correct sample storing were in place.

8.3.3 Analytical methods

For the analysis of sIgA the procedures as outlined in Chapter 3.7 were followed. The CV of this method based on analyses of duplicate samples was 2.8 ± 2.7%.

8.3.4 Data processing and statistical analyses

The PASW 18.0 statistical package (SPSS Inc., Chicago IL, USA) was used for all statistical analyses. Means and SD were computed for normally distributed variables, medians and quartiles for all other variables. Furthermore, CVs were calculated. For each sample, salivary data (sIgA secretion rate and concentration) were then matched to the individual average 7-day training load before sample collection. A logarithmic transformation was applied to these salivary data in order to weigh increases by a certain factor the same as decreases by the same factor. Slopes of the linear regression "log-transformed salivary data *vs.* individual training load" were calculated for each participant individually, and the slopes were tested with a Wilcoxon statistic against a fixed value of Zero; a Bonferroni correction was applied for multiple comparisons. The same procedure was applied to the "saliva flow rate *vs.* sIgA concentration" and "saliva flow rate *vs.* sIgA secretion rate" relationships.

Further, median salivary data were compared between participants contracting at least one URS and participants not contracting any URS during the study period, using the Mann-Whitney test. Finally, all salivary data were pooled, expressed as a percent deviation from the individual median, and analysed with Mann-Whitney tests to explore the impact of URS occurrence within two weeks after sample collection on salivary data. The same procedures were applied to investigate the impact of resting status on salivary data; however, due to the low occurrence of samples with a rating of "better than normal" (N = 3), these samples were excluded from this analysis. Statistical significance for all analyses was accepted at $P < 0.05$.

8.4 Results

Over the five-month study period, 127 saliva samples were collected. The within-individuals variability of sIgA secretion rate was high, with a mean CV of 54%, the CV for between-individuals variability was 76% (Figure 8.2). The CV for sIgA concentration was 55% (within-individuals) and 88% (between-individuals). Eighty percent of the sIgA secretion rate data of participants D (incomplete spinal lesion level) and I (female) were found to be within the 10^{th} and 90^{th} percentile of the studied population, which was male with a complete spinal lesion level. Therefore, their data was entered for all analyses.

A significant negative relationship was found between sIgA secretion rate and individual training load, with the average slope of the individual regression lines differing significantly from Zero ($P = 0.04$, Figure 8.3). However, on average, training load accounted for only 8 (quartiles: 4, 18) % of the variance. Furthermore, a trend was found between training load and sIgA concentration ($P = 0.06$), again accounting for 8 (quartiles: 2, 20) % of the variance.

A negative relationship was found between individual saliva flow rate and sIgA concentration, with the average slope of the individual regression lines differing significantly from Zero ($P = 0.002$, Figure 8.4). Furthermore, negative but non-significant relationships were detected when comparing average data of saliva flow rate and sIgA concentration between participants ($P = 0.43$, Figure 8.4). Finally, no significant relationship between individual saliva flow rate and sIgA secretion rate was found ($P = 0.72$).

Three players reported a minimum of one episode of URS during the study period (participants A, F and K). However, their median sIgA secretion rate did not differ from the other players ($P = 0.19$, Figure 8.2). Furthermore, sIgA responses did not differ between samples with no subsequent episode of URS when compared with samples with a subsequent episode of URS within two weeks ($P > 0.05$). Likewise, no differences in sIgA responses were found when comparing samples of the different resting status ratings ($P > 0.05$, Table 8.2). Finally, over the 5 month study period, players spent 20 ± 12 days abroad for competitions and used planes for transportation on 6.1 ± 3.7 occasions.

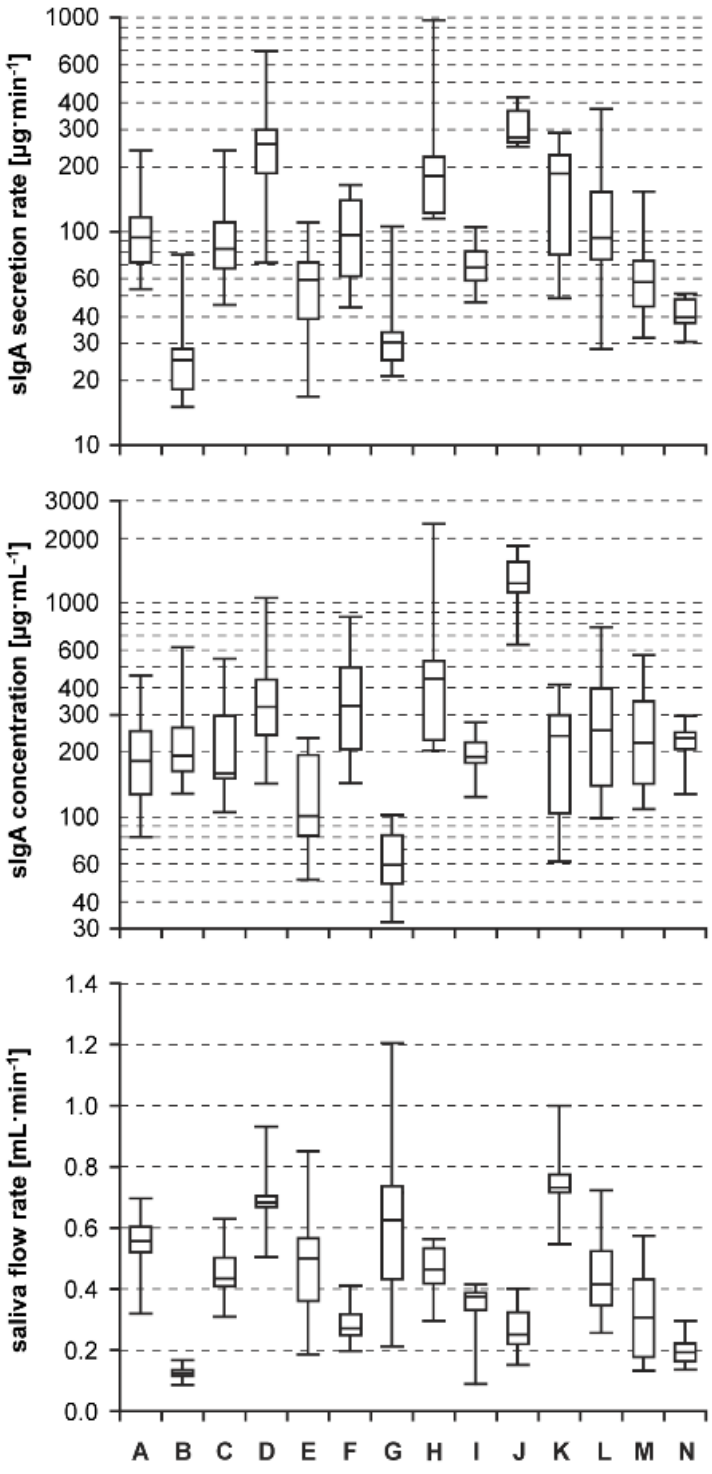

Figure 8.2 Resting sIgA secretion rate, sIgA concentration and saliva flow rate over the five month study period.

A – N, participant codes. Participants A, F, and K contracted a minimum of one episode of upper respiratory symptoms during the study period. Values are displayed as box plots showing median, quartiles, and minimum/maximum.

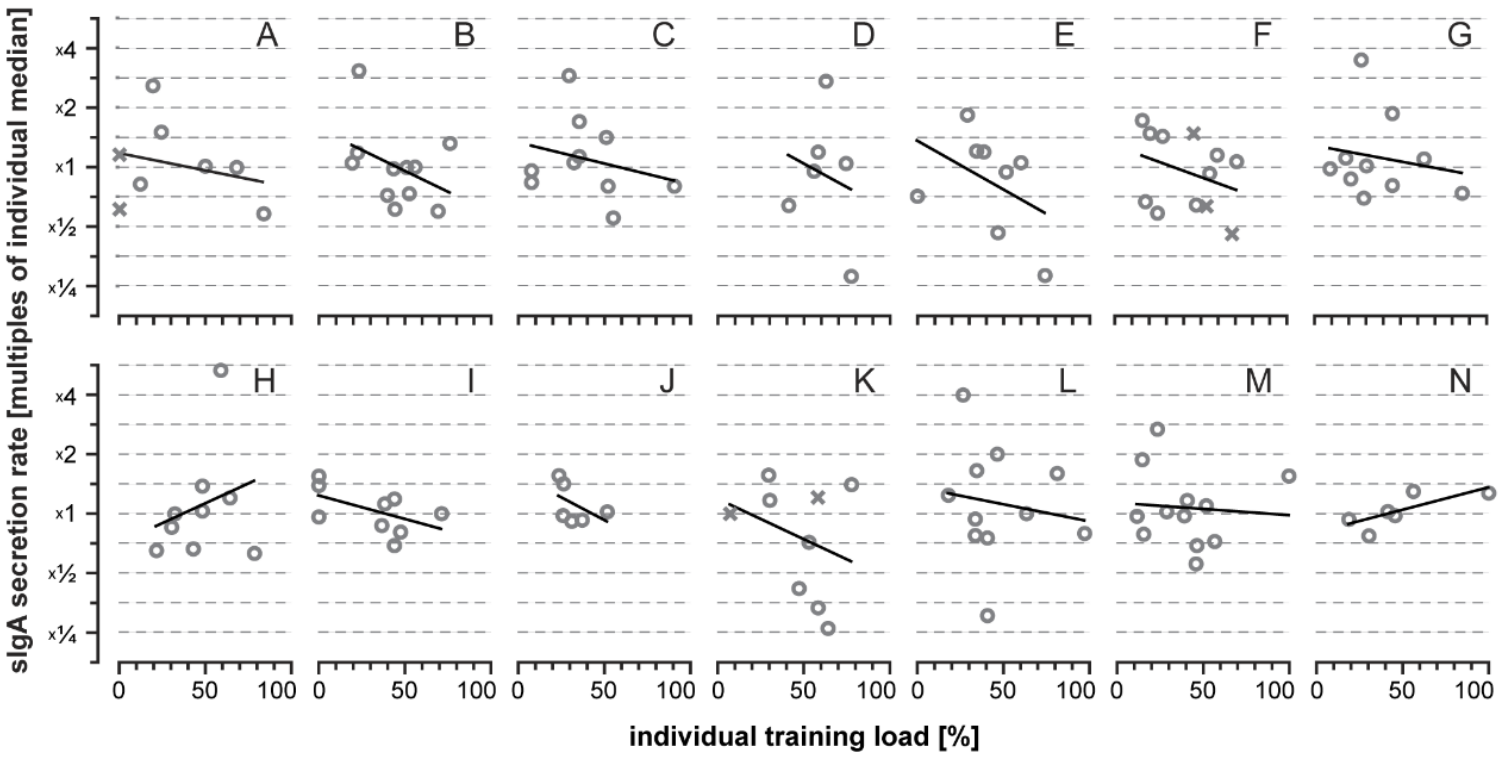

Figure 8.3 Resting sIgA secretion rate vs. individual training load over the five month study period.

Data points and individual regression lines. **A – N**, participant codes; **circles**, samples with no subsequent upper respiratory symptoms (URS), **crosses**, samples with subsequent URS.

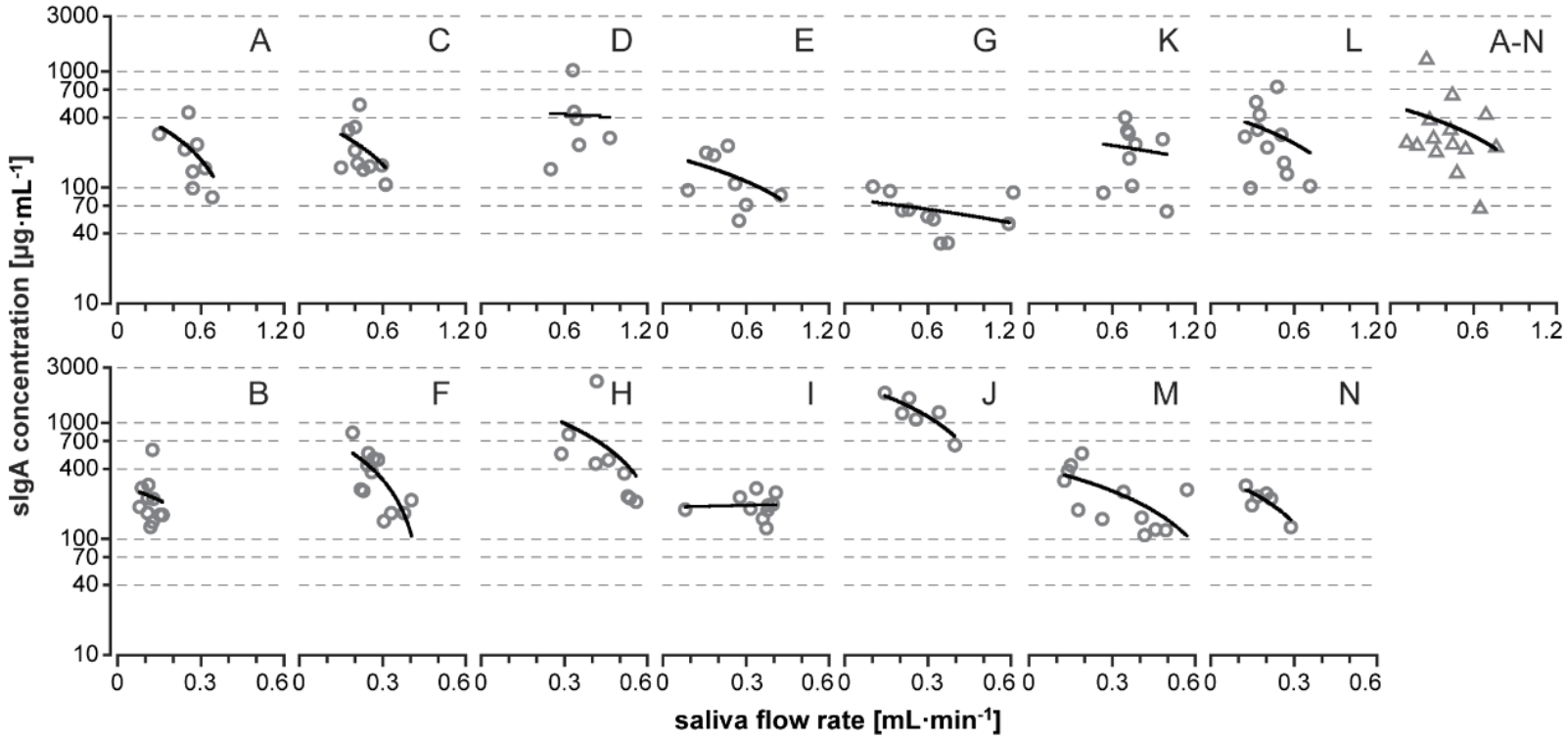

Figure 8.4 Resting sIgA concentration vs. saliva flow rate over the five month study period.

Data points and linear regression lines. **A – N**, participant codes; **circles**, raw data; **triangles**, (top right panel), average data shown for all individuals. Please note the different scale of the x-axis in the top and bottom charts and the presentation of sIgA concentration on a logarithmic scale, which leads to a distortion of linear regression lines.

Table 8.2 Influence of resting status and the occurrence of URS within two weeks on salivary data.

Resting status	sIgA secretion rate (% from individual median)	sIgA concentration (% from individual median)	Saliva flow rate (% from individual median)
"same as normal" (N = 92)	101 (80, 128)	100 (71, 149)	100 (85, 118)
"worse than normal" (N = 30)	92 (72, 120)	98 (74, 121)	100 (91, 120)
"better than normal" (N = 3) missing data (N = 2)	excluded from analysis	excluded from analysis	excluded from analysis
URS within two weeks			
No (N = 120)	100 (77, 128)	100 (73, 140)	100 (85, 118)
Yes (N = 7)	100 (62, 118)	100 (64, 125)	106 (94, 112)

Numbers are median (quartiles).

8.5 Discussion

8.5.1 Main findings

The main finding of this study is that resting sIgA secretion rate is associated with training load in TETRA athletes, which is in line with findings in AB athletes (Cunniffe *et al.*, 2011; Neville *et al.*, 2008). However, the present study cannot confirm a relationship between decreased sIgA responses and the occurrence of subsequent URS. Saliva flow rate further explained some of the variation observed in sIgA concentration on an intra-individual basis.

As pointed out in the discussions of the two preceding studies (Chapters 6.5 and 7.5), it is commonly accepted that the sympathetic nervous system may at least partly be responsible for the changes in salivary markers, such as sIgA (Bishop & Gleeson, 2009; Sari-Sarraf *et al.*, 2007), and that both sympathetic and parasympathetic stimulation results in increases

of sIgA secretion (Proctor & Carpenter, 2007). TETRA individuals represent a model with no centrally mediated sympathetic nervous control (Krassioukov, 2009), as centrally mediated sympathetic stimuli do not activate the decentralised part below the level of lesion (Corbett *et al.*, 1971). Furthermore, salivary gland innervation originates from the upper thoracic segments and is therefore disrupted in TETRA individuals as well (Proctor & Carpenter, 2007). Therefore, it is important to note that with respect to salivary gland function, the studied population is distinctively different to the AB populations studied earlier. However, it seems that these differences in TETRA athletes do not have a major impact on resting sIgA secretion rate and sIgA concentration when compared with the AB population. Even though between and within subject variability of sIgA responses were high, they were in a similar range and of a similar variability when compared with AB athletes (Neville *et al.*, 2008). The main modulation of resting sIgA is therefore likely to stem from variations in parasympathetic activity, as this remains normal in TETRA individuals (Krassioukov, 2009) and has also been shown to be related to periods of heavy exercise (Pichot *et al.*, 2000). Another possible modulator is sympathetic reflex activity, which may be triggered by circulating metabolites related to stress or fatigue (Karlsson *et al.*, 1998). Since this reflex activity is still intact in the TETRA population, it results in a remaining, but qualitatively altered neural function (Karlsson *et al.*, 1998). This is supported by the suggestion that sympathetic reflex activity may be a possible reason for the qualitatively altered sIgA secretion rate response following acute exercise in this population (Chapter 6.5).

It should be noted that the participants of this study were highly trained. The training loads observed in this population were comparable to those observed previously in AB participants in studies investigating sIgA responses to chronic exercise (Cunniffe *et al.*, 2011; Neville *et al.*, 2008; Gleeson *et al.*, 2000). In contrast, many studies conducted with TETRA populations reporting depressed markers of immunity have been obtained in patient and/or marginally trained populations (Campagnolo *et al.*, 2008; Klokker *et al.*, 1998). Given the positive long-term effects of exercise on immune function and health (Gleeson *et al.*, 2011b), it must therefore be considered that long term training in TETRA individuals may have an impact on these markers, through mechanisms like an altered sympathovagal balance as a result of chronic exercise. Therefore, the trained TETRA population may exhibit a different response when compared to less trained TETRA

populations. However, this assumption requires further investigation and a direct comparison between the two groups.

This study cannot confirm a cause and effect relationship between low sIgA responses and subsequent URS. However, the negative relationship between training load and sIgA secretion rate may explain some of the increase in URS following periods of hard training, as previously observed in both SCI (Furusawa *et al.*, 2007) and AB athletes (Nieman, 1997). Based on the impairments of the TETRA population, it is concluded that the impact of exercise on sIgA secretion rate does not seem to be influenced by sympathetic central drive, and again parasympathetic or sympathetic reflex activity can be suggested as possible modulators. On the other hand, altered sympathetic outflow in this specific population cannot be ruled out as underlying reason for the lack of a relationship between sIgA responses and the occurrence of URS. However, it is important to note that even though a relationship between sIgA responses and URS occurrence has been shown in AB populations, it was rather weak ($R^2 = 0.17$), which is in line with our findings (Neville *et al.*, 2008). Moreover, others were not able to show any relationships between sIgA and URS occurrence (Cunniffe *et al.*, 2011; Gleeson *et al.*, 2000). It can therefore be concluded that this relationship is not clear and potentially blurred by the additional stresses required for the occurrence of URS. It has been suggested earlier that the relationship between exercise and URS is affected by poorly known individual determinants such as genetic factors, fitness, or nutritional status (Moreira *et al.*, 2009). Factors concealing a clear relationship may further include pathogen exposure, environmental factors, or psychological strategies (Pyne *et al.*, 2000b), which were not controlled for in the present study and therefore potentially differed between participants. It should further be noted that the studied athletes spent a considerable amount of time abroad due to their sport and therefore used planes for transportation rather frequently, which may represent additional stressors (Waterhouse *et al.*, 2004). As a final point, it should also be acknowledged that the presence of URS is not necessarily indicative of the presence of upper respiratory tract infection and may reflect airway inflammation rather than viral or bacterial infection (Spence *et al.*, 2007). Nevertheless, regardless of cause, the deleterious effects of URS on training and competition are of main concern to athletes and coaches.

The occurrence of URS in the study period was comparably low (Cunniffe *et al.*, 2011; Neville *et al.*, 2008), another possibility is therefore that insufficient statistical power did not reveal a significant relationship between increased URS incidence following occurrence of low sIgA responses. Sampling in winter may have resulted in more recorded URS (Cunniffe *et al.*, 2011; Neville *et al.*, 2008). This again implies that a decreased sIgA response alone is not enough for subsequent episodes of URS. Additional stresses as stated above (Pyne *et al.*, 2000b) may be necessary to obtain a higher occurrence of URS, which is more likely to happen during the winter months (Neville *et al.*, 2008). It is also worth mentioning that there are still very few studies that have been able to show a direct link between exercise-induced immune depression and increased incidence of confirmed illness in athletes, which therefore needs addressing in future studies, although it must be recognised that this is difficult (Gleeson, 2007).

The variation in saliva flow rate was pronounced in the studied population, notably, some very low saliva flow rates were recorded (for example participants B, F, J, N) when compared with AB athletes (Cunniffe *et al.*, 2011). This may be population-related and due to altered innervation of the salivary glands, which also affects saliva flow rate (Proctor & Carpenter, 2007). However, it appears that the production of sIgA is not affected to the same extent, because on both an intra- (negative significant relationship) and an inter- (negative non-significant relationship) individual comparison, it is evident that sIgA concentration is higher in samples with a low saliva flow rate. In line with previous research in AB populations (Bishop & Gleeson, 2009), it may therefore be that secretion rate is the more appropriate measure for sIgA than concentration in TETRA individuals, as it takes the pronounced alterations of saliva flow rate in this population into account.

8.5.2 Practical applications

This study found depressions in sIgA secretion rate during periods of an increased training load in TETRA elite athletes. Given the higher susceptibility for URS in TETRA individuals, extra care should be taken during periods of heavy training to prevent illness. Counter-measures include training (i.e., allow for adequate recovery), environmental (i.e., limit exposure to adverse conditions), psychological (i.e., monitor stresses), behavioural (i.e., reduce exposure to common infections), and clinical considerations (i.e., conduct

pathology testing) (Pyne *et al.*, 2000b). It is possible that some of these strategies have already been adopted by the participants on an individual basis, hence resulting in the low occurrence of URS in the present study. This may have been achieved by successfully avoiding pathogen exposure, and/or by adopting strategies to prevent changes in sIgA reaching levels low enough to increase the risk of contracting URS. However, to investigate the impacts of low sIgA responses on URS in the studied population, further studies to be conducted in the winter months should be encouraged. Also, if feasible, a larger cohort should be recruited. This may result in a higher occurrence of URS, which then would improve the statistical power of the outcomes.

8.5.3 Limitations

Self-reporting URS has been questioned earlier (Gleeson, 2007), and it is appreciated that as a result of self-reporting, some episodes of URS may have been missed or mis-classified. There is further error potential by self-reporting training load, where the accuracy of training load relies on the participants' recollection. A further source of error is the home-collection of a part of the saliva samples. By providing detailed instructions participants should have been prepared optimally to provide valid samples, illness and training reports. However, for future studies, collaboration with trained medical staff for the definition of URS would potentially increase the validity of the outcomes. Alternatively, using a validated URS questionnaire such as the Wisconsin Upper Respiratory Symptom Survey (Barrett *et al.*, 2005) could increase the accuracy of the definition of URS. Also, if feasible, hydration status should be assessed, as it may have an impact on oral-mucosal respiratory immunity (Oliver *et al.*, 2007). This has been done in study 4 (Chapter 7), as the data of study 4 was analysed at a later time point than the data of the present study. For future studies, analysing more parameters relating to oral-respiratory mucosal immunity (i.e., α-amylase, lysozyme, lactoferrin) may shed more light on the interaction of oral immune markers and URS (Walsh *et al.*, 2011). Markers of sympathetic nervous activity (i.e., chromogranin A) may provide further information about a mechanistic explanation of findings related to oral-respiratory mucosal immune function. Finally, the inclusion of a control group would strengthen the outcomes of any follow-up study.

8.6 Conclusions

Training load in TETRA wheelchair rugby athletes was negatively associated with sIgA secretion rate, as previously observed in AB athletic populations. Furthermore, the range and variation of sIgA secretion rate and concentration are comparable to the existing AB literature. It is therefore concluded that a trained TETRA population's chronic oral-mucosal immunological response to exercise is comparable to AB athletic populations. However, the non-significant relationship between sIgA and URS occurrence questions the use of sIgA as a prognostic tool for the early detection of upcoming URS in the studied population.

9

General discussion

9.1 Summary of the main findings

The main findings of this thesis are summarised in Figure 9.1. All findings contribute to the principal aim of this thesis, which was to broaden and develop the understanding of exercise physiology and exercise immunology in wheelchair athletes. Special attention was given to the TETRA subgroup, which differs distinctively from the PARA and NON-SCI subgroups with respect to autonomic innervation, and therefore, potentially the mucosal immune responses. This is the reason why two experimental studies (studies 4 and 5) investigated this particular participant group only.

While studies 1 and 2 draw comparisons with literature found in the AB population and provide recommendations about how to improve future testing, protocol design development and exercise prescription for wheelchair athletes, studies 3 – 5 offer a basis to discuss basic human physiology and how adaptable it may be following a spinal cord injury.

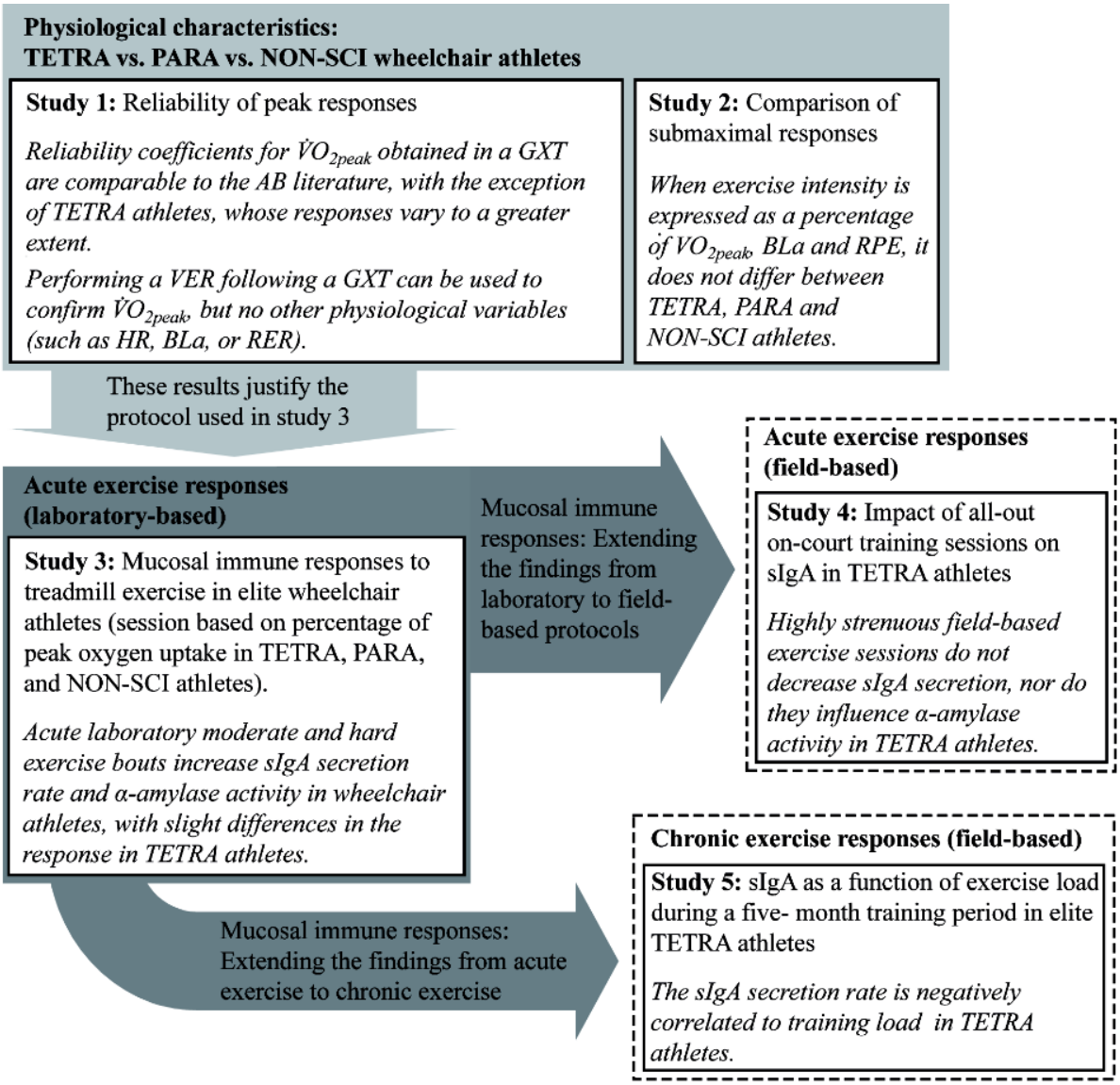

Figure 9.1 Schematic of the main findings of this thesis.

Main results are given in italic script.

9.2 Mucosal immune function: Proposed mechanisms

Highly strenuous exercise, which has been associated with depressions in mucosal immune function (Nieman *et al.*, 2002; Walsh *et al.*, 2002; MacKinnon & Jenkins, 1993; Mackinnon *et al.*, 1993), does not decrease sIgA secretion in TETRA athletes (study 4), possibly due to their abolished central sympathetic function. However, the observed decreases in saliva flow rate may be attributed to increased sympathetic activation

(MacKinnon & Jenkins, 1993), which would be expected during intense exercise, implying a remaining sympathetic function in TETRA athletes despite their central lesion. In line with this, the results of study 3 imply a similar mucosal immune response between subgroups. Secretions of sIgA and α-amylase at rest, during and after acute exercise were comparable between TETRA, PARA and NON-SCI athletes, with only slightly different responses in the TETRA subgroup when compared with the PARA and NON-SCI subgroups: The slightly larger exercise-induced rise in sIgA secretin rate in TETRA resulted in the same post exercise sIgA secretion rate following exercise, implying an adequate mucosal immune response. Finally, chronic sIgA responses to exercise in TETRA athletes seem to follow the same pattern as previously observed in AB populations (study 5; Neville *et al.*, 2008; Fahlman & Engels, 2005).

9.2.1 Sympathetic, parasympathetic, and hormonal influence on mucosal immune function

These findings suggest that the possible impacts of a central sympathetic lesion can be counteracted to maintain a mucosal immune response similar to the AB population. A hyper-responsiveness of α-adrenoreceptors has been suggested earlier, as the amount of infused noradrenaline required to cause foot vein restriction is decreased in TETRA individuals when compared to AB controls (Arnold *et al.*, 1995). This hyper-responsiveness in TETRA individuals may compensate for some of the lack of the centrally mediated neural drive. Salivary glands are innervated by both the sympathetic and the parasympathetic nervous system (Proctor & Carpenter, 2007). It is therefore also possible that parasympathetic activity can compensate for the loss of sympathetic activity in TETRA individuals – a situation fundamentally different to the adrenal glands, which cannot fall back on the parasympathetic nervous system in the event of sympathetic dysfunction (Schmid *et al.*, 1998c) (Figure 9.2). The central government of the adrenal glands is therefore completely abolished in TETRA individuals, and as a consequence, the plasma concentrations of the adrenal gland products adrenaline and noradrenaline at rest and during exercise are drastically reduced in this population when compared with individuals with intact adrenal gland innervation (Yamanaka *et al.*, 2010; Schmid *et al.*, 1998b; Schmid *et al.*, 1998c). In contrast, drastic depressions in salivary parameters were not observed in TETRA athletes in this thesis. However, the slightly different acute sIgA

secretion rate responses to exercise in TETRA athletes (study 3) imply that the sympathetic nervous system is involved in the fine-tuning of mucosal immune function.

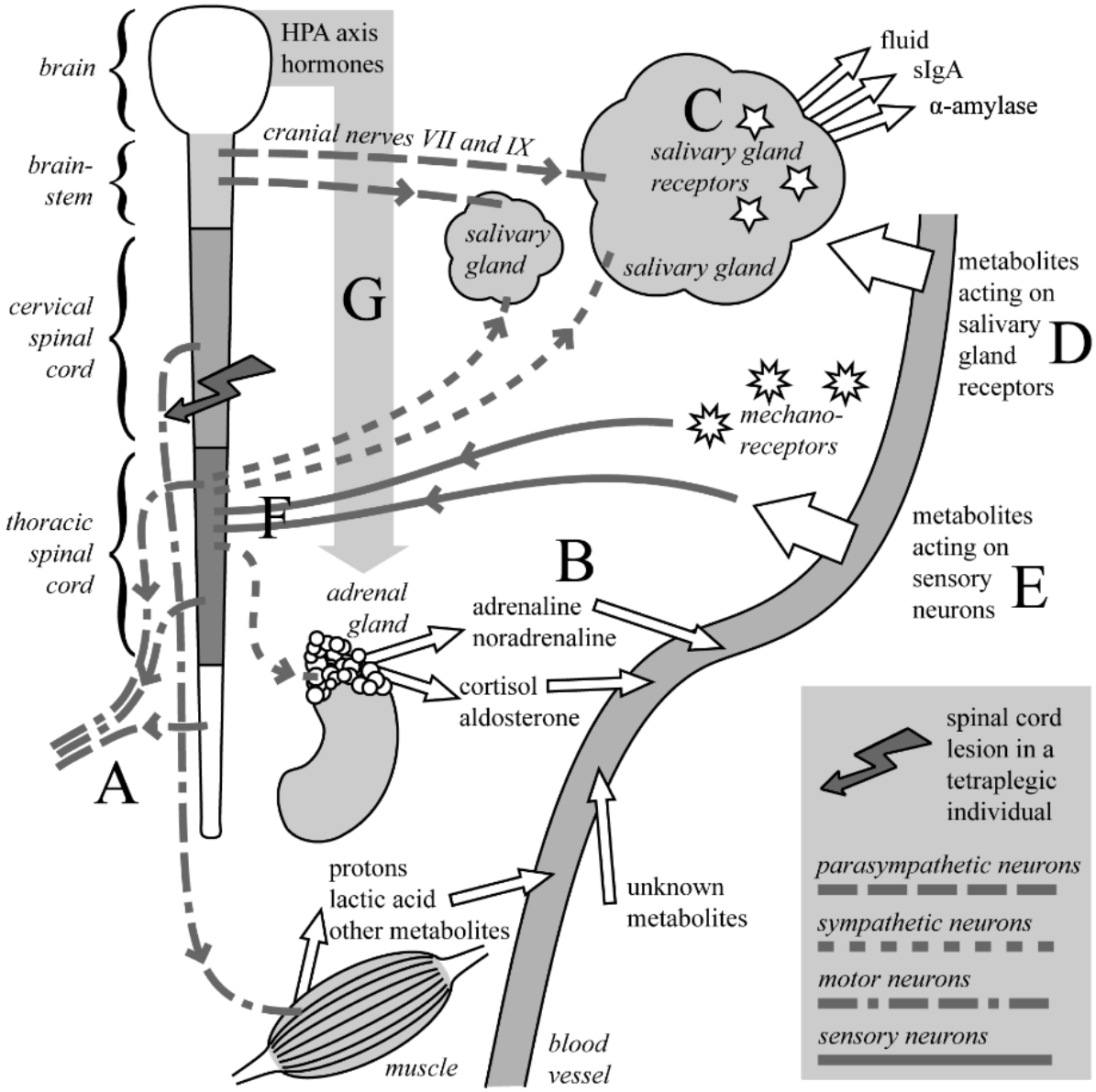

Figure 9.2 Innervation and metabolites influencing salivary gland function.

Capital letters address the situation in tetraplegic individuals with a complete lesion. **A**, paralysis of muscles innervated below the level of lesion; **B**, decreased output due to sympathetic innervation lesion; **C**, potential hyper-responsiveness of salivary gland receptors; **D**, **E**, potential compensatory mechanism of circulating metabolites due to sympathetic function loss; **F**, potential reflex activity in response to sensory input; **G**, potential compensatory mechanism of HPA axis due to sympathetic function loss. HPA, hypothalamic pituitary adrenal. Information compiled from various sources (Walsh *et al.*, 2011; Krassioukov, 2009; Proctor & Carpenter, 2007; Schmid *et al.*, 1998b; Arnold *et al.*, 1995; Victor *et al.*, 1988).

As an alternative explanation, parasympathetic activity may play the more important role than sympathetic activity in governing salivary gland function. In this case, loss of sympathetic function would not result in a fundamentally different mucosal immune response, which would be in line with the findings in TETRA athletes of this thesis. Finally, it should also be considered that the actions of the hypothalamic pituitary adrenal (HPA) axis have an impact on mucosal immune function. Through the action of adrenocorticotropic hormone (ACTH), cortisol secretion is governed by this hormonal system (Webster *et al.*, 2002). Cortisol, in turn, is known to have immunomodulatory effects, as cortisol secretion rate correlates inversely with sIgA secretion rate during chronic exercise (He *et al.*, 2010). Therefore, actions normally governed by the sympathetic nervous system may be compensated by the HPA axis in TETRA athletes. The sympathetic, parasympathetic, and hormonal influences on salivary gland function are summarised in Figure 9.2.

9.2.2 Spinal reflex activity

Despite no central innervation of the adrenal glands in TETRA athletes, adrenaline and noradrenaline seem still to be produced to a certain extent in this population (Schmid *et al.*, 1998b; Schmid *et al.*, 1998c). It has been suggested that "the occurrence of […] increases in catecholamines during physical exercise in tetraplegics is most likely caused by the condition of spinal reflexes, uninhibited by supraspinal centres" (Schmid *et al.*, 1998c). Even though the salivary glands are structurally different to the adrenal glands, they may as well be influenced by spinal reflexes, which modify their secretory behaviour (Figure 9.2).

Reflex sympathetic activity is likely to be driven by afferent signals from mechanoreceptors (Vissing *et al.*, 1991), and likewise, muscle acidosis can increase sympathetic outflow in resting muscles by reflex pathways, as shown for peroneal nerve sympathetic activity during handgrip exercise (Victor *et al.*, 1988). These mechanisms may have a profound impact on salivary gland function in the highly trained individuals as studied in this thesis, since the fitness level of these participants allow high degrees of strain, stimulating mechanoreceptors to a great extent. Importantly, the BLa following field-based training in TETRA athletes (study 4) was found at a level comparable to values found in the AB literature, despite a largely reduced muscle mass in the studied population

due to their disability. Therefore, the potential to trigger sympathetic outflow by muscle metabolites and mechanical strain represents a valid option in TETRA athletes.

Triggering a response independent of supraspinal centres by processing afferent information on a spinal level is not a novel concept. As an example, it has been debated extensively in the field of pain perception, resulting in the gate-control system theory (Melzack & Wall, 1965). This theory states that afferent signals are processed on a spinal level, thus providing practitioners with a tool to modify them with the help of sensory stimuli (for example, the pain of a hurting body part may be reduced by massaging the skin around this body part). The mechanisms for mucosal immune function control by spinal reflexes proposed in this thesis are based on the same concept. It differs from the gate-control system theory with respect to the direction of the signal, as the gate-control system theory is interested in signals to the brain (triggering pain), whilst in this thesis the signals back into the periphery (triggering secretion of immune parameters) are of principal interest. However, the modulation of signals on a spinal level without the need of central command is the core of both concepts.

In SCI individuals, electrical and mechanical skin stimuli impulses below the lesion, deep breaths, and bladder stimulation all evoke bursts of efferent neuron activity, resulting in cutaneous vasoconstriction (Stjernberg *et al.*, 1986; Wallin & Stjernberg, 1984). It has been concluded that these are triggered by neural bursts containing sympathetic impulses of spinal origin (Stjernberg *et al.*, 1986). Furthermore, therapeutic electrical stimulation can induce peripheral sympathetic nerve activity (Mikami *et al.*, 2005). These studies provide more evidence for the occurrence of reflex mechanisms in SCI individuals, and their proposed influence on mucosal immune function.

In this context, it is important to note that intact reflex arches must remain to allow these mechanisms to be functional. An injury affecting efferent and afferent nerves, or the spinal cord itself where these signals are processed, would not allow a signal to travel to the spinal cord, be processed and sent back. This type of injury would result in a denervated (areflexic) spinal segment among segments that retain their reflex function (Jacobs & Nash, 2004). In the unlikely event of an injury affecting the entire area relevant to sympathetic

innervation (T1 – L1), sympathetic reflex pathways would be abolished. Importantly, none of the participants of the studies presented in this thesis had such an injury.

9.2.3 The good news for tetraplegic athletes

The cause for depressions in immune markers during periods of heavy training remains controversial. It has been suggested that these depressions may be caused by a shortage of metabolic components during exercise: The resource-limitation hypothesis postulates that there is an energetic or nutritional cost associated with the immune system, which may be compromised during periods of heavy training (Raberg *et al.*, 1998). Conversely, it is possible that depressing immune function provides a mechanism to reduce the potential of an autoimmune response evoked by an exercise challenge (O'Kennedy, 2000; Raberg *et al.*, 1998). By not being able to send the (potentially exercise-induced) central command to cause immune function depression (as in TETRA individuals with sympathetic dysfunction), an overshoot in parameters of the immune function may be expected. Indeed, the greater rise in sIgA secretion rate following moderate exercise (study 3) and the absence of depressions of sIgA secretion rate following exhaustive exercise (study 4) imply that TETRA athletes may even benefit from sympathetic dysfunction, providing them with a mechanism to increase their mucosal protection against invading pathogens.

Given the low URS incidence following exercise in TETRA athletes reported in study 5, the results of this thesis underpin the positive impact of exercise on immune function in this population. Because respiratory illnesses (Brown *et al.*, 2006) and the risk for pulmonary complications (Lucke, 1998) are a major issue in the SCI community, the results of this thesis stress both the value (improved mucosal immune function following acute exercise) and the caveat (depressions of sIgA during periods of heavy training) of exercise in the health promotion of this population.

9.2.4 Practical applications and limitations

The results of this thesis imply that the sIgA responses at rest and during exercise in TETRA athletes are not largely different to population groups with intact autonomic

innervation. It is important to note the positive effects of acute exercise on markers of mucosal immunity, even when these exercise sessions were highly strenuous (studies 3 and 4). Acute exercise can therefore be regarded as a means to improve mucosal immune function and should be promoted, especially in populations that may be more susceptible to infections (Brown *et al.*, 2006).

Interestingly, even though only descriptive, URS episodes over 5 months of training were limited to three of the 14 TETRA athletes, with a combined number of five URS episodes (study 5). This does not seem high when compared with the AB literature, where 102 URS episodes were found in 38 athletes throughout a year (Neville *et al.*, 2008). This implies that with regard to mucosal immune function, TETRA athletes should be at no disadvantage when compared with AB athletes. Possibly, their athletic lifestyle helps maintaining adequate immunity and therefore results in a lower susceptibility for infections compared with their sedentary or patient counterparts (Brown *et al.*, 2006). Even though speculative at this stage, this again provides further evidence for the benefits of physical activity in this population.

Still, the limitations of the immuno-protective effects of exercise must be recognised. It should be noted that when the training load of TETRA athletes is high, their mucosal immune response is depressed (study 5), which is in line with AB results. Therefore, precautions should be made to limit the risk of URS, as described in detail by Pyne *et al.* (2000b). Some precautions seem to have been naturally taken already by the studied participant group, which may have resulted in the low URS incidence. Indeed, anecdotal evidence from conversations with the studied athletes confirms this assumption, even though it must be accepted that it was not possible to scientifically evaluate these comments.

It is important to note the limitations of URS monitoring and the link between sIgA and URS in the scientific literature, which would certainly also apply to the studied subgroup of wheelchair athletes. Some findings are simply controversial, whilst some may even appear discouraging to regard low levels of sIgA as a risk factor for URS. For example, it has been postulated that sIgA is not a reliable indicator of upper respiratory tract infection in collegiate female soccer athletes because of the missing link between sIgA concentration

and URS, studied in 12 athletes and 8 controls during a 13 week fall season (Vardiman *et al.*, 2011). This confirms previous research, examining 42 students in times of mental stress: No relationship between sIgA concentration and URS incidence were found following an examination period, even though sIgA concentration rate was depressed until the 6th and last day of the monitoring period after the examination (Deinzer & Schuller, 1998). Likewise, URS in 14 amateur ultramarathon runners was not related to sIgA concentration measured in the 1-month period before and 14-day period after the race (Peters *et al.*, 2010). Similar outcomes were found when tackling the problem from another perspective: When IgA was administered as a precautionary measure as a nasal spray, URS in 14 world-class canoeists did not decrease significantly despite an increase in sIgA concentration during treatment (Lindberg & Berglund, 1996). It must be noted that the statistical power of studies showing a relationship between sIgA and URS is generally high, with 38 athletes studied during 50 weeks of training (Neville *et al.*, 2008), or 75 college football players and 25 controls during a 12-month period (Fahlman & Engels, 2005). Nonetheless, a smaller-scale study with 20 participants observed over a 12-week moderate exercise training period has found a relationship between increased sIgA concentration and decreased number of days with sickness (Klentrou *et al.*, 2002).

Laboratory data further documenting controversial responses are the changes observed in sIgA, which have been shown to both remain constant (Davison, 2011; Walsh *et al.*, 1999) or decrease (Nieman *et al.*, 2002; Mackinnon *et al.*, 1993) as a result of acute heavy exercise. Furthermore, the relationship between exercise load and URS risk is not clear cut. In a study of 170 experienced marathon runners, only 3% reported URS during the week after a July marathon race event (Nieman, 2000), implying a comparably low risk of contracting URS after a highly strenuous activity. Conversely, it has been reported that there is an up to five times increase in risk for picking up an infection in the weeks following a competitive ultra-endurance running event (Nieman *et al.*, 1990).

A final limitation for the practical use of sIgA is its variability, and sIgA has been regarded as only a marginal predictor of short-term risk due to a high degree of analytical and biological variability (Gleeson, 2003). The use of one-off measures of sIgA is therefore unlikely to be informative, unless the individual is found to be IgA-deficient (Gleeson, 2003). The findings described in this paragraph call for caution when considering sIgA as a

tool to predict upcoming URS. Therefore, the practical value of this thesis regarding mucosal immune function does not lie in the formulation of critical values of immune parameters, but rather the understanding of immune function physiology.

9.3 Novel findings in the exercise physiology of wheelchair athletes

Another principal aim of this thesis was to investigate the reliability of variables derived in peak testing, particularly $\dot{V}O_{2peak}$. Despite decades of research in disability sport, there appears to be a lack of these data. Knowledge about the accuracy of the determination of $\dot{V}O_{2peak}$ (and any physiological measures in general) is not only important for within-group comparisons, but also to establish individual changes over time. Hence, the systematic variation of a parameter (for example the increase in $\dot{V}O_{2peak}$ caused by a potential training programme) must be larger than random variation of that same parameter (caused by technical limitations of measurement equipment or natural day-to-day variation of an individual). Only then one can be sure that a "real" change has occurred, which is not caused by random variation, coining the term of the "smallest meaningful change".

The smallest meaningful change of $\dot{V}O_{2peak}$ in PARA and NON-SCI wheelchair athletes is comparable to the AB literature (study 1). It is encouraging that performing a VER reliably confirms the $\dot{V}O_{2peak}$ obtained in a GXT in all tested subgroups, even though the protocol can probably be optimised for athletes with a high $\dot{V}O_{2peak}$. Nonetheless, the results of this thesis support the value of including a VER into a peak testing procedure. It is of great practical significance that in TETRA wheelchair athletes, larger relative changes are needed to document "real" changes in $\dot{V}O_{2max}$. Possible causes have been discussed extensively in Chapter 4.5; however, the main practical outcome is to use the smallest meaningful changes found in study 1 and implement them as criteria into physiological testing or study evaluation for each subgroup separately.

Another practical outcome of this thesis is to question whether $\dot{V}O_{2peak}$ in wheelchair athletes is the right key variable to document changes in aerobic performance. This is due to the between days variability of $\dot{V}O_{2peak}$, which may be greater than the relatively small changes as a result of training in already highly trained groups. This issue is not limited to the findings of this thesis and was reported in AB populations earlier, where $\dot{V}O_{2peak}$

remained unchanged despite improvements of other performance-related parameters (Legaz Arrese *et al.*, 2005). Therefore, especially in TETRA athletes, whose aerobic capacity is reduced, using $\dot{V}O_{2peak}$ as a longitudinal marker of fitness status is probably not appropriate.

As a result, alternatives to $\dot{V}O_{2peak}$ should be further explored in wheelchair athletes. BLa monitoring and determination of lactate thresholds may be meaningful procedures to monitor this group of individuals longitudinally (study 2; Thomas *et al.*, 2008). For example, it has been shown in AB athletes that $\dot{V}O_2$ at lactate threshold increased significantly throughout a triathlon season, whereas $\dot{V}O_{2peak}$ remained unchanged (Kohrt *et al.*, 1989). Likewise, other parameters, such as velocity (Grant *et al.*, 1997) or power (Bishop *et al.*, 1998) at lactate threshold, seem to be sensitive in predicting exercise performance. Other procedures may also prove useful, such as tests to exhaustion at a relatively high power output (potentially chosen as a percentage of $\dot{V}O_{2peak}$ or maximum power output) (Leicht *et al.*, 2010), or the determination of the power output or $\dot{V}O_2$ at lactate minimum between two exhaustive bouts (Perret *et al.*, 2012).

The results of study 2 mainly impact on exercise prescription and study design in the field of wheelchair sport. Keeping all the limitations known from AB sport in mind, exercise intensity can be prescribed or monitored at the same levels of $\%\dot{V}O_{2peak}$, BLa and RPE in all studied subgroups. This is of special significance when planning research using exercise intensity as an independent variable in cross-sectional designs with various disability subgroups, as examined in study 3. The limitations mentioned include the relatively large variability within participant subgroups, which has been observed earlier, for example when determining maximum fat oxidation at an intensity expressed as $\%\dot{V}O_{2peak}$ (Achten & Jeukendrup, 2004). Therefore, it must be appreciated that when prescribing exercise based on $\%\dot{V}O_{2peak}$ on an individual level, physiological responses potentially vary substantially from the group mean. This suggests that better alternatives may exist for individually prescribing exercise. For example, analysis of BLa enables the determination of various thresholds, such as fixed lactate levels as described in study 2, or the aerobic and anaerobic threshold (Faude *et al.*, 2009). Alternatively, breath-by-breath analysis of a GXT can be used to determine ventilatory thresholds (de Lira *et al.*, 2010; Coutts & McKenzie, 1995), which again may prove beneficial for exercise prescription on an individual level.

The use of RPE to regulate exercise intensity is an established field in AB populations (Kang *et al.*, 2009; Kang *et al.*, 2003; Dunbar *et al.*, 1992) and has recently also received attention in wheelchair non-athletic (Al-Rahamneh & Eston, 2011a; Al-Rahamneh *et al.*, 2010; Lewis *et al.*, 2007; Grange *et al.*, 2002), and, to a smaller extent, in wheelchair athletic populations (Paulson *et al.*, 2012; Goosey-Tolfrey *et al.*, 2010b). The results of study 2 may provide a basis to further this field in wheelchair athletes, as this study showed how RPE relates to BLa and $\%\dot{V}O_{2peak}$, which could serve as pilot data to improve exercise prescription in wheelchair athletes. It has been suggested earlier that the range of 50 – 80% $\dot{V}O_{2peak}$ is the recommended exercise intensity for PARA persons to improve endurance capacity (Jacobs & Nash, 2004). This range is spanned by an average RPE of around 9 – 14 in all subgroups investigated. It may therefore be an option to investigate training plans based on these RPE data rather than $\%\dot{V}O_{2peak}$ or other traditional intensity markers (e.g., such as $\%HR_{peak}$). This may prove especially useful in TETRA athletes with a blunted HR response, as HR has not been found a useful tool to regulate exercise intensity in this population (Valent *et al.*, 2007b; McLean *et al.*, 1995).

9.4 Future directions

The design of a VER following a GXT seems to be suitable for athletes managing to maintain the VER for 100 s. However, a high proportion of athletes with a high $\dot{V}O_{2peak}$ appeared not to be able to maintain the prescribed exercise intensity long enough for the VER to return useful results. Therefore, future studies may look into the design of a VER, which can be adapted by PARA and NON-SCI throughout the aerobic fitness spectrum for them to be able to achieve a VER-$\dot{V}O_{2peak}$ which confirms the GXT-$\dot{V}O_{2peak}$. This may be achieved by either reducing the gradient or the speed of the VER used in the protocol as outlined in Chapter 4.5.

In the field of physiological testing in wheelchair-athletes, the number of participants was reasonably high in the studies 1 – 3 and 5. Furthermore, they were adequately powered, even though it has to be acknowledged that the effect sizes assumed were quite high (Batterham & Atkinson, 2005). It has been recognised that one of the problems in research concerning SCI individuals is the fact that the intervention groups (and control groups if present) are almost always rather small and heterogeneous, and the statistical power of the

studies is thus limited (Valent *et al.*, 2007a). Therefore, future work may also look into either confirming the findings or (in the long term probably more meaningful) analysing and comparing studies in meta-analyses or reviews.

The mean $\dot{V}O_{2peak}$ for the participants in the studies of this thesis (as determined in studies 1 – 3) was found to be in the range previously found in highly trained wheelchair athletes (Campbell *et al.*, 2004; Goosey-Tolfrey *et al.*, 2003; Campbell *et al.*, 1997; Bhambhani *et al.*, 1994). The high levels of wheelchair sport experience and training volume (as determined in all studies) further highlight the elite nature of the cohort recruited. Even though the selection of homogenous athletic participant groups is a strength of this thesis, the conclusions cannot be extended to other population groups. It may therefore be of interest to investigate whether exercise impacts on mucosal immune function in a similar enhancing way in patient or recreationally-trained SCI individuals. This is of major importance, as respiratory problems are likely to be of greater significance in patient populations (Brown *et al.*, 2006) when compared with the studied athletic population (study 5).

In the field of exercise immunology in SCI populations, the link between depressed sIgA and higher URS incidence has yet to be established. Even though in study 5 data were collected during five consecutive months, few players suffered from URS in this period and the significance of the findings presented would be improved with a higher proportion of athletes suffering from URS. Low incidences of URS during a study period of a similar length have been reported previously (West *et al.*, 2010). To solve this limitation in observational studies, researchers would need to monitor a group of individuals until an adequate number have presented URS. However, the practicalities of such research are difficult, as experienced when collecting data for study 5, as for obvious reasons individuals cannot be forced to present URS. An option to increase the likelihood of increasing URS incidence is to collect samples during the winter months, when URS incidence has been observed to be higher (Neville *et al.*, 2008).

It would further be of interest to investigate how long markers of the mucosal immune system remain elevated following acute exercise. The last saliva sample in the protocols used in the acute exercise studies was obtained 30 min after exercise. Extending the

observational time span would help define the period of increased mucosal protection following exercise in wheelchair athletes, and specifically athletes with a compromised autonomic nervous system.

Another interesting area to follow is the measurement of adrenaline or noradrenaline following exercise, and to compare this with salivary α-amylase in TETRA athletes and a control group with intact autonomic adrenal gland innervation. This would help to establish the relationship between catecholamines and α-amylase, whether these parameters correlate independently of autonomic innervation, and whether α-amylase indeed can be used as a marker of sympathetic nervous system activation (Nater & Rohleder, 2009).

Further work in TETRA individuals may look into the comparison of blood and salivary derived stress markers for each marker individually (such as IgA, cortisol, adrenaline) and their relationship to exercise. It is possible that this relationship differs to the AB population due to autonomic function loss. Therefore, the suitability of these markers for monitoring/predicting purposes may differ from the AB population.

The completeness of a spinal cord injury is usually described by the degree of motor and sensory dysfunction (Freeman Somers, 2010), and there do not seem to exist any standard tests to determine the degree of autonomic dysfunction. Structures involved in motor and sensory function (which includes ascending and descending tracts) are distributed over the whole cross-section of the spinal cord (Marieb & Hoehn, 2007). A motor and sensory complete lesion therefore implies that any autonomic function below the level of lesion is abolished. However, there may be a slight chance that despite motor and sensory lesions some structures of the autonomic nervous system are unaffected and therefore functioning normally. It is difficult to prove *complete* loss of centrally mediated autonomic output (for example, depressed HR_{peak} is only proof of dysfunction of neurons supplying the heart and therefore, only *partial* loss), even more as studies 3 – 5 imply the possibility of autonomic reflex activity, which may counter-act central dysfunction. Future work may therefore investigate ways to examine autonomic nervous drive and try to identify whether it originates centrally or via reflex pathways. This knowledge could be used to rule out any potential contribution of central autonomic drive towards autonomic reflex activity in

TETRA individuals due to a potential incomplete lesion. This would strengthen results of future studies investigating autonomic function in TETRA individuals.

This thesis provides evidence for the positive impacts of exercise on oral immune function in wheelchair athletes. The findings also suggest that despite central autonomic function loss, mucosal immune markers in TETRA athletes are regulated adequately to defend their host at rest and during exercise. Furthermore, the expected high levels of physical capacity can be observed in all studied athletic subgroups. In summary, exercise can be regarded as a means to improve both physical capacity and mucosal immune function in this population. It is therefore of great practical relevance to extend the insights of this thesis to a wider population within wheelchair sport and the SCI community.

10

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