

**Paratriathletes' physiological and thermoregulatory response to
training load and competition**

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A Doctoral Thesis

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Abstract

Paratriathlon is a multi-impairment, endurance sport which made its Paralympic Games debut in 2016. Athletes' impairments typically include but, are not limited to, spinal cord injury; cerebral palsy, or other neurological disorders; amputations or visual impairments. However, despite athletes displaying impairments that present several considerations for coaches and practitioners, there has been very little research in the sport. Specifically, there is little understanding of how athletes' impairments may impact their physiological response to acute or chronic changes in training load. Similarly, it is not known how consequences of athletes' impairments affect thermoregulation and the ability to adapt to the heat. Thus, this thesis aimed to elucidate these unknown areas whilst bridging the knowledge gap to research in able-bodied triathlon.

The first two studies of this thesis investigated paratriathletes' response to changes in training load, longitudinally (Chapter four) and more acutely (Chapter five). Specifically, Chapter four noted paratriathletes' mucosal immune function, represented by salivary secretory immunoglobulin A, displayed an inverse relationship with weekly training duration, but not measures of training load. Furthermore, upper respiratory illness incidence was not related to mucosal immunity. In Chapter five, it was shown that paratriathletes are resilient to large changes in training load in the form of a two-week intensified training period. A 14-d overseas training camp did not negatively affect hormonal, immunological or wellness measures whilst self-perceived sleep, stress and recovery parameters were improved. One explanation is that the camp environment minimised external life stresses and coaches' careful management of training load reduced the likelihood of overreaching.

In Chapter six, the thermoregulatory strain of paratriathlon competition in the heat was characterised. It was shown, via the use of ingestible sensors, that paratriathletes' core temperature reached levels significantly higher than previous research in able-bodied triathletes. Furthermore, trends for category-specific responses are presented, namely between those in PTWC and PTVI, highlighting the differences between impairment groups. Self-reported heat illness symptomatology was also greater than previous research in able-bodied athletes. Acknowledging the thermal strain paratriathletes face during competition in hot environments, Chapter seven sought to present the effectiveness of an ecologically valid preparatory heat acclimation strategy. Utilising a mixed active and passive intervention, controlling the relative intensity of exercise by heart rate, it was shown that paratriathletes are

capable of partial heat acclimation through thermoregulatory adaptations. However, the breadth of adaptations was less than able-bodied triathletes.

These were the first studies of paratriathletes' physiological and thermoregulatory response to training load and competition in the heat. It was shown that paratriathletes of a high training level are robust to acute changes in training load whilst training load had no relationship with mucosal immunity, despite a high illness incidence. However, paratriathletes are at heightened risk of thermoregulatory strain when competing in the heat, as shown by high core temperatures and self-reported heat illness symptomatology. Nonetheless, strategies can be utilised to induce thermoregulatory adaptations in this cohort. This provides valuable information for coaches and practitioners working with paratriathletes as they seek to minimise training time-loss and ameliorate the strain of competition in the heat.

Key words: Disability, triathlon, mucosal immune function, overreaching, thermoregulation, heat acclimation

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Preface

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Abbreviations

AB	Able-bodied	SD	Standard deviation
AeLT	Aerobic lactate threshold	sIgA	Salivary secretory immunoglobulin A
AnLT	Anaerobic lactate threshold	SR	Secretion rate
ANOVA	Analysis of variance	sRPE	Session rating of perceived exertion
BLa	Blood lactate concentration	sT	Salivary testosterone
CI	Confidence intervals	T_c	Core temperature
CP	Cerebral palsy	TL	Training load
CV	Coefficient of variation	TRIMP	Training impulse
ETL	External training load	TS	Thermal sensation
GPS	Global positioning system	T_{sk}	Mean skin temperature
GXT	Graded exercise test	URI	Upper respiratory tract illness
HA	Heat acclimation	USG	Urine specific gravity
HR	Heart rate	VI	Visual impairment
HST	Heat stress test	$\dot{V}O_2$	Rate of oxygen uptake
IT	Intensified training	$\dot{V}O_{2peak}$	Peak rate of oxygen uptake
ITL	Internal training load		
MAP	Maximum aerobic power output		
OR	Overreaching		
PO	Power output		
POMS	Profile of mood states		
PV	Plasma volume		
RESTQ-S	Recovery-stress questionnaire for sports		
RH	Relative humidity		
RHR	Resting heart rate		
RPE	Rating of perceived exertion		
sC	Salivary cortisol		
sC:T	Salivary cortisol:testosterone ratio		
SCI	Spinal cord injury		

1

Introduction

1.1 Paratriathlon

Paratriathlon is a variant of triathlon modified for individuals with a physical impairment (Mujika *et al.*, 2015). Athletes compete over the triathlon sprint distance of 750 m swim, 20 km non-drafting cycle and 5 km run. The sport consists of six categories for competition based on individuals' physical impairment (International Triathlon Union, 2017); see Table 1.1 for more information.

Athletes in the PTWC category predominantly rely on a wheelchair for daily activities and so require the use of a handcycle and racing wheelchair for cycle and run sections of the race, respectively. Categories PTS2, PTS3, PTS4 and PTS5 consist of ambulant athletes with those most impaired in the PTS2 category and the least impaired classified as PTS5. Individuals in these categories may require the use of prostheses or adaptations to bicycles. Athletes in the PTVI category require the use of an able-bodied (AB) guide during races and ride a tandem bicycle (Table 1.1). Race durations for paratriathlon are typically 60 to 90 min, depending on the athletes' impairment severity and course demands (Table 1.2). With competitions held globally, athletes are frequently exposed to challenging environments such as high ambient temperatures and humidity. For example, at the 2020 Paralympic Games in Tokyo, athletes may face temperatures greater than 30°C with a relative humidity (RH) of 70% or more (Japan Meteorological Agency, 2017).

Table 1.1: Paratriathlon categories, example impairments and race modalities. Information taken from International Triathlon Union (ITU, 2017).



















Category	Example impairment	Swim	Bike	Run
PTWC	Spinal cord injury, unilateral or bilateral above the knee amputation, severe impairment of muscle power or range of motion. There are two subcategories: H1 (most impaired) and H2 (least impaired).			
PTS2	Unilateral above the knee amputation, double below the knee amputation, severe limitation to upper and lower limb muscle power caused by cerebral palsy or other neurological disorders.			
PTS3	Partial brachial plexus, moderate multiple limb impairments, significant loss of power in upper and lower limb muscles via impairments such as cerebral palsy or other neurological disorders.			
PTS4	Below the knee amputation, above the elbow amputation, mild cerebral palsy, moderate impairment to muscle power.			
PTS5	Below the elbow amputation, below the knee amputation, partial loss of arm muscle power, lower limb deficiency or mild neurological impairments.			
PTVI	Total (sub category B1) or partial (B2 or B3) visual impairment.			

Table 1.2: Paratriathlon individual modality and overall (including transitions and category factoring) race durations from 2017 ITU World Triathlon Grand Final winners. An able-bodied comparison is provided from junior races at the same event (ITU, 2018).

	Female				Male			
	Swim	Bike	Run	Overall	Swim	Bike	Run	Overall
PTWC	15:43	43:42	16:02	1:20:28	10:34	33:49	11:16	1:02:18
PTS2	13:53	41:09	23:45	1:23:30	11:34	35:32	18:46	1:09:55
PTS3	N/A	41:11	20:12	1:23:25	10:42	37:11	19:05	1:11:22
PTS4	14:55	38:42	19:59	1:18:18	11:18	33:33	15:52	1:04:15
PTS5	11:44	36:17	18:13	1:09:49	11:09	33:04	14:42	1:01:20
PTVI	12:50	35:10	18:25	1:13:48	10:18	33:06	15:43	1:05:21
AB	10:16	31:11	17:39	1:01:22	08:56	29:39	15:23	0:55:54

AB – Able-bodied.

The sport of paratriathlon made its Paralympic Games debut in Rio de Janeiro, September 2016. As noted by Mujika *et al.* (2015), the inclusion of the sport at the Paralympic Games has set clear performance targets which have resulted in a rise in professional paratriathletes. Despite the growing status of paratriathlon, there remains very little published literature concerning the sport. To date, the case study of a single, male, below-knee amputee paratriathlete’s training habits by Mujika *et al.* (2015) remains the only source of peer-reviewed information. However, whilst the study of Mujika *et al.* (2015) provided a starting point for research in paratriathlon, the report lacks applicability to many as the paratriathlete profiled was competing in longer, Olympic distance races (1500 m swim, 40 km cycle, 10 km run) not the sprint distance typical of paratriathlon.

The lack of published literature centred on paratriathlon opposes that of Olympic triathlon. Amongst other areas, the physiological profile (Suriano & Bishop, 2010), training habits (Mujika, 2014) and strategies to improve performance in the heat (Schmit *et al.*, 2017) have all been investigated in the AB variant of the sport. To date, however, none of these topics have been studied in paratriathlon. Furthermore, it is not known how transferable the findings from Olympic triathlon are to paratriathlon due to certain consequences of physical disabilities such as spinal cord injury (SCI), cerebral palsy (CP), amputation or visual impairment (VI). Thus, it is pertinent for those working in the sport to have a better understanding of the aforementioned topics and to learn how transferable findings are between AB and Paralympic

triathletes. Specifically, there is a need to understand how this population responds to training from a physiological perspective and how training can be manipulated to attenuate any performance decrement and thermoregulatory strain when competing in the heat.

1.2 Aims and objectives of the thesis

The overarching aim of this thesis was to better characterise the physiology of paratriathletes, bridging the knowledge gap concerning AB and Paralympic athletes. This is with particular focus on paratriathletes' physiological response to training load (TL) and strategies to improve performance in the heat. These topics were identified in consultation with paratriathlon coaches and athletes as areas for significant impact. This is due to the dearth of research concerning the sport, the large TLs undertaken by athletes with associated risks of training unavailability and the challenging environments in which athletes must compete. The objectives were thus:

- To determine how athletes' mucosal immune system adapts to TL over a prolonged period and how this relates to illness incidence.
- To describe the responses of common, non-invasive, hormonal, immunological, physiological and psychological markers of overreaching (OR) to a period of intensified training (IT).
- To characterise the thermoregulatory strain imposed by paratriathlon racing in the heat.
- To analyse the effectiveness of an ecologically valid heat acclimation (HA) strategy and assess how adaptations may differ to AB athletes.

1.3 Organisation of the thesis

A comprehensive literature review (*Chapter two*) was initially conducted. This primarily concerned the physiological consequences of Paralympic athletes' physical impairments and how this may influence their response to TL or performance in the heat. Furthermore, an overview of previous research regarding TL, mucosal immunity, thermoregulation and HA is provided. Methods employed in multiple experimental chapters are described in *Chapter three*. This is succeeded by four experimental chapters (*Chapters four to seven*).

Chapter four investigated the responses of paratriathletes' mucosal immune system to changes in TL over a nine-month, prospective study period. Additionally, illness incidence was analysed to determine the impact of mucosal immunity changes. *Chapter five* sought to determine the physiological responses to more acute fluctuations in TL than the previous chapter, specifically a 14-day period of IT. *Chapter six* characterised the thermoregulatory strain imposed by competitive paratriathlon in the heat. Finally, considering the results of the

preceding chapter, *Chapter seven* examined the effectiveness of a novel, heart rate (HR)-controlled, HA intervention on thermoregulatory variables and determined how adaptations may differ to AB athletes. A general discussion of key findings, contribution to scientific literature, practical applications and future directions of this work is then presented in *Chapter eight*. A summary of the experimental chapters is shown in Figure 1.1.

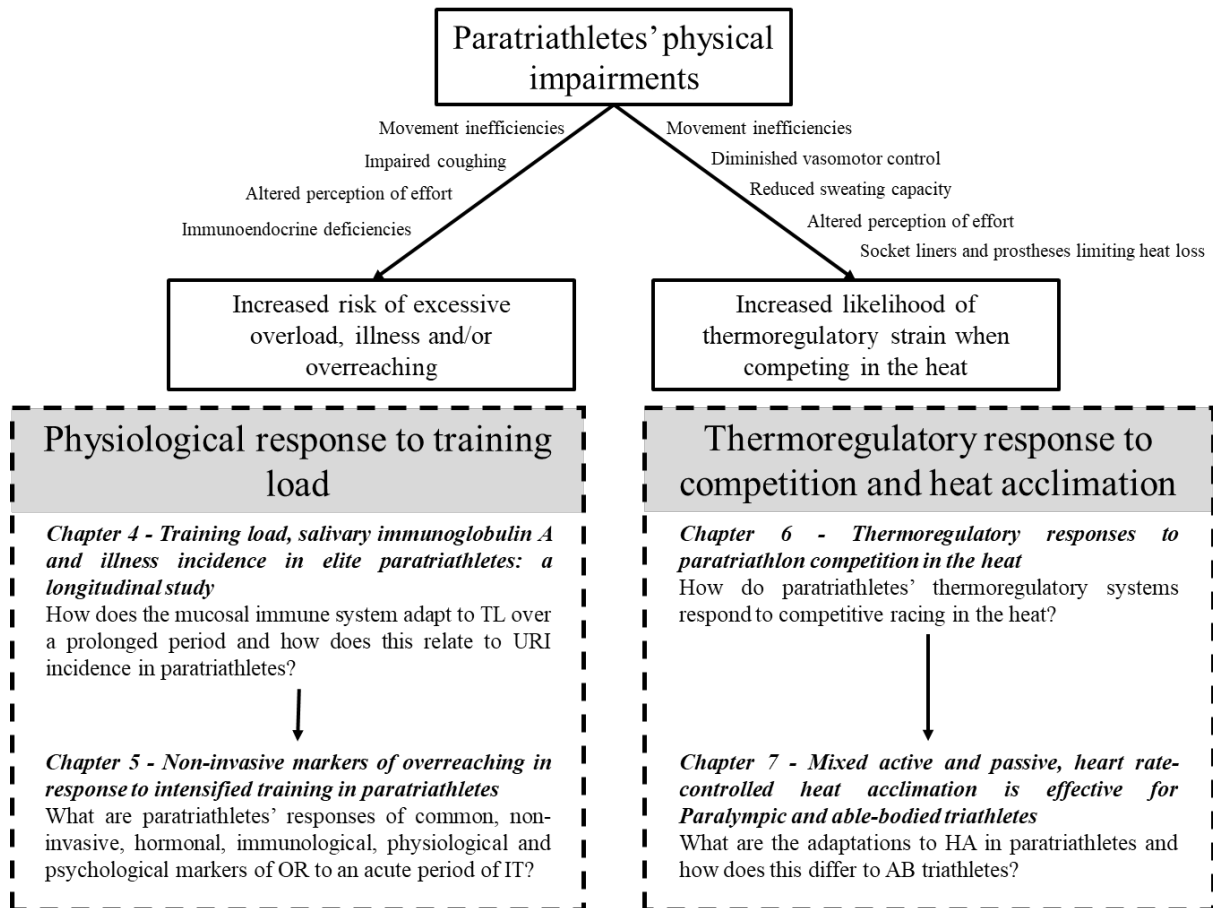


Figure 1.1: Schematic representation of experimental studies of this thesis. TL – Training load. URI – Upper respiratory tract illness. OR – Overreaching. IT – Intensified training. HA – Heat acclimation. AB – Able-bodied.

2

Literature review

2.1 The Paralympic athlete

Paratriathlon is a multi-disability sport with athletes' impairments typically consisting of: SCI, CP or other neurological disorders, amputations, or VI. Accordingly, coaches and practitioners working in the sport must have an appreciation of the many consequences of athletes' impairments that may affect performance or training capabilities and how they may differ to AB athletes. The following sections will outline these consequences before discussing how they may relate to paratriathlon training and/or performance.

2.1.1 Spinal cord injury

The spinal cord, enclosed within the vertebral column, is the major channel through which afferent and efferent information is relayed between the brain and body (Kirschblum *et al.*, 2011). Damage to the spinal cord, and subsequent injury, can occur through the application of excessive forces (e.g., motor vehicle collision) or via degenerative and congenital disorders. SCI can be defined as either tetraplegia or paraplegia depending on the level of injury. Tetraplegia refers to injuries of the cervical region of the spinal cord and results in a loss of function in all four limbs. Paraplegia, meanwhile, refers to injuries of the thoracic, lumbar or sacral regions of the spinal cord and causes a loss of function in the trunk, organs and legs below the lesion level. Commonly, paratriathletes with SCI compete in the PTWC category (Figure 2.1). However, athletes with an incomplete SCI can also compete in ambulant paratriathlon categories depending upon functional capacity assessed during classification.



Figure 2.1: Paratriathlete with a spinal cord injury competing in the PTWC category. Image courtesy of British Triathlon (2016a).

Spinal cord injury is the most extensively researched physical impairment with respect to athletic performance (Paulson & Goosey-Tolfrey, 2017). A great deal is known from wheelchair racing and ball sports such as wheelchair rugby, wheelchair basketball and wheelchair tennis concerning the physiological disparities of athletes with SCI compared to AB athletes (Bhambhani, 2002; Goosey-Tolfrey & Leicht, 2013). These include, amongst others: lowered active muscle mass; lower stroke volume as a consequence of an impaired muscle pump in the lower limbs; a potentially lowered maximum HR (Paulson & Goosey-Tolfrey, 2017). All consequences of SCI are proportional to lesion level and completeness such that athletes with a high level, complete lesion display greater impairment whereas those with a lower level, incomplete lesion will display physiological responses to exercise more akin to AB athletes (Bhambhani, 2002).

Athletes with an SCI display mechanical limitations such as impaired trunk function, as a result of muscle paralysis, that reduce coughing efficiency (Brown *et al.*, 2006). Furthermore, this population group may be prone to immunoendocrine deficiencies through a decentralisation of the autonomic nervous system (Nash, 2000). Additionally, muscle paralysis commonly necessitates the use of a wheelchair for daily ambulation, an inefficient movement, resulting in significant use of the upper limbs during daily life and training (Webborn & Van de Vliet, 2012). Furthermore, athletes with an SCI display diminished muscle mass in comparison to AB athletes, resulting in greater peripheral fatigue (Iturricastillo *et al.*, 2016).

This may interact with athletes' perceptions of effort during, or after, exercise as peripheral cues outweigh central cues. Injury to the spinal cord also results in a lack of afferent input to the thermoregulatory centre with a loss of vasomotor control and sweating capability below the lesion level (Freund *et al.*, 1984). This is further exacerbated by a loss of venous muscle pump to redistribute blood from paralysed muscle (Hopman *et al.*, 1992). A supplementary consequence of significant muscle paralysis is that athletes commonly display high proportions of body fat (Goosey-Tolfrey *et al.*, 2016; Graham-Paulson *et al.*, 2017). For example, in their case study of a PTWC H1 paratriathlete with an SCI, Graham-Paulson *et al.* (2017) reported a body fat of 25.4%, as assessed via dual-energy X-ray absorptiometry. This is considerably higher than $15.1 \pm 5.6\%$ shown by Mueller *et al.* (2013) in their descriptors of male AB triathletes, employing similar methods.

2.1.2 Cerebral palsy

Cerebral palsy is a postural and movement disorder caused by central brain injury at a young age which results in altered neuromuscular physiology and diminished exercise capacity (Runciman *et al.*, 2016). CP presents three main impairment profiles: hemiplegia where one side of the body is affected; diplegia where two limbs are affected (typically the lower limbs); quadriplegia where all four limbs are affected (Bax, 1964). Athletes with CP signify a large proportion of the Paralympic athlete population, representing 20% of the British Paralympic team at the 2012 London Games (T. Paulson, personal communication, January 2018), including participation in paratriathlon. In paratriathlon, athletes with CP may compete in the PTWC category if they are wheelchair users, whilst ambulant athletes with CP compete in PTS2 to PTS5, depending on their impairment severity (Figure 2.2). Despite athletes with CP's commonality in Paralympic sport, and their impairment presenting several areas for consideration (Runciman *et al.*, 2016), research is only now emerging to help support athletes.



Figure 2.2: Paratriathlete with right-side hemiplegia cerebral palsy competing in the PTS3 category. Image courtesy of British Triathlon (2016a).

Runciman *et al.* (2016) studied the pacing strategies of T37 (true ambulant hemiplegia) and T38 (minimal, yet significant, impairment in any limbs) sprint athletes with CP in comparison to an AB group over a distance-deceived (1000 + 600 m) and non-deceived shuttle run test (1600 m). During the distance-deceived trial, participants were under the assumption the trial would be completed at 1000 m, thus paced themselves accordingly. The authors noted that athletes with CP commonly underperformed in comparison to the AB group through overly conservative pacing as a potentially protective mechanism. Specifically, the CP athletes displayed a flatter pacing profile during the distance deceived trial. Runciman *et al.* (2016) also found that for the same relative intensity, athletes with CP tended to report lower ratings of perceived exertion (RPE) values in comparison to the AB group. As such, there are potential considerations regarding pacing and perception of effort during longer duration tasks in this impairment group.

Athletes with CP display significant movement inefficiencies (Blauwet *et al.*, 2017). Specifically, the presence of athetosis, spasticity, or ataxia, may all limit muscular coordination and increase the energy cost of movements (Blauwet *et al.*, 2017). As such, the internal workload required to produce a set absolute intensity is significantly greater in athletes with CP, thus increasing the susceptibility to excessive overload. Moreover, it has been shown,

albeit in children, that the metabolic heat production for an absolute external workload is significantly elevated in this population group due to the aforementioned movement inefficiencies (Maltais *et al.*, 2004). Additionally, athletes' high muscular tone impairs venous return via muscle pumps and negatively impacts on blood flow distribution (Kloyiam *et al.*, 2011).

2.1.3 Amputation

Although athletes with an amputation represent a large proportion of Paralympic athletes, winning 22% of British medals at the Rio 2016 Paralympic Games (T. Paulson, personal communication, January 2018) very little research has focused on the physiology of these athletes with most literature centred on biomechanical properties (Sagawa *et al.*, 2010). In paratriathlon, and other Paralympic sports, athletes with an amputation may compete in wheelchair or ambulant classes, depending on the level of their impairment. Athletes with an amputation contend with the same issue of reduced active muscle mass as athletes with an SCI, as evidenced by dual-energy X-ray absorptiometry comparisons to AB individuals (Sherk *et al.*, 2010). Similarly, wheelchair athletes with an amputation face the common issue of excessive upper limb use, due to the associated large loads of daily locomotion and sport performance during the inefficient movement of chair propulsion (Webborn & Van de Vliet, 2012). Likewise, ambulant upper limb amputees display a similar propensity for upper-body overuse syndromes due to excessive loads as a result of residual limb overcompensation (Østlie *et al.*, 2011) which is likely further exacerbated by the swimming component of paratriathlon training. Furthermore, similar to athletes with CP, ambulant athletes with an amputation also suffer from movement inefficiencies (Blauwet *et al.*, 2017; Ward & Meyers, 1995). The energy cost of ambulation at a set external workload is significantly greater for amputees than AB individuals with ascending levels of amputation creating increased metabolic demand (Ward & Meyers, 1995).



Figure 2.3: A wheelchair-dependent and an ambulant paratriathlete with a lower-limb amputation competing in the PTWC and PTS2 categories, respectively. Images courtesy of British Triathlon (2016a).

A considerable uniqueness to athletes with an amputation is a lower body surface area to volume ratio as the result of missing limbs (Epstein *et al.*, 1983; Webborn, 1996). A lower body surface area diminishes the capacity for evaporative and convective heat loss, increasing heat storage in this population. Furthermore, socket liners and prostheses act as an insulating barrier, limiting heat dissipation (Klute *et al.*, 2007) and promoting stump skin injuries (Klute *et al.*, 2007; Legro *et al.*, 1999). Moreover, skin grafts on amputees, or other athletes, removes a portion of the skin capable of sweating and cutaneous vasodilation (Crandall & Davis, 2010).

2.1.4 Visual impairment

Individuals with a VI may participate in paratriathlon, in the PTVI category, and other Paralympic sports, whilst displaying a range of impairment severities (Figure 2.4). Athletes can be categorised in one of three levels: B1 are considered blind athletes (from no light perception in either eye, up to light perception but unable to recognise the shape of a hand at any distance or direction); B2 are considered to have severely impaired vision (from ability to recognise the shape of a hand, up to a visual acuity of 20/600 or a visual field of less than 5° in the best eye); B3 are considered to have moderate to poor vision (visual acuity above 20/600 to 20/200, or a visual field of less than 20° and more than 5° in the best eye) (Lieberman, 2005). VI includes athletes with albinism, an impairment caused by genetical mutations that can result in

diminished melanin synthesis affecting the skin, eyes and hair (Bothwell, 1997). Whilst athletes with a VI may be physiologically similar to AB athletes, they still require several considerations as a consequence of their impairment.



Figure 2.4: Paratriathlete with a visual impairment (right), competing in the PTVI (B3) category, and her able-bodied guide (left). Image courtesy of British Triathlon (2016b).

Firstly, athletes with a VI are restricted in their ability to utilise visual feedback (Taylor *et al.*, 2016) that may have implications in motor learning, proprioception and developing robust techniques (e.g., swimming or running) to tolerate TLs (Magno e Silva *et al.*, 2013). Additionally, restricted visual feedback has been proposed to impact on the ability to monitor personal pacing (Taylor *et al.*, 2016). It has been shown that swimmers with a VI display significant alterations in the pacing profile of 400 m performance compared to AB athletes, suggested to be related to an undermined pace awareness (Taylor *et al.*, 2016). Furthermore, compromised visual feedback not only relates to sporting movements but will also present difficulties in self-monitoring hydration through urine colour or volume (Webborn & Van de Vliet, 2012). Finally, athletes with albinism are more prone to sunburn in situations of high radiant load due to the lack of skin pigmentation (Bothwell, 1997).

2.1.5 Normative endurance physiology

Due to the several aforementioned consequences of Paralympic athletes' impairments, their physiological parameters relating to endurance sporting performance may be significantly

disparate to AB athletes. Whilst research detailing physiological descriptors of Paralympic athletes is accessible, there is only a finite amount of literature available to draw normative data from (Table 2.1).

Table 2.1: Normative endurance physiology parameters in Paralympic athletes, represented relative to able-bodied athletes.

Impairment	Study	n	Age (y)	Sex (♂/♀)	Parameter	Value	%AB
SCI	Sousa <i>et al.</i> (2016)	1	N/A	♂	Swimming $\dot{V}O_{2peak}$	47.8 ml·kg ⁻¹ ·min ⁻¹	83 - 96
	Baumgart <i>et al.</i> (2018)	432	N/A	♂ and ♀	Handcycling $\dot{V}O_{2peak}$	36.0 ± 4.3 ml·kg ⁻¹ ·min ⁻¹	52 - 59
					Racing wheelchair $\dot{V}O_{2peak}$	39.6 ± 1.9 ml·kg ⁻¹ ·min ⁻¹	53 - 63
	Fischer <i>et al.</i> (2015)	7	41 ± 4	♂	Handcycling AeLT	137 ± 26 W	52
					Handcycling AnLT	162 ± 25 W	48
					Handcycling MAP	178 ± 34 W	46
	Zeller <i>et al.</i> (2017)	1	54	♀	Handcycling AnLT	181 W	75
	de Groot <i>et al.</i> (2018)	10	40 ± 12	♂	Handcycling MAP	220 W	78
					Handcycling MAP	159 ± 29 W	41
	Perret <i>et al.</i> (2012)	8	33 ± 12	7 ♂, 1 ♀	Racing wheelchair AnLT	16.6 ± 3.0 km·h ⁻¹	87 - 100
					Racing wheelchair MAV	21.2 ± 4.0 km·h ⁻¹	101 - 117
Amputation (non-ambulant)	Knechtle <i>et al.</i> (2004)	2	32 - 37	N/A	Handcycling $\dot{V}O_{2peak}$	38.4 - 38.8 ml·kg ⁻¹ ·min ⁻¹	55 - 63
	Couttis (1990)	3	26 - 28	♂	Racing wheelchair $\dot{V}O_{2peak}$	47.5 - 67.2 ml·kg ⁻¹ ·min ⁻¹	64 - 90

♂ - Male. ♀ - Female. AB - Able-bodied. SCI - Spinal cord injury. $\dot{V}O_{2peak}$ - Peak rate of oxygen uptake. AeLT - Aerobic lactate threshold. AnLT - Anaerobic lactate threshold. MAP - Maximum aerobic power output. MAV - Maximum aerobic velocity.

Table 2.1 continued: Normative endurance physiology parameters in Paralympic athletes, represented relative to able-bodied athletes.

Impairment	Study	n	Age (y)	Sex (♂/♀)	Parameter	Value	%AB
Amputation (ambulant)	Menaspà <i>et al.</i> (2012)	1	40	♂	Cycling $\dot{V}O_{2peak}$	61.3 ml·kg ⁻¹ ·min ⁻¹	88
					Cycling AnLT	255 W	76
					Cycling MAP	315 W	82
	Mujika <i>et al.</i> (2015)	1	37	♂	Cycling AeLT	166 W	62
					Cycling AnLT	190 W	57
					Cycling MAP	251 W	65
					Running AeLT	12.5 km·h ⁻¹	68
	Mengelkoch <i>et al.</i> (2017)	3	28 ± 8	♂	Running AnLT	12.9 km·h ⁻¹	68
					Running MAV	16.8 km·h ⁻¹	80
					Running $\dot{V}O_{2peak}$	55.4 ± 6.0 ml·kg ⁻¹ ·min ⁻¹	74
de Groot <i>et al.</i> (2012)	20	29 ± 11	16 ♂, 4 ♀	Running MAV	10.9 km·h ⁻¹	52	
				Cycling $\dot{V}O_{2peak}$	42.5 ± 13.2 ml·kg ⁻¹ ·min ⁻¹	57 - 61	
				Cycling MAP	219 ± 88 W	57 - 78	

♂ - Male, ♀ - Female, AB - Able-bodied, $\dot{V}O_{2peak}$ - Peak rate of oxygen uptake, AeLT - Aerobic lactate threshold, AnLT - Anaerobic lactate threshold, MAP - Maximum aerobic power output, MAV - Maximum aerobic velocity.

Table 2.1 continued: Normative endurance physiology parameters in Paralympic athletes, represented relative to able-bodied athletes.

Impairment	Study	n	Age (y)	Sex (♂/♀)	Parameter	Value	%AB
Visual impairment	Malwina <i>et al.</i> (2015)	13	41 ± 13	♂	Cycling $\dot{V}O_{2peak}$	45.8 ± 6.9 ml·kg ⁻¹ ·min ⁻¹	66
Able-bodied	Suriano & Bishop (2010)	N/A	N/A	♂ and ♀	Swimming $\dot{V}O_{2peak}$	38.1 – 57.7 ml·kg ⁻¹ ·min ⁻¹	/
	Schabert <i>et al.</i> (2000)	10	17 – 34	5 ♂, 5 ♀	Cycling $\dot{V}O_{2peak}$	61.3 ± 4.6 (♀) – 69.9 ± 4.5 (♂) ml·kg ⁻¹ ·min ⁻¹	/
					Cycling MAP	282 ± 19 (♀) – 385 ± 14 (♂) W	/
					Running $\dot{V}O_{2peak}$	63.2 ± 3.6 (♀) – 74.7 ± 5.3 (♂) ml·kg ⁻¹ ·min ⁻¹	/
					Running MAV	18.0 ± 0.9 (♀) – 20.9 ± 0.9 (♂) km·h ⁻¹	/
	Bernard <i>et al.</i> (2009)	8	27 ± 5	5 ♂, 3 ♀	Cycling AeLT	187 ± 13 (♀) - 226 ± 19 (♂) W	/
					Cycling AnLT	242 ± 14 (♀) – 336 ± 23 (♂) W	/
	Knoepfli <i>et al.</i> (2004)	9	24 ± 4 (♂) – 29 ± 5 (♀)	6 ♂, 3 ♀	Running AeLT	16.2 ± 0.9 (♀) - 18.5 ± 0.4 (♂) km·h ⁻¹	/
					Running AnLT	16.5 ± 1.0 (♀) - 19.1 ± 0.7 (♂) km·h ⁻¹	/

♂ - Male. ♀ - Female. AB - Able-bodied. $\dot{V}O_{2peak}$ - Peak rate of oxygen uptake. AeLT - Aerobic lactate threshold. AnLT - Anaerobic lactate threshold. MAP - Maximum aerobic power output. MAV - Maximum aerobic velocity.

For athletes with an SCI, the recent meta-analysis of Baumgart *et al.* (2018) provided information regarding sport-specific peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) values in Paralympic sports. In handcycling, the paratriathlon equivalent of cycling for PTWC athletes, relative $\dot{V}O_{2\text{peak}}$ values of $36.0 \pm 4.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (95% confidence intervals [CI]: 27.4 to $44.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) were reported. For wheelchair racing, the paratriathlon equivalent of running for PTWC athletes, values of $39.6 \pm 1.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (95% CI: 35.9 to $43.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) were found. Whilst data is extremely scarce with regards to para-swimming, in the study of Sousa *et al.* (2016), a male swimmer with paraplegia displayed a relative $\dot{V}O_{2\text{peak}}$ of $47.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

Some research exists regarding the physiological descriptors beyond $\dot{V}O_{2\text{peak}}$ in athletes with an SCI. Fischer *et al.* (2015) reported aerobic (AeLT) and anaerobic lactate threshold (AnLT) values of $137 \pm 26 \text{ W}$ and $162 \pm 25 \text{ W}$, respectively, in their study of male handcyclists with paraplegia. Additionally, Fischer *et al.* (2015) presented maximum aerobic power output (MAP) values of $178 \pm 34 \text{ W}$ which is higher than $159 \pm 29 \text{ W}$ stated by de Groot *et al.* (2018) in their study of trained handcyclists also with paraplegia. In a case study of a female athlete, Zeller *et al.* (2017), reported peak AnLT and MAP values of 181 and 220 W, respectively. Perret *et al.* (2012) presented a maximum lactate steady state, associated with AnLT, of $16.6 \pm 3.0 \text{ km}\cdot\text{h}^{-1}$ and a maximum aerobic velocity of $21.2 \pm 4.0 \text{ km}\cdot\text{h}^{-1}$ during treadmill wheelchair racing in well-trained athletes with an SCI. For wheelchair users with an amputation, Knechtle *et al.* (2004) reported relative $\dot{V}O_{2\text{peak}}$ values of 38.4 and $38.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in two handcyclists whilst Coutts (1990) presented three male wheelchair racers with an amputation displaying a relative $\dot{V}O_{2\text{peak}}$ of 47.5 to $67.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

Regarding ambulant athletes with an amputation, a finite number of case studies exist detailing physiological characteristics. In their study, Menaspà *et al.* (2012) described the physiology of an elite, male, para-cyclist with a transfemoral amputation. The athlete had an AnLT of 255 W, $\dot{V}O_{2\text{peak}}$ of $61.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and MAP of 315 W. Mujika *et al.* (2015) noted the changes in physiological parameters over 19 months in a male long-distance paratriathlete with a below the knee amputation. Peak AeLT, AnLT and MAP from a cycling graded exercise test (GXT) were 166 W, 190 W and 251 W, respectively. Whilst from a running GXT, the highest velocity at AeLT, AnLT and maximum aerobic velocity was $12.5 \text{ km}\cdot\text{h}^{-1}$, $12.9 \text{ km}\cdot\text{h}^{-1}$ and $16.8 \text{ km}\cdot\text{h}^{-1}$, respectively. Furthermore, Mengelkoch *et al.* (2017), whilst studying three individuals with a transfemoral amputation during running, reported $\dot{V}O_{2\text{peak}}$ and maximum aerobic velocity values of $55.4 \pm 6.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $10.9 \pm 1.7 \text{ km}\cdot\text{h}^{-1}$, respectively

Research concerning the physiological parameters of individuals with CP has mainly been confined to children and non-athletes (Runciman *et al.*, 2016). Nonetheless, de Groot *et al.* (2012) presented data for cycling $\dot{V}O_{2\text{peak}}$ and MAP in athletes with CP from mixed sporting backgrounds. Testing on three separate occasions, the authors noted the highest $\dot{V}O_{2\text{peak}}$ was $42.5 \pm 13.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with a MAP of $219 \pm 88 \text{ W}$. Beyond this, data is severely limited. Lastly, due to athletes with a VI being physiologically similar to AB athletes, it can be assumed there is no impairment-specific interaction on physiological parameters. This has been partly confirmed by the study of Malwina *et al.* (2015) whereby no significant differences in $\dot{V}O_{2\text{peak}}$ were evident between tandem cyclists with or without a VI (45.8 ± 6.9 vs $52.2 \pm 9.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), although both values are below well-trained triathletes (Suriano & Bishop, 2010).

Conversely to research in Paralympic athletes whereby inferences on normative physiological markers are typically sought from individual paratriathlon modalities, adequate information exists concerning the physiology of AB triathletes. Although data is limited regarding aerobic capacity during swimming, $\dot{V}O_{2\text{peak}}$ values of 49.9 to 57.7 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ or 38.1 to 45.3 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ have been reported for male and female AB triathletes, respectively (Suriano & Bishop, 2010). Schabort *et al.* (2000), in their study of 10 elite triathletes (5 male, 5 female), all of whom were members of the same national team, presented data concerning maximal physiological measures during cycling and running. The authors stated that cycling $\dot{V}O_{2\text{peak}}$ and MAP was $69.9 \pm 4.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $385 \pm 14 \text{ W}$ for males and $61.3 \pm 4.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $282 \pm 19 \text{ W}$ for females, respectively. Also, running $\dot{V}O_{2\text{peak}}$ and maximum aerobic velocity were $74.7 \pm 5.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $20.9 \pm 0.9 \text{ km}\cdot\text{h}^{-1}$ for males and $63.2 \pm 3.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $18.0 \pm 0.9 \text{ km}\cdot\text{h}^{-1}$ for females, respectively. Bernard *et al.* (2009) reported cycling AeLT and AnLT in elite triathletes of $266 \pm 19 \text{ W}$ and $336 \pm 23 \text{ W}$ for males and $187 \pm 13 \text{ W}$ and $242 \pm 14 \text{ W}$ for females, respectively. Finally, Knoepfli *et al.* (2004) presented the running physiology of nine Swiss national team triathletes. It was shown that AeLT occurs at 18.5 ± 0.4 or $16.2 \pm 0.9 \text{ km}\cdot\text{h}^{-1}$ for males and females, respectively, whilst AnLT occurs at $19.1 \pm 0.7 \text{ km}\cdot\text{h}^{-1}$ or 16.5 ± 1.0 (Knoepfli *et al.*, 2004).

As such, clear disparities exist between the levels of physiological parameters in Paralympic and AB athletes competing in triathlon or its respective sporting modalities. These differences are likely to be influenced by Paralympic athletes' physical impairments and their associated consequences. These include, but are not limited to: muscle paralysis, missing limbs, ataxia, atrophy, spasticity or coordination impairments. Consequently, coaches and

practitioners working with Paralympic athletes must have an awareness of the reported reduced absolute and relative physiological characteristics. However, caution should be applied when interpreting normative values of Paralympic athletes' aerobic fitness due to the commonly outdated nature of the aforementioned published research and sub-elite characteristics of study participants.

2.1.6 Summary

In summary, there are several consequences of Paralympic athletes' impairments that must be considered by those working within multi-impairment sports, such as paratriathlon. Figure 2.5 summarises these points for each impairment group. The following sections will then discuss how these consequences effect paratriathlon training and competition, with particular focus on athletes' physiological response to TL and thermoregulation during competition in the heat.

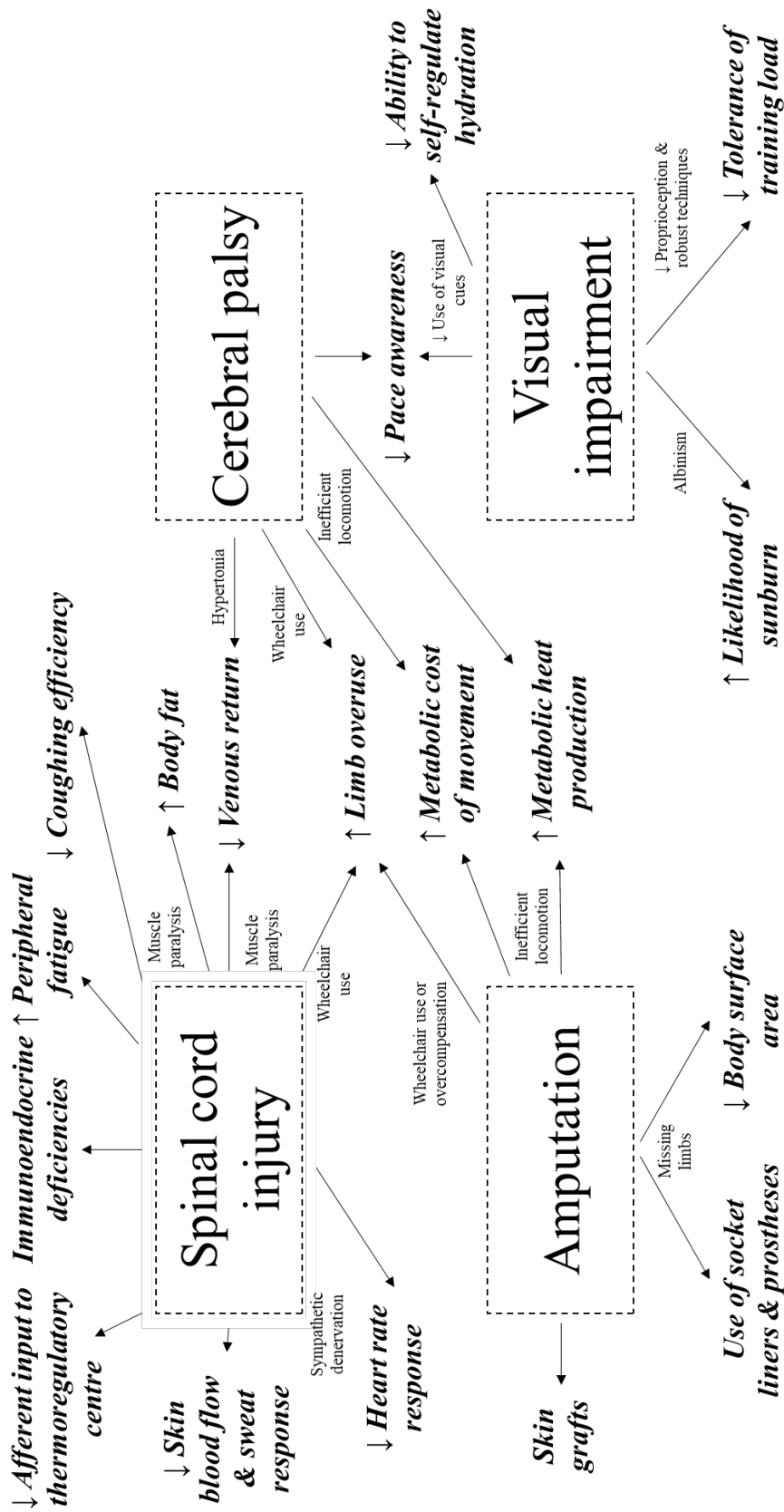


Figure 2.5: Model of impairment consequences and considerations for paratriathlon training and performance.

2.2 Training load

Management of TLs is fundamental to coaches and practitioners to prevent both under- and overtraining in an attempt to optimise performance outcomes and manage injury and/or illness risk in AB and Paralympic sport (Borresen & Lambert, 2009; Paulson *et al.*, 2015). To manipulate training for performance enhancement, training must first be quantified (Mujika, 2013). Methods of TL quantification have been extensively researched in AB sport with Mujika (2017) producing a review of varying methods, as discussed below.

Several physiological, psychological or external measures have been utilised in the quantification of internal (ITL) or external (ETL) training load. ITL has been described as the relative physiological and psychological stress imposed by training (Halson, 2014) with ETL defined as the work completed by the athlete, measured independently of his or her internal characteristics (Wallace *et al.*, 2009). Whilst ITL relies upon the use of physiological or psychological parameters such as HR or RPE, ETL incorporates external data such as power output (PO) or session duration. Each measure used in ITL quantification is based upon assumptions regarding exercise intensity and physiological toll imposed. Firstly, HR is the most commonly used parameter and is employed based on the principle of a linear relationship between HR and exercise intensity (Hopkins, 1991; Rodríguez-Marroyo *et al.*, 2012). HR can subsequently be used in several methods of ITL quantification, namely the calculation of training impulse (TRIMP) scores (Banister *et al.*, 1991; Lucía *et al.*, 2003).

The principle of calculating a training impulse was first proposed by Banister *et al.* (1991) (Banister's TRIMP). Banister's TRIMP can be calculated from training session duration and individually pre-determined resting, maximum and average exercising HR. This was subsequently adapted by Lucía *et al.* (2003), considering only the time spent exercising rather than total session duration in their model, Lucía's TRIMP. Multiplying the time spent in three specific, HR-defined training zones (zone 1 = below ventilatory threshold/AeLT, zone 2 = between ventilatory threshold/AeLT and respiratory compensation point/AnLT, zone 3 = above respiratory compensation point/AnLT) with associated weighting coefficient (k) for each ($k=1$ for zone 1, $k=2$ for zone 2, $k=3$ for zone 3) a TRIMP score could be produced (Impellizzeri *et al.*, 2004; Lucía *et al.*, 2003). The authors used this method in calculating TL of road cyclists whilst it has also been used to calculate ITL in AB triathlon (Mujika, 2014), soccer (Impellizzeri *et al.*, 2004) and endurance running (Esteve-Lanao *et al.*, 2005). In a study investigating the relationship between HR derived measures of ITL and rate of oxygen uptake

($\dot{V}O_2$), the criterion measure of ITL, Wallace *et al.* (2014) concluded that TRIMP methods were valid methods of quantifying physiological load with large correlations noted. Nonetheless, it is worth noting that TRIMP measures may be limited by generic weighting coefficients and the influence small changes in HR may have on TL calculated (i.e., 1 beat·min⁻¹ can be the difference between zone 2 and zone 3) (Borresen & Lambert, 2008; Wallace *et al.*, 2014)

In an attempt to simplify TL quantification, the use of subjective measures of exercise intensity have been employed. The use of RPE as a measure of TL is based on the assumption athletes can inherently monitor the physiological stress of exercise (Mujika, 2017). RPE can be used in the production of a session-RPE (sRPE) score, first proposed by Foster *et al.* (2001), in which an average RPE for a single training session, based on the category ratio 10 scale of Borg *et al.* (1985), is multiplied by training duration in minutes. sRPE has been shown to compare favourably with HR derived methods of ITL quantification for endurance sports (Foster *et al.*, 2001; Rodríguez-Marroyo *et al.*, 2012) with Wallace *et al.* (2014) finding a large relationship between sRPE and $\dot{V}O_2$. It has, therefore, been proposed as a valid and practical alternative to using HR (Borresen & Lambert, 2009; Wallace *et al.*, 2014).

As well as quantifying TL internally using measures such as HR and RPE, ETL quantification has been well researched. Typical measures used when quantifying ETL include distance covered, training duration and pace (Borges *et al.*, 2014; Borresen & Lambert, 2008). Additionally, cycling PO has been proposed as a method for quantifying ETL (Jobson *et al.*, 2009). Cejuela-Anta and Esteve-Lanao (2011) further present an ETL quantification method when computing TLs for AB triathletes by summing swim, bike and run training minutes multiplied by modality weighting factors of 0.75, 0.5 and 1, respectively. These factors were proposed based on previous research regarding the metabolic cost of triathlon modalities, although it is unclear how appropriate these factors may be across paratriathlon categories (Cejuela-Anta & Esteve-Lanao, 2011). The use of ETL is limited by the inability to present actual physiological strain imposed by training (Borges *et al.*, 2014; Impellizzeri *et al.*, 2004; Wallace *et al.*, 2014). It has been suggested that both ETL and ITL be used concurrently when monitoring athletes' training (Borges *et al.*, 2014; Paulson *et al.*, 2015; Wallace *et al.*, 2014).

Whilst methods of TL quantification have been assessed in many AB sports (Mujika, 2017) there has been little published research concerning TL in Paralympic sport. This is despite Paralympic athletes representing a population potentially at greater need of TL quantification and monitoring. For example, athletes with an SCI are particularly prone to

overuse injuries and illnesses (Derman *et al.*, 2018a; Derman *et al.*, 2018b), with the latter resulting from coughing inefficiencies and/or immunoendocrine deficiencies (Brown *et al.*, 2006; Nash, 2000). PTWC athletes with an SCI, CP or an amputation are at heightened risk of shoulder overload due to the stresses of daily wheelchair use (Derman *et al.*, 2018a; Webborn & Van de Vliet, 2012). This is further aggravated by paratriathlon training modalities whereby the upper limbs are in use extensively. Moreover, ambulant athletes with CP or an amputation display significant movement inefficiencies, increasing the metabolic cost of locomotion (Blauwet *et al.*, 2017; Ward & Meyers, 1995), augmenting the susceptibility to excessive TLs. Athletes with VI, meanwhile, are restricted in their ability to utilise visual feedback (Taylor *et al.*, 2016) and may thus have issues tolerating TLs (Chapter 2.1.4). However, quantifying TL in groups of athletes with physical impairments raises certain issues that must be considered. This includes potentially limited sympathetic responses to exercise in athletes with an SCI, limiting the use of HR as a training tool (Paulson *et al.*, 2015). Furthermore, there may be limits to the use of sRPE in some populations due to altered perceptions of effort, such as athletes with CP (Runciman *et al.*, 2016).

Whilst Fulton *et al.* (2010) and Edmonds *et al.* (2015) described the training characteristics of Paralympic swimmers, the study of Paulson *et al.* (2015) was the first to assess methods of TL quantification in disability sport. Paulson *et al.* (2015) established the relationships between measures of ITL (Banister's TRIMP, Lucia's TRIMP, sRPE) and ETL in a group of wheelchair rugby athletes. It was found that both HR and sRPE measures were a valid tool in TL quantification by displaying large correlations to ETL. However, Iturricastillo *et al.* (2016), whilst computing match load rather than TL, observed only moderate association between objective, HR-derived methods and subjective measures using sRPE in a group of wheelchair basketballers. Further, the authors noted that associations between measures were highly variable amongst athletes with mixed disabilities (Iturricastillo *et al.*, 2016). However, it is worth noting the disparate athlete populations from the aforementioned studies. Paulson *et al.* (2015) mostly investigated athletes with tetraplegia whilst Iturricastillo *et al.* (2016) studied athletes with either paraplegia or no SCI. More recently, de Groot *et al.* (2018) aimed to establish relationships between ITL and ETL in handcyclists with paraplegia. Utilising 12 weeks of training data, the authors noted large correlations between the ETL of Training Stress ScoreTM and the ITL of Banister's TRIMP or sRPE. Therefore, these methods were both advocated for handcyclists with paraplegia. From a paratriathlon perspective, although Mujika *et al.* (2015) attempted to describe the training habits of a single paratriathlete using a HR

derived TRIMP (Lucía *et al.*, 2003), the athlete in question was competing in long duration events, not the common sprint distance. As such, research should build on that of Mujika *et al.* (2015) to study the conventional sprint distance events.

2.3 Training load and mucosal immunity

It is well accepted that athletes' TLs must be of a sufficient level to stimulate a positive training adaptation (Borresen & Lambert, 2009; Mujika, 2017). Nonetheless, it has also been acknowledged that high TL increases the likelihood of illness or injury in AB athletes (Foster, 1998). Specifically, the most common medical complaint is URI (illnesses of the upper respiratory tract not limited to infections) (Derman *et al.*, 2018b), with evidence of high training stress suppressing the ability of the innate immune system to combat pathogens and increasing URI incidence (Cunniffe *et al.*, 2011; Fahlman & Engels, 2005; Foster, 1998; Gleeson *et al.*, 1999b; Gleeson *et al.*, 2012; Gleeson *et al.*, 2013; Hellard *et al.*, 2015). Although research is lacking with regard to Paralympic athletes, there is some evidence of a similar relationship in wheelchair rugby players with tetraplegia (Leicht *et al.*, 2012). Contraction of URI in AB athletes is likely to have a direct effect on impairing performance (Van Tonder *et al.*, 2016) or limit training availability (Cunniffe *et al.*, 2011), with this effect presumably similar in Paralympic athletes. Thus, URIs are an unwanted outcome of insufficiently managed TLs making any information of the relationship between TL and URI valuable for coaches and practitioners.

Though the interaction between training-imposed physiological stress and immune function is multi-faceted (Neville *et al.*, 2008; Pyne & Gleeson, 1998; Walsh *et al.*, 2011), one area which has received a great deal of attention is the suppression of mucosal immunity. Over 90% of infections involve the mucosa (Brandtzaeg, 2003) and a key antibody in host defence of the upper respiratory tract, and the first line of defence in mucosal immunity, is salivary secretory immunoglobulin A (sIgA). sIgA has several roles in host defence (Walsh *et al.*, 2011). Firstly, sIgA acts to prevent pathogen adherence and penetration to the mucosal epithelium. Furthermore, the antibody neutralises viruses within epithelial cells during transcytosis and aids the secretion of locally formed immune complexes across mucosal epithelial cells (Walsh *et al.*, 2011). The role of sIgA in defence against URI has been well researched with studies cross-sectionally documenting an inverse relationship between sIgA concentration and URI risk, highlighting its importance in proper immune function (Mackinnon *et al.*, 1987; Nieman *et al.*, 2002). More recently, longitudinal studies of sIgA and illness incidence in competitive athletes have been investigated. From research tracking AB

athletes over prolonged periods, there is evidence that lower sIgA levels relates to URI incidence. This has been shown to be true over a range of sports such as American football (Fahlman & Engels, 2005), swimming (Gleeson *et al.*, 1999b), cycling (Gleeson *et al.*, 2017), soccer (Moreira *et al.*, 2014; Mortatti *et al.*, 2012), sailing (Neville *et al.*, 2008) and ice hockey (Orysiak *et al.*, 2017).

In athletes, the proposed mechanism through which depressions in sIgA and resultant URI incidence occur is through chronically high TLs with insufficient recovery (Pyne & Gleeson, 1998). Specifically, it has been speculated that prolonged sympathetic nervous system activation and elevated cortisol, via regular intense exercise, may downregulate sIgA synthesis. Furthermore, sIgA secretion may be attenuated through the inhibitory effects of sympathetic nervous system activation and cortisol on the expression of polymeric immunoglobulin receptor, which aids transcytosis through the epithelium (Walsh *et al.*, 2011). Support for the notion of high TL negatively effecting sIgA levels has come from the findings of several authors (Fahlman & Engels, 2005; Moreira *et al.*, 2014; Neville *et al.*, 2008). As such, it is proposed that high TL suppress mucosal immunity, i.e. sIgA, which, in turn, heightens the risk of URI.

Although authors have presented a negative relationship between TL and sIgA (Fahlman & Engels, 2005; Moreira *et al.*, 2014; Neville *et al.*, 2008), this has not been unequivocally accepted by all, with some reports showing no correlation (Edmonds *et al.*, 2015; Gleeson *et al.*, 1999b; Gleeson *et al.*, 2012). Additionally, with the multi-faceted nature of immunity, not all studies have shown relationships between sIgA and URI incidence (Cunniffe *et al.*, 2011). However, these discrepancies may be partly explained by differences in the representation of sIgA (e.g., concentration or secretion rate [SR]) (Bermon *et al.*, 2017) or training data. Training has been characterised using methods such as the ETL of distance covered (Gleeson *et al.*, 1999b; Edmonds *et al.*, 2015) or varying representations of ITL from sRPE (Cunniffe *et al.*, 2011), metabolic equivalents estimated via questionnaire (Gleeson *et al.*, 2012) or arbitrary assessment of training difficulty (Orysiak *et al.*, 2017), which are likely to all have differing degrees of association to mucosal immunity. It is noteworthy that there is a paucity of studies utilising objective measures of ITL, such as HR, with many studies relying upon athlete, coach or researcher subjective evaluation of TL. Greater use of ITL, in conjunction with external means, has recently been recommended by Kellmann *et al.* (2018) in their consensus statement on recovery and performance in sport. The use of ITL will provide greater insight into the biological stress of TL. Furthermore, the high inter- and intra-individual variation commonly

shown in sIgA (Cunniffe *et al.*, 2011; Gleeson *et al.*, 1999b) may cloud some relationships, which may be further exacerbated by low sample sizes and inadequate sampling frequency (Bermon *et al.*, 2017; Neville *et al.*, 2008).

Despite the great deal of attention interactions between TL and mucosal immunity have received in the AB population, there is a scarcity of research in Paralympic athletes. This is despite athletes potentially being at heightened risk of URI due to the propensity for excessive overload, and therefore TLs, caused by movement inefficiencies (Chapter 2.2). Furthermore, Paralympic athlete populations may display reduced coughing efficiency (Brown *et al.*, 2006) which may be further exacerbated by immunoendocrine deficiencies (Nash, 2000). Leicht *et al.* (2012) were the first to present a negative correlation between TL and sIgA SR in physically impaired athletes. The authors studied a group of wheelchair rugby players with an SCI over a period of five months and noted the effects of TL on mucosal immunity were not different to those from the AB literature (Leicht *et al.*, 2012). Conversely, Edmonds *et al.* (2015) found no changes in sIgA concentration over 14 weeks of periodised training in a group of swimmers of mixed disabilities. The discrepancies in results between the two studies may be possibly explained by different analytic methods, athlete populations and method of TL quantification (Bermon *et al.*, 2017).

Although there has been no published literature concerning quantified TLs in paratriathlon, the case study of Mujika *et al.* (2015) provides a starting point from which to safely assume paratriathletes may be undergoing large TLs. As well as high training volumes (Mujika *et al.*, 2015), paratriathletes are likely to be training multiple times per day, which further increases any risk of illness (Hellard *et al.*, 2015). Hence, it may be possible that paratriathletes are at risk of mucosal immune function suppression and subsequent URI as a consequence of physical impairments and high TLs. Research should seek to elucidate the effects of TL on mucosal immunity in paratriathlon whilst shedding light on the equivocal findings in Paralympic sport (Edmonds *et al.*, 2015; Leicht *et al.*, 2012). Knowledge in this area will allow those working within paratriathlon to gain insight into how a mixed disability group responds to TL for future management of immunosuppression and URI risk.

2.4 Intensified training load

As stated in Chapter 2.3, athletes' TL must be of a sufficient level to stimulate training adaptations (Borresen & Lambert, 2009; Mujika, 2017). However, it is commonplace for coaches and athletes to plan intentional, acute, overload of training in an attempt to maximise

beneficial outcomes. Typically, periods of IT involve progression of session duration, intensity of frequency (Hough *et al.*, 2013), thus elevating TL above normal levels and are often undertaken during training camps. During periods of IT, it is possible for athletes to become overreached, whether it be functional or non-functional. In fact, some coaches view OR as a deliberate part of the training process due to the potential for improved performance after a taper period (Coutts *et al.*, 2007a). Functional OR is defined as an accumulation of training and/or non-training stress resulting in short-term decrement in performance capacity with or without related physiological and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take from several days to several weeks (Meeusen *et al.*, 2013). If IT were to continue for athletes displaying functional OR, the development of non-functional OR or even overtraining syndrome is possible whereby any recovery in performance may take several weeks, months or years (Meeusen *et al.*, 2013) (Figure 2.6). It is well accepted that to prevent the onset of non-functional OR an adequate training-recovery balance be established (Bresciani *et al.*, 2011; Coutts *et al.*, 2007a; Halson *et al.*, 2003; Killer *et al.*, 2017; Rietjens *et al.*, 2005). As such, it is important for coaches and practitioners to be able to monitor athletes for any signs of potential OR prior to significant performance decrement.

Process	Training (overload)	Intensified training →		
	Acute fatigue	Functional overreaching	Non-functional overreaching	Overtraining syndrome
Recovery	Day(s)	Days - weeks	Weeks - months	Months - years
Performance	Increased	Temporary decrease (e.g. training camp)	Stagnation or decrease	Decrease

Figure 2.6: Presentation of the differing stages of overload, intensified training and overreaching. Adapted from Meeusen *et al.* (2013).

As the definitions of Meeusen *et al.* (2013) allude to, the most accurate way of assessing OR in athletes is through testing performance, preferably at a maximal, sport-specific level (Meeusen *et al.*, 2013). However, the use of maximal performance tests brings a certain level of disruption to athletes' training routine either by invoking further fatigue or through excessive

rest and subsequent detraining (Le Meur *et al.*, 2013). As such, efforts have been made to identify markers of potential OR, after IT, that are less taxing to athletes (Coutts *et al.*, 2007b).

To date, several hormonal, immunological, physiological and psychological parameters have been studied in relation to IT. Two of the most commonly assessed variables are the responses of resting cortisol and testosterone. Cortisol, a major catabolic hormone, is secreted from the adrenal cortex, via the hypothalamic–pituitary–adrenal axis and increases in response to stressors (Papacosta & Nassis, 2011). Testosterone, meanwhile, is the primary steroid hormone within the androgen family and its secretion is regulated by the hypothalamic–pituitary–gonadal axis (Papacosta & Nassis, 2011). Due to the steroid structure of both hormones, they freely diffuse into saliva which can offer a less invasive medium for sampling than plasma (Papacosta & Nassis, 2011). Salivary cortisol (sC) and testosterone (sT) represent the biologically active portion of the body’s hormones with significant correlations to, and higher sensitivity than, plasma concentration. The use of cortisol and testosterone together can represent the body’s catabolic:anabolic balance (Hough *et al.*, 2013). It had been proposed that after periods of IT there is an increase in the biologically active, free cortisol with a concomitant decrease in free testosterone; thus, representing a more catabolic state as the body fails to maintain homeostasis with a challenging stress-recovery balance (Lehmann *et al.*, 1993). However, several studies assessing both plasma and sC and sT responses to IT have displayed mixed results. This includes reports of decreases in cortisol (Hedelin *et al.*, 2000) or testosterone (Bresciani *et al.*, 2011; Hough *et al.*, 2015), increases in cortisol (Papacosta *et al.*, 2013; Svendsen *et al.*, 2016), or no meaningful change (Coutts *et al.*, 2007b; Hough *et al.*, 2013; Jürimäe *et al.*, 2004; Rietjens *et al.*, 2005; Robson-Ansley *et al.*, 2006; Slivka *et al.*, 2010; Steinacker *et al.*, 2000). It is worth noting, however, that there has been great variation in: training protocols; training status of participants; measurement methods and control for diurnal variations of hormones in the aforementioned studies (Hough *et al.*, 2013). Whilst sC and sT have commonly been assessed in AB athletes with respect to IT, this has not been explored in Paralympic endurance athletes. This is despite recent evidence stating there is no significant difference in the responses of the aforementioned parameters to acute exercise (Leicht *et al.*, 2017) and, as explained in Chapter 2.2, athletes being at greater risk of OR as a consequence of their physical impairments.

Immunosuppression is another aspect of athletes’ responses to IT that has received attention. It has been well documented that individuals are more susceptible to illness after periods of IT (Walsh *et al.*, 2011) and when overreached (Hauswirth *et al.*, 2014).

Accordingly, several authors have sought to utilise athletes' leukocyte profile as a potential tool in the diagnosis of OR (Bresciani *et al.*, 2011; Coutts *et al.*, 2007b; Halson *et al.*, 2003; Rietjens *et al.*, 2005; Robson-Ansley *et al.*, 2006; Svendsen *et al.*, 2016). However, as with hormonal parameters, results have been inconclusive. This led to Coutts *et al.* (2007b) proposing that sIgA may be a more sensitive marker in response to IT. As described in Chapter 2.3, sIgA production and secretion is impaired during high training stress through the effects of circulating cortisol and catecholamines, thus placing the body at risk of URI (Walsh *et al.*, 2011). Several authors have found depressions in IgA during periods of high TLs in AB (Fahlman & Engels, 2005; Moreira *et al.*, 2014; Neville *et al.*, 2008) and Paralympic athletes (Leicht *et al.*, 2012). Despite this, in studies whereby athletes become overreached, there has yet to be support for the use of sIgA as an early marker of OR (Halson *et al.*, 2003; Robson-Ansley *et al.*, 2006; Slivka *et al.*, 2010). However, there may be some effect of high inter- and intra-individual variation commonly reported in sIgA (Cunniffe *et al.*, 2011; Gleeson *et al.*, 1999b) or differing representation of results (SR vs. concentration). Furthermore, as with sC and sT, variation in sIgA measures has been shown to be similar between AB and Paralympic athletes (Leicht *et al.*, 2012) yet evidence is lacking to ascertain the responses to IT.

Several physiological factors have also been proposed as potential markers of OR in athletes undergoing IT. These include resting (RHR), submaximal and maximal exercising HR (Meeusen *et al.*, 2013). The proposal for using HR comes from the negative effect training stress has on the autonomic nervous system which may result in a concomitant alteration in HR (Bosquet *et al.*, 2008). It has been suggested that IT results in an elevation of RHR but a lowering of submaximal and maximal HR during exercise (Bosquet *et al.*, 2008). However, the suitability of testing HR during exercise for detection of OR has been questioned with this typically recorded during incremental tests to exhaustion (Hedelin *et al.*, 2000; Killer *et al.*, 2017; Le Meur *et al.*, 2013). The use of maximal tests in possibly overreached athletes has already been questioned due to further disruption to training. Furthermore, in their meta-analysis of the appropriateness of HR to detect OR, Bosquet *et al.* (2008) state that findings are still equivocal concerning RHR.

Sleeping habits have recently been assessed in relation to IT and OR. Sleep is a vital part of recovery where many biological regenerative processes occur, additionally there are close links between sleep, immune function and mood state (Hauswirth *et al.*, 2014; Killer *et al.*, 2017). A recent review concluded that sleep quality is typically impaired during training camps (Gupta *et al.*, 2017) with Hauswirth *et al.* (2014) evidencing this by presenting greater wake

times and movement, as measured by wrist actigraphy, during a period of IT. However, it has been shown that sleep quantity may increase during IT as greater TL augments the requirement for sleep in professional rugby league players (Thornton *et al.*, 2018). As such, sleep responses to IT may be specific to the parameter measured. Interestingly, whilst sleep quality was objectively impaired in the study of Hausswirth *et al.* (2014), subjective sleep quality was not altered. Recently, Caia *et al.* (2018) state that although athletes may be able to accurately perceive sleep duration, subjective sleep quality is a poorer indicator of sleep efficiency in comparison to actigraphy. It is worth noting that the responses of any sleep parameters to IT has yet to be studied in athletes with a physical impairment. This is despite the review of Gupta *et al.* (2017) stating that levels of insomnia symptoms can be up to 70% greater in Paralympic athletes which may relate to thermoregulatory impairments or altered melatonin secretion in athletes with an SCI (Scheer *et al.*, 2006). For example, Silva *et al.* (2012), in their study of 27 Paralympic track and field athletes, reported 83.3% of athletes presented excessive daytime sleepiness and had poor sleep quality, albeit from subjective assessments.

Whilst the evidence for the use of cortisol, testosterone, sIgA, HR and sleep are yet to show uniformity, one area in the responses to IT that has received a great deal of support is psychological measures. Typically, psychological measures are in the form of self-report questionnaires of mood state, such as the Profile of Mood States (POMS), or subjective stress and recovery such as the Recovery-Stress Questionnaire for Sport (RESTQ-S). POMS is a 65-item questionnaire that provides a method of assessing transient, fluctuating mood states. It includes five negative affect scales: fatigue, depression, tension, anger, confusion and a positive affect scale, vigour. Participants are given a score for each of the six mood states. A total mood disturbance score is calculated by summation of the negative scales and subtraction of the positive (Bresciani *et al.*, 2011). RESTQ-S is a questionnaire consisting of 76 items that indicate how often the respondent participated in various activities during the past three days and nights. The measure includes twelve scales which assess various stressing agents of a general nature and general recovery activities and seven additional sports-specific scales. The scores of the stress-related scales are summated to obtain a total stress score. The same procedure is used for the recovery-oriented scales, resulting in a total recovery score. Scores can also be obtained for general stress, sport-specific stress, general recovery and sport-specific recovery (Bresciani *et al.*, 2011). Several authors have shown support for the use of psychological questionnaires and subjective wellness measures to detect OR after IT (Bresciani *et al.*, 2011; Buchheit *et al.*, 2013; Coutts *et al.*, 2007a; Coutts *et al.*, 2007b; Halson *et al.*,

2003; Hough *et al.*, 2013; Jürimäe *et al.*, 2004; Killer *et al.*, 2017; Le Meur *et al.*, 2013; Papacosta *et al.*, 2013; Robson-Ansley *et al.*, 2006) with suggestions that psychological changes are the first manifestation of OR (Bresciani *et al.*, 2011).

Although the effects of IT and markers of OR have been studied in many sports such as Australian Rules Football (Buchheit *et al.*, 2013), cycling (Halsen *et al.*, 2003; Killer *et al.*, 2017; Rietjens *et al.*, 2005; Slivka *et al.*, 2010), canoeing (Hedelin *et al.*, 2000), rowing (Jürimäe *et al.*, 2004; Steinacker *et al.*, 2000) and judo (Papacosta *et al.*, 2013), the sport of triathlon has enabled researchers to deliberately overreach athletes (Coutts *et al.*, 2007a; Coutts *et al.*, 2007b; Hausswirth *et al.*, 2014; Hough *et al.*, 2015; Le Meur *et al.*, 2013; Robson-Ansley *et al.*, 2006). This is partly due to triathletes' habitually high TLs and, therefore, 'at risk' status of OR (Le Meur *et al.*, 2013). Despite the focus IT has received in triathlon, there is no evidence in paratriathlon; this is in spite of reports that paratriathletes may be undertaking large TLs with potential IT periods (Mujika *et al.*, 2015). Furthermore, there has been no published literature concerning IT and markers of OR in any Paralympic sport despite the knowledge of athletes' training habits (de Groot *et al.*, 2018; Fulton *et al.*, 2010; Mujika *et al.*, 2015; Paulson *et al.*, 2015; Zeller *et al.*, 2017). As Zeller *et al.* (2017) state, to avoid the risk of overtraining, it is important that scientific knowledge in the area of disability sport be developed. As such, it is pertinent to assess the hormonal, immunological, psychological and physiological responses to IT in paratriathletes. This is due to physical disabilities placing Paralympic athletes at greater risk of excessive overload (Chapter 2.2), thus OR, and the potential for performance impairment. Therefore, coaches and practitioners working in Paralympic sport require knowledge on athletes' responses to IT to maximise training adaptations without negative consequences.

2.5 Thermoregulation during performance in the heat

Whilst coaches and practitioners working within high performance endurance sports aim to manage athletes' TL, to minimise the likelihood of training unavailability through URI or OR, the overarching goal is for athletes to perform to their maximum potential in competitive situations. However, it is common for competitive events (e.g., 2016 Rio de Janeiro Olympic and Paralympic Games, 2020 Tokyo Olympic and Paralympic Games, Paratriathlon World Cups) to be held in environments that challenge athletes' endurance exercise performance due to high ambient temperatures and/or humidity (Table 2.2).

Table 2.2: Historic weather data at selected paratriathlon races (Weather Underground, 2018).

Date	Event	Location	Temperature (°C)	Relative humidity (%)
10 th July 2015	ETU European Championships	Geneva, Switzerland	29	28
18 th July 2015	ITU World Paratriathlon Event	Iseo, Italy	35	31
1 st August 2015	ITU World Paratriathlon Event	Rio de Janeiro, Brazil	31	52
11 th September 2016	Paralympic Games	Rio de Janeiro, Brazil	28	66
8 th October 2017	ITU World Paratriathlon Cup	Sarasota, Florida, USA	30	80

It is well understood that endurance exercise performance and capacity are impaired in hot and humid environmental conditions (Galloway & Maughan, 1997). Specifically, even in 21°C, cycling time to exhaustion at 70% $\dot{V}O_{2peak}$ is shorter relative to 11°C (Galloway & Maughan, 1997), with several studies also reporting a diminished endurance performance with added thermal load (Ely *et al.*, 2009; Friesen *et al.*, 2018; Levels *et al.*, 2014; Périard & Racinais, 2016). Endurance events, such as triathlon or paratriathlon, require high relative intensities to be sustained for extended periods of time (Table 1.2). This increases the likelihood of athletes developing: substantial hypohydration; cardiovascular strain; elevated whole-body (core and skin) temperature; reliance upon carbohydrate metabolism; perception of effort and thermal strain, resulting in fatigue, under-performance or even heat illness (Daanen *et al.*, 2018; Guy *et al.*, 2014; Périard *et al.*, 2015; Périard & Racinais, 2016).

Specifically, prolonged exercise in the heat results in an elevated sweat rate to increase evaporative heat losses, and cutaneous vasodilation for heat dissipation via non-evaporative pathways (radiation, convection, conduction) (Racinais *et al.*, 2015a; Taylor & Cotter, 2006). Such processes result in augmented fluid losses and peripheral blood flow which reduces central blood volume (Périard & Racinais, 2016). This leads to elevated cardiovascular strain during exercise, specifically a decreased stroke volume as a result of increased HR and reduced

cardiac filling time (Chou *et al.*, 2018), and a consequential progressive decrease in $\dot{V}O_{2\text{peak}}$ (Périard & Racinais, 2016). Furthermore, increased skin blood flow requirements challenge the cardiovascular system to maintain blood pressure despite the metabolic demands of active musculature for oxygen and nutrient delivery (Corbett *et al.*, 2014). As the body is challenged in its ability to dissipate heat, and heat gain from exercise in hot environments exceeds heat loss, elevations in core (T_c) and skin (T_{sk}) temperature occur.

Raised T_c and T_{sk} , with a narrowing of the temperature gradient between the two parameters, has been attributed to well documented performance decrements in the heat. This is through alterations of the central and peripheral nervous systems via decreased neural drive to maintain an external workload (Racinais *et al.*, 2017). Additionally, raised body temperatures are related to the development of heat illness during exercise in the heat (Laursen *et al.*, 2006). Thus, due to the influence of body temperature on health and performance, there has been great interest in determining the thermoregulatory strain imposed by endurance exercise in the heat. Whilst laboratory research has well defined the responses of AB athletes' thermoregulatory systems to exercise in challenging environments (Galloway & Maughan, 1997), it has been acknowledged that these findings lack applicability to many athletes due to significant disparities to field-based performance. This includes greater airflow, and therefore heat dissipation in sports such as cycling (Laursen *et al.*, 2006), or fluid availability and ease of consumption when running (Lee *et al.*, 2010). Furthermore, a great deal of research into thermoregulatory responses to exercise has been performed in military populations which results in significantly different exercise demands to endurance athletes such as continuous low-intensity exercise superimposed by bouts of heavy lifting (Veltmeijer *et al.*, 2015). Subsequently, researchers have attempted to typify the thermoregulatory strain imposed in real world athletic events. This is in accordance with the recommendations of Bergeron *et al.* (2012) in their International Olympic Committee consensus statement whereby the authors challenge researchers to characterise the sport- and event-specific thermal strain profiles of international level athletes competing in the heat using ingestible temperature sensors.

Due to event durations, and the relative intensity of effort, several studies have presented the thermoregulatory responses to triathlon races of varying lengths, including half-Ironman (1.9 km swim, 90 km cycle, 21.1 km run) and Ironman (3.8 km swim, 180 km cycle, 42.2 km run) events, as well as triathlon's constituent sport modalities. However, there is no evidence of the thermoregulatory strain imposed by competition in paratriathlon. Research has shown that AB triathletes' T_c can reach $38.4 \pm 0.7^\circ\text{C}$ (Baillot & Hue, 2015) or $38.8 \pm 0.7^\circ\text{C}$

(Del Coso *et al.*, 2014) at the end of half-Ironman races in the heat (27-29°C). Furthermore, by acquiring in-race T_c readings, Baillot and Hue (2015) presented a trend in T_c changes during racing. The authors showed that T_c : rose during swimming as a consequence of a limited capacity for heat dissipation in warm water; plateaued on the bike due to significant convective cooling through a high cycling velocity and drafting legality increasing air flow; rose again when running as a result of limited convective cooling and a high metabolic rate (Baillot & Hue, 2015). Moreover, a similar trend has been presented in laboratory studies of sprint (Peeling & Landers, 2007) and Olympic distance events (Kerr *et al.*, 1998). Whilst these studies built on research confined to post-race T_c measurement, data is still restricted to infrequent T_c sampling during field-based research. Moreover, there is no field-based research detailing the thermoregulatory strain of sprint distance triathlon events.

Research from AB athletes has attempted to determine risk factors and correlates to in-race T_c changes. The majority of data suggest T_c changes are unrelated to: fluid losses estimated from body mass changes (Baillot & Hue, 2015; Byrne *et al.*, 2006; Laursen *et al.*, 2006; Lee *et al.*, 2010; Sharwood *et al.*, 2004; Veltmeijer *et al.*, 2015); event finishing time (Baillot & Hue, 2015; Byrne *et al.*, 2006; Laursen *et al.*, 2006; Veltmeijer *et al.*, 2015); or, anthropometric characteristics (Baillot & Hue, 2015; Byrne *et al.*, 2006; Veltmeijer *et al.*, 2015). However, as no study has researched Paralympic athletes, it is not possible to discern any relationship between physical impairment and thermal strain imposed, despite Paralympic athletes being at elevated risk of heat-related performance impairments.

Athletes with an SCI display well acknowledged impairments in skin blood flow and sweat responses below their lesion level (Freund *et al.*, 1984; Griggs *et al.*, 2014), reduced venous return (Hopman *et al.*, 1992), whilst the high body fat percentages mentioned in Chapter 2.1.1 will further increase the heat storage for a given workload (Kenney, 1985). Consequently, the finite literature regarding thermoregulation in Paralympic athletes has focused on those with an SCI. In their case study of a male handcyclist with an SCI, Abel *et al.* (2006) presented a peak rectal temperature of 40.4°C at the end of a 42 km race (1:48:54 h) in temperate (20.0 to 22.0°C) conditions. Moreover, the athlete's T_c increased continuously throughout exercise, highlighting the level of thermoregulatory disruption. In a larger scale study, Griggs *et al.* (2017) revealed peak T_c values of $39.3 \pm 0.5^\circ\text{C}$ for wheelchair rugby players with an SCI and $38.8 \pm 0.3^\circ\text{C}$ for non-SCI players at the end of a competitive wheelchair rugby match in 18.4 to 20.9°C and 31.1 to 45.1% RH. Additionally, Griggs *et al.* (2017) stated that peak T_c and rate of T_c change were significantly greater in athletes with an SCI in comparison to non-SCI,

indicating impairment-specific responses. Similarly, Veltmeijer *et al.* (2014) found greater increases in T_c in wheelchair tennis players with an SCI ($0.6 \pm 0.1^\circ\text{C}$; $n=2$) than players without an SCI ($0.3 \pm 0.1^\circ\text{C}$; $n=4$) during simulated match play in 21.2 to 24.8°C and 51.8 to 61.4% RH. However, research is even more limited in other impairment groups, despite athletes also being at risk of thermal strain.

Due to the greater metabolic heat production of locomotion in athletes with CP (Maltais *et al.*, 2004), they are at elevated risk of thermal strain and performance impairment than AB athletes (Leprêtre *et al.*, 2016). Furthermore, this will be exacerbated by their high muscular tone impairing venous return (Kloyiam *et al.*, 2011), increasing the cardiovascular strain and relative intensity of exercise (James *et al.*, 2018). Moreover, impaired pace awareness in athletes with CP (Runciman *et al.*, 2016) may increase their risk of thermoregulatory strain. When competing in the heat, AB athletes typically progressively down-regulate their intensity of effort to redistribute work in a manner that allows them to complete the required task in the context of the accumulating heat strain (Tucker *et al.*, 2006). This not only relates to physiological adjustments but also behavioural alterations to account for the cognitive interpretation of the environment, thermal state, or perceived effort (Flouris & Schlader, 2015; Schmit *et al.*, 2016). If athletes with CP are unable to effectively process the aforementioned factors, they may increase the risk of heat illness and/or performance impairment as a consequence of maintaining an inappropriate workload for the environmental context.

As a consequence of missing limbs, athletes with an amputation display limited body surface area for evaporative and convective heat loss, increasing heat storage in this population (Epstein *et al.*, 1983; Webborn, 1996). This also leads to a closer coupling of T_c and T_{sk} which is further exacerbated by socket liners and prostheses limiting heat dissipation (Klute *et al.*, 2007). Moreover, skin grafts remove a portion of the skin capable of sweating and cutaneous vasodilation (Crandall & Davis, 2010), augmenting the impairment in thermoregulatory capacity. Consequently, athletes with an amputation may experience considerable thermal discomfort whilst exercising in the heat due to elevated T_{sk} . Also, similar to athletes with CP, those with an amputation display significant gait asymmetries (Ward & Meyers, 1995), elevating metabolic heat production for a given workload. The thermoregulatory challenge posed by individuals with an amputation was described by the case study of Andrews *et al.* (2011). The authors showed that an individual with a lower limb amputation completed a 16 km run in 12.0°C slower, yet with a greater T_c (38.4°C), than a non-amputee (37.9°C). This was

proposed to be due to the aforementioned effects of amputation on movement inefficiencies, metabolic cost of locomotion and impaired heat loss.

As athletes with a VI are limited in their capability to use visual feedback, this can directly impact on pacing (Taylor *et al.*, 2016). As previously mentioned for athletes with CP, an inability to self-pace and downregulate the intensity of effort when competing in the heat will increase the thermoregulatory toll and, thus, negatively impact performance in athletes with VI. Furthermore, difficulties in self-monitoring hydration through urine colour or volume may cause issues when training or competing in the heat (Webborn & Van de Vliet, 2012). Ensuring proper hydration is a well acknowledged method of alleviating heat stress when competing in thermoregulatory challenging environments (Périard *et al.*, 2015). Therefore, if athletes with a VI cannot self-regulate their hydration based on visual feedback, they increase their risk of thermoregulatory strain in hot environments. Finally, athletes with albinism are prone to sunburn in situations of high radiant load (Bothwell, 1997). This exacerbates thermal sensation (TS) and limits thermoregulation during exercise through a locally mediated effect on sweat gland responsiveness and capacity (Bergeron *et al.*, 2012; Pandolf *et al.*, 1992).

Consequently, whilst limited literature exists regarding thermoregulation in Paralympic sports, this has been constrained to specific, wheelchair sports and impairments (e.g. SCI). As such, due to the greatly disparate event demands and athlete impairment characteristics, comparisons to multi-impairment sports such as paratriathlon are limited. Thus, it is pertinent to better describe the thermoregulatory strain profiles of Paralympic endurance sports, i.e. paratriathlon, using ingestible temperature pills, in line with the recommendations of Bergeron *et al.* (2012).

2.6 Heat acclimation

As explained in Chapter 2.5, endurance exercise performance is impaired in environments that significantly stress athletes' thermoregulatory systems, yet, competitive events are regularly held in such conditions. Thankfully, performance impairments can be ameliorated through chronic heat exposures and processes of HA or heat acclimatisation (Daanen *et al.*, 2018). HA refers to repeated exposure to artificial environments, such as the use of heat chambers or hot water immersion, whereas heat acclimatisation concerns exposure to natural environments including warm-weather training camps. Whilst both forms of chronic heat exposure invoke similar adaptations, this thesis will primarily focus on HA due to the logistical constraints, such

as travel and variable weather conditions, faced by athletes and support staff utilising heat acclimatisation.

Heat acclimation has been extensively researched due to its application to military, occupational and athletic settings (Gibson *et al.*, 2015) and has been described as the most important intervention one can adopt to reduce physiological strain and optimise performance in the heat (Racinais *et al.*, 2015a). It is well understood that HA results in beneficial thermoregulatory, cardiovascular and perceptual adaptations that lead to attenuated impairments when performing in hot and humid conditions (Daanen *et al.*, 2018). Specifically, HA has been shown to: reduce T_c and T_{sk} ; improve cardiovascular stability; increase whole-body sweat rate and lower the threshold for sweating onset; decrease sweat electrolyte content; increase skin blood flow; induce plasma volume (PV) expansion; decrease muscle glycogen use and blood lactate concentration (BLa) (Daanen *et al.*, 2018; Guy *et al.*, 2014; Périard *et al.*, 2015; Tyler *et al.*, 2016).

To induce beneficial adaptations, HA typically consists of 5 to 16 d of heat exposure, with individual exposures of 1 to 2 h in temperatures $\geq 35.0^\circ\text{C}$ (Daanen *et al.*, 2018; Stanley *et al.*, 2015; Zurawlew *et al.*, 2015). It has been proposed that heat exposures must be of sufficient duration and thermal strain to increase T_c , T_{sk} and sweat rate, which appear to be the main drivers for adaptation in AB athletes, above a set threshold (Casadio *et al.*, 2017; Taylor *et al.*, 2014; Tyler *et al.*, 2016; Zurawlew *et al.*, 2015). It has commonly been reported that a significant proportion of HA adaptations occur within the first week of chronic heat exposures (Garrett *et al.*, 2011). Hence, short-term HA (<8 d) have been regularly researched as it may be more applicable to elite athletes as is potentially less disruptive to training regimes (Garrett *et al.*, 2012). Nonetheless, there is evidence that moderate-term HA (8-14 d) is required for full adaptation of several parameters such as sweat rate and end-exercise T_c and HR, which may be particularly meaningful for the preparation of athletes competing in endurance events such as triathlon or paratriathlon (Daanen *et al.*, 2018; Guy *et al.*, 2014; Tyler *et al.*, 2016). As such, although moderate-term HA induces greater thermoregulatory adaptations, strategies must be sought to minimise interference with athletes' training regimes to be efficacious.

Commonly, HA protocols have consisted of exercise at a fixed external workload over the study period (Febbraio *et al.*, 1994; Houmard *et al.*, 1990; Lorenzo *et al.*, 2010; Nielsen *et al.*, 1997; Schmit *et al.*, 2018; Wingfield *et al.*, 2016). However, it has been speculated that this approach results in diminishing adaptations during the intervention as the relative thermal

strain imposed gradually lessens (Garrett *et al.*, 2012; Taylor *et al.*, 2014; Tyler *et al.*, 2016). Consequently, isothermic approaches have been employed, based on the seminal work of Fox *et al.* (1963). During isothermic HA, participants maintain a set thermal strain, commonly a T_c of $\sim 38.5^\circ\text{C}$ (Garrett *et al.*, 2012; Gibson *et al.*, 2015; Neal *et al.*, 2016), over the study period whilst external workload gradually increases concurrent to thermoregulatory adaptation. This provides a series of sufficiently overloading thermal stimuli to invoke continued adaptations (Taylor *et al.*, 2014). It is worth noting, however, that isothermic HA presents challenges that limit its applicability to the elite athlete setting. Due to the requirement to continually measure T_c , there are issues with the financial cost of telemetric T_c pills, potential discomfort from rectal or oesophageal temperature instrumentation and inaccuracies of tympanic temperature (Taylor *et al.*, 2014). Moreover, rectal temperature assessment may result in autonomic dysreflexia in athletes with an SCI (Price & Campbell, 1999). Thus, alternative methods to regulate exercise intensity during HA have been proposed. One such method is by controlling the relative intensity and using HR to dictate the external workload performed (Périard *et al.*, 2015). Utilising a HR associated with a percentage of $\dot{V}O_{2\text{peak}}$, AnLT or maximum HR in temperate conditions would provide a constant cardiovascular stimulus as athletes acclimate (Périard *et al.*, 2015). Support for this proposed regimen comes from evidence of unchanged HR during isothermic HA (Garrett *et al.*, 2012; Magalhães *et al.*, 2010; Zurawlew *et al.*, 2015). Furthermore, in the team-sport domain, initial evidence suggests HR-controlled HA can be effective at stimulating thermoregulatory and performance improvements (Philp *et al.*, 2017). As such, the use of HR rather than T_c may provide greater real-world application to elite athletes looking to acclimate to the heat (Périard *et al.*, 2015; Tyler *et al.*, 2016).

Despite isothermic HA providing a constant thermal strain for adaptation, its applicability for athletes, especially pre-competition, has been questioned (Ruddock *et al.*, 2016; Zurawlew *et al.*, 2015). This is due to the impact excessive exercising heat stress may have on athletes' fatigue and ITL (Casadio *et al.*, 2017). This is particularly pertinent in the multi-modal sport of triathlon or paratriathlon whereby coaches and athletes must balance the distribution of swim, bike and run training. Therefore, the efficacy of passive HA has been studied. Passive HA denotes exposing the body to environmental heat stress without the additive exercise strain, examples of which include the use of hot water immersion (Ruddock *et al.*, 2016; Zurawlew *et al.*, 2015; Zurawlew *et al.*, 2018) or sauna exposure (Scoon *et al.*, 2007; Stanley *et al.*, 2015). These methods have been employed immediately after exercise in temperate environments, thus allowing individuals the opportunity to train without impairing

session intensity whilst also invoking HA. Furthermore, prior exercise results in elevated T_{c} , T_{sk} and sweat rate before the commencement of passive HA, thus reducing the required heat exposure duration. Passive HA has been shown to induce PV expansion (Scoon *et al.*, 2007; Stanley *et al.*, 2015; Zurawlew *et al.*, 2015) and decrease: resting T_{c} ; end-exercise T_{c} , T_{sk} and RPE; T_{c} at sweating onset (Zurawlew *et al.*, 2015; Zurawlew *et al.*, 2018) with some evidence of improved endurance performance (Scoon *et al.*, 2007; Zurawlew *et al.*, 2015). Nonetheless, the use of passive HA has also been questioned as it is unclear if adaptations are similar to that of active HA (Daanen *et al.*, 2018). Moreover, it seems logical that prior to competition in hot environments, athletes should be perceptually aware of the added strain of exercise and heat stress. This is particularly relevant in endurance sports whereby familiarisation with the heat, even without HA, induces pacing alterations to protect performance (Schmit *et al.*, 2016).

Acknowledging the respective merits of active and passive HA, the use of combined methods has been proposed as an approach to optimally acclimate in a time efficient manner (Guy *et al.*, 2014). Ruddock *et al.* (2016) utilised a mixed, active and passive HA protocol in their case study of a soccer referee prior to the 2014 FIFA World Cup in Brazil. The athlete performed a mix of isothermic, intermittent running and post-exercise hot water immersion over an 18-day period. This approach was sufficient to induce a 7.1% expansion of PV whilst increasing whole body sweat rate and decreasing resting and exercising tympanic temperature, exercising HR and $\dot{V}O_2$ with a concomitant improvement in repeated sprint runs. As such, from this preliminary evidence, it appears mixed active and passive HA may be capable of inducing beneficial thermoregulatory adaptations. Additionally, due to the structure of the protocol, the logistical and physical demands of the athlete were reduced but potentiating stimuli for adaptation were maintained (Ruddock *et al.*, 2016). This approach may be particularly appealing to elite athletes pre-competition, whereby best practise guidelines are rarely attainable within the confines of physical preparation (Casadio *et al.*, 2017).

The effectiveness of HA has been studied in a range of athletes including rowers (Garrett *et al.*, 2012), runners (Houmard *et al.*, 1990; James *et al.*, 2018; McCleave *et al.*, 2017; Zurawlew *et al.*, 2015), cyclists (Lorenzo *et al.*, 2010; Racinais *et al.*, 2015b; Stanley *et al.*, 2015), AB triathletes (Schmit *et al.*, 2018) and a soccer referee (Ruddock *et al.*, 2016). To date, however, only the study of Castle *et al.* (2013) has investigated Paralympic athletes. The small group of target shooters with tetraplegia ($n=2$) or paraplegia ($n=3$) undertook a seven-day consecutive HA intervention consisting of 20 min moderate intensity, isothermic arm crank ergometry and 40 min rest in 33.4°C and 64.8% RH. As HA resulted in a decrease in resting

and exercising aural temperature, a decrease in RPE and TS and a small increase ($1.5 \pm 0.6\%$) in PV, this was the first evidence of beneficial adaptations in Paralympic athletes. Nonetheless, due to the lack of change in exercising HR or whole body sweat rate, the responses were deemed only partial acclimation (Castle *et al.*, 2013). As no sweat responses were noted during HA, as a consequence of athletes' impairments, it was speculated that the temperature of cerebral-spinal fluid perfusing the hypothalamus may be of greater importance for adaptation than peripherally derived, neural, thermal afferents (Castle *et al.*, 2013). This proposal is of particular relevance for athletes with impairments to peripheral thermal afferents, such as the Paralympic athlete population.

However, more recently in non-athletes with an SCI, Trbovich *et al.* (2016) found no adaptations to a HA period in a larger group. Undergoing a similar protocol to Castle *et al.* (2013), the participants ($n=10$) performed seven consecutive days of moderate intensity arm cranking for 30 min before resting for 15 min, all in 35.0°C (Trbovich *et al.*, 2016). The authors found no significant change in exercising aural temperature, T_{sk} , HR or PV. Similarly, solely utilising passive acclimation of hot water immersion, Gass and Gass (2001) demonstrated no significant change in thermoregulatory parameters in individuals with paraplegia yet improvements were evident in an AB group. Specifically, five consecutive days of 60 min immersion in 39.0°C water did not decrease oesophageal temperature to a statistically significant level. However, it is worth noting that the participants in the aforementioned studies were not well trained, which possibly limited their adaptive timeframe to the protocol as aerobic fitness may influence adaptation speed (Cheung & McLellan, 1998). As such, research is currently limited to allow firm conclusions to be drawn on the adaptive potential of athletes with an SCI to periods of prolonged heat exposure whilst there is no research of HA in other impairment groups.

Despite the study of Castle *et al.* (2013), research is still severely limited for Paralympic athletes. As described in Chapter 2.1, Paralympic athletes are likely to be at heighten risk for performance decrements in the heat as a consequence of: a loss of vasomotor control and sweating capability (Freund *et al.*, 1984); reduced venous return (Hopman *et al.*, 1992; Kloyiam *et al.*, 2011); movement inefficiencies increasing metabolic heat production (Blauwet *et al.*, 2017; Ward & Meyers, 1995); impaired pace awareness (Runciman *et al.*, 2016; Taylor *et al.*, 2016); limited surface area for heat loss via missing limbs or skin grafts (Crandall & Davis, 2010; Epstein *et al.*, 1986; Webborn, 1996); limb insulation from socket liners (Klute *et al.*, 2007); or a propensity for hypohydration (Webborn & Van de Vliet, 2012) or sunburn

(Bothwell, 1997). Furthermore, as many Paralympic sports, including paratriathlon, involve athletes of mixed impairments, HA protocols that are capable of inducing adaptations across multi-impairment groups are required. Additionally, similar to elite AB athletes, HA protocols must be capable of feasibly invoking adaptation without significant disruption to pre-competition training regimes. Therefore, research is needed to build on the noted literature gaps regarding the efficacy of HA for Paralympic athletes (Price, 2015).

2.7 Summary

Paratriathlon is a variant of triathlon modified for individuals with a physical impairment. It is a multi-impairment sport with disabilities including, but not limited to: SCI, CP, amputation or VI. As the sport made its Paralympic Games debut in the 2016 Rio de Janeiro Games, there has been a recent increase in the professionalism and training and competition demands of athletes. Nonetheless, the case study of Mujika *et al.* (2015) remains the only source of peer-reviewed knowledge of the sport; this is vastly disparate to AB triathlon.

In AB athletes, a great deal is known regarding: immunological adaptations to TL (Walsh *et al.*, 2011); responses of markers of OR to IT (Meeusen *et al.*, 2013); thermoregulation during competition in the heat (Lee *et al.*, 2010) and strategies of HA with associated adaptations (Guy *et al.*, 2014). However, very little is known about these topics in Paralympic athletes and the transferability of research from AB athletes is far from clear. This is despite this population displaying physical impairments that increase the likelihood of training overload, and thus potential immunosuppression or OR, and/or increased thermal load and altered thermoregulation during performance in challenging environments. As such, this thesis aims to bridge the knowledge gap concerning AB and Paralympic athletes and provide the first resource which coaches and practitioners working in multi-impairment sports, such as paratriathlon, can utilise to maximise training and performance. This will be achieved by answering the following questions:

- How does the mucosal immune system adapt to TL over a prolonged period and how does this relate to URI incidence in paratriathletes?
- What are paratriathletes' responses of common, non-invasive, hormonal, immunological, physiological and psychological markers of OR to an acute period of IT?
- How do paratriathletes' thermoregulatory systems respond to competitive racing in the heat?

- Is an ecologically valid HA protocol transferable between paratriathletes and AB triathletes?

3

Laboratory testing procedures

Several of the following experimental chapters required the use of similar procedures. To avoid repetition, methods used in multiple chapters (Chapters 4 to 7) are detailed below and are subsequently only described in brief, where necessary, later in this thesis.

3.1 Cycling graded exercise tests

In Chapters 4 and 7, participants completed either a cycling or handcycling GXT for the determination of AeLT, AnLT, maximum HR, MAP and $\dot{V}O_{2peak}$. This required the undertaking of a submaximal and maximal GXT; these protocols are described below.

3.1.1 Submaximal cycling graded exercise test

Firstly, after providing informed consent, and screening for medical contraindications via health questionnaire, participants' body mass was recorded using electronic scales (Detecto 6550, Webb City, Missouri, USA). All cycling GXTs were performed on the Cyclus 2 ergometer (RBM elektronik-automation GmbH, Leipzig, Germany) whereby participants could use their personal bike or handcycle to prevent configuration unfamiliarity (Figure 3.1). The bike frame was placed on an electronic brake with the chain over the pinion driving the braking mechanism. After a self-selected warm-up, and 5 min passive recovery, participants began the submaximal GXT at a PO assumed to be below their AeLT based on reported training history or previous test results (bike = 100 to 170 W; handcycle = 60 W). Thereafter, PO increased 25 W (females), 30 W (males), or 20 W (handcycle athletes) every three minutes. HR was recorded continuously via telemetric HR monitor (RS400, Polar, Kempele, Finland). Likewise, gas exchange variables were collected throughout the test for determination of $\dot{V}O_2$ (Metalyzer® 3B, Cortex Biophysik GmbH, Leipzig, Germany). Before each test, the gas analyser was calibrated using a two-point gas calibration ($O_2 = 17.0\%$, $CO_2 = 5.0\%$ against room air) and volumes with a 3-l syringe at flow rates of $0.5\text{--}3.0\text{ l}\cdot\text{s}^{-1}$, according to the manufacturer's recommendations.



Figure 3.1: Examples of a cycling graded exercise test using road bike (left) or handcycle (right) and the Cyclus 2 ergometer.

At the end of every three-minute stage, a 20 μl capillary blood sample was collected from the earlobe and analysed by a lactate analyser (Biosen C-Line, EKF Diagnostics, Magdeburg, Germany) for BLa. The lactate analyser was calibrated before each test using a lactate standard solution of 12 $\text{mmol}\cdot\text{l}^{-1}$. The GXT was terminated when participants' BLa exceeded 4.0 $\text{mmol}\cdot\text{l}^{-1}$. AeLT was determined via log-log transformation of $\dot{V}\text{O}_2$ and BLa (Beaver *et al.*, 1985). Two linear regression lines were fitted to the horizontal and ascending parts of the BLa– $\dot{V}\text{O}_2$ relationship data, and the $\dot{V}\text{O}_2$ at the intersection of the regression lines was defined as the aerobic BLa threshold (Figure 3.2) (Beaver *et al.*, 1985; Leicht *et al.*, 2014). AnLT was defined as the PO associated with a BLa 1.5 $\text{mmol}\cdot\text{l}^{-1}$ greater than that at AeLT (Dickhuth *et al.*, 1999).

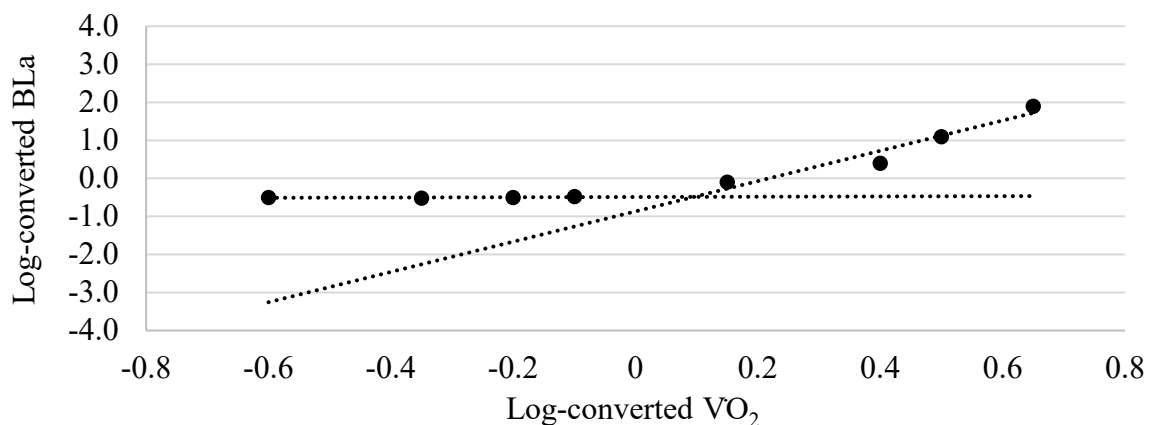


Figure 3.2: Example data illustrating the determination of aerobic lactate threshold from the intercept of the linear regression lines of log-converted rate of oxygen uptake ($\dot{V}\text{O}_2$) and blood lactate concentration (BLa).

3.1.2 Maximal cycling graded exercise test

After a 15-20 min recovery period from the submaximal GXT, during which participants were permitted passive or active recovery at a low PO (\leq starting workload), and when BLA returned to $<2.0 \text{ mmol}\cdot\text{l}^{-1}$, participants began the maximal GXT. The maximal GXT began at the PO of participants' AeLT for two minutes, after which PO increased 5 W every 15 s until volitional exhaustion. MAP was defined as the average PO during the final 60 s of the maximal GXT, $\dot{V}O_{2\text{peak}}$ was defined as the highest $\dot{V}O_2$ value recorded over a 30 s period (Figure 3.3) whilst maximum HR was defined as the highest HR value over a 5 s epoch.

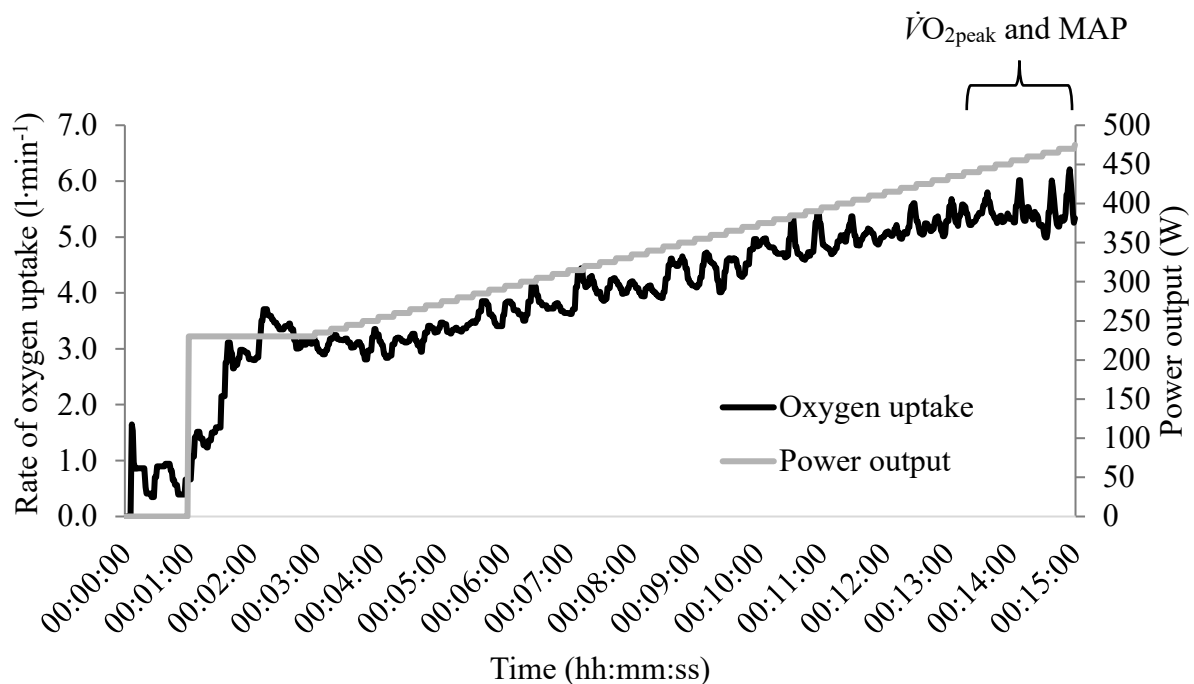


Figure 3.3: Example data illustrating the determination of peak rate of oxygen uptake ($\dot{V}O_{2\text{peak}}$) and maximal aerobic power output (MAP).

3.2 Use of cycling power meters

In Chapters 4 and 7, mobile cycling power meters (Vector, Garmin, Olathe, Kansas, USA) were utilised by ambulant paratriathletes and/or AB triathletes. To assess their reliability and validity against the Cyclus 2, a separate study was performed (Appendix A). In summary, the Garmin Vector power meter was found to be reliable, despite systematically underestimating Cyclus 2 power output by 8 W.

3.3 Salivary secretory immunoglobulin A analysis

In Chapters 4 and 5, saliva samples were collected and then analysed, via sandwich enzyme-linked immunosorbant assay (ELISA), for secretory immunoglobulin A concentration. These procedures are subsequently detailed.

Immunoplates (Corning® Costar®, SAFC Biosciences Ltd, Andover, UK) were initially coated with 0.1% rabbit anti-human capture IgA (Dako UK, Ely, UK) in 0.05M carbonate/bicarbonate (pH 9.6) and incubated at 4°C for ~72 h before being washed and treated with blocking solution (2% bovine serum albumin [Fraction V, SAFC Biosciences Ltd] in phosphate-buffered saline. Immunoplates were incubated with blocking solution for 60 min at room temperature before being washed and, subsequently, purified colostrum IgA (SAFC Biosciences Ltd) standard samples were applied. Depending on the participants' average saliva flow rate (calculated from sample volume and collection duration), samples were diluted in phosphate-buffered saline by 1:200, 1:500, 1:750 or 1:1000 ratios. 50 µl of saliva samples were applied to each plate well in duplicate and incubated overnight at 4°C. Plates were again washed prior to the application of polyclonal rabbit anti human-IgA detection antibody (Dako UK) and incubated at room temperature for 90 min. Immunoplates were washed again and a colouring substrate (OPD substrate, Dako UK) was added with the sample absorbance determined via spectrophotometry at 490 nm (Opsys MR, Dynex Technologies Inc, Chantilly, Virginia, USA) after eight minutes incubation in the dark. All samples from each participant were analysed in duplicate on the same immunoplate to prevent any inter-assay variation. Duplicate samples with a coefficient of variation (CV) $\geq 10\%$ resulted in exclusion from analysis. The mean CV for sIgA concentration was 3.2%.

3.4 Upper respiratory tract illness quantification

In Chapters 4 and 5, upon the provision of a saliva sample for sIgA determination (Chapter 3.3), participants were provided with a retrospective illness questionnaire to quantify URI presence. The questionnaire used has previously been employed by Gleeson *et al.* (2012) in AB populations. The questionnaire captured a total of 14 URI symptoms: fever, headache, sore throat, scratchy throat, catarrh, runny nose, blocked nose, cough, sneezing, joint pain unrelated to normal training, muscle soreness unrelated to normal training, weakness/fatigue, loss of appetite, loss of sleep. Considering the preceding seven days, participants were asked to record the number of days they experienced each symptom and the severity of each symptom on a three-point scale (1 = light, 2 = moderate, 3 = severe). The number of days each symptom persisted was multiplied by the severity rating and summed to provide an overall quantitative

symptom score; a score ≥ 12 indicated the presence of URI. A single URI episode was taken as a weekly symptom score ≥ 12 with at least one week from another week with a score ≥ 12 . Consequently, successive weeks with a score ≥ 12 would be classed as one URI episode (Gleeson *et al.*, 2012).

3.5 Core temperature measurement

In Chapters 6 and 7, T_c is measured in paratriathletes and/or AB triathletes. The specifics of which are detailed forthwith.

In this thesis, two different devices were utilised in the measurement of T_c . Firstly, in Chapter 6, the BodyCap e-Celsius Performance (Caen, France) equipment was used. The e-Celsius is a 1.7 g, 17.7 mm by 8.9 mm pill which must be previously activated and a handheld monitor (e-Viewer Performance). This device was chosen due to the study design whereby T_c was recorded throughout field-based paratriathlon racing. The device permits temperature measurement and storage at pre-defined epochs before subsequent wireless data download to the monitor. Thus, T_c could be captured during the race without the necessity for a constant close proximity of a receiving monitor. The equipment has recently been shown to display a standard error of measurement of 0.039°C compared to highly sensitive and calibrated wired temperature probes across a range of temperatures (35 to 44°C) with an intra-class coefficient of 1.00 (Bongers *et al.*, 2018). Furthermore, by retesting the protocols, an intra-class coefficient of 1.00 was found, showing the device's reliability. However, in Chapter 7 the CorTemp (HQInc., Palmetto, Florida, USA) was used. This is due to the laboratory setting of this experimental study not necessitating remote T_c measurement thus the CorTemp monitor could be in constant close proximity to the participant. This equipment has been readily used previously (Baillot & Hue, 2015; Byrne *et al.*, 2006; Griggs *et al.*, 2017; Laursen *et al.*, 2006; Lee *et al.*, 2010; Peeling & Landers, 2007; Veltmeijer *et al.*, 2015). In the aforementioned study of Bongers *et al.* (2018), the CorTemp showed a standard error of measurement of 0.028°C and, as with e-Celsius Performance, an intra-class coefficient of 1.00. Again, the CorTemp displayed an intra-class coefficient of 1.00 across repeat testing. In both Chapter 6 and Chapter 7, participants were asked to ingest the relevant temperature sensor 6 hours pre-race or pre-testing, respectively. This is in line with the recommendations of Byrne and Lim (2006) to avoid both temperature fluctuations in the upper gastrointestinal tract and sensor expulsion before data collection.

4

Training load, salivary immunoglobulin A and illness incidence in elite paratriathletes: A longitudinal study

This chapter has been accepted for publication in a slightly modified form as:

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4.1 Abstract

Purpose: To longitudinally explore the relationship between TL measures, sIgA and URI in elite paratriathletes.

Methods: Seven paratriathletes participated in the study. Participants provided weekly saliva samples for the measurement of sIgA. Samples were collected over 23 consecutive weeks (February – July) and a further 11 consecutive weeks (November – January). sIgA was subsequently compared to individuals' weekly training duration, ETL and ITL utilising time spent in pre-determined HR zones. Correlations were assessed via regression analyses. URI was quantified via weekly self-report symptom questionnaire.

Results: There was a significant negative relationship between participants' individual weekly training duration and sIgA SR ($p=0.028$) with changes in training duration accounting for 12.7% of the variance (quartiles: 0.2%, 19.2%). There was, however, no significant relationship between ETL or ITL and sIgA parameters ($p \geq 0.104$). There was no significant difference in sIgA when URI was present or not ($p \geq 0.228$); likewise, there was no difference in sIgA when URI occurred within two weeks of sampling or not ($p \geq 0.120$).

Conclusions: Paratriathletes' weekly training duration significantly affects sIgA SR, yet there was no relationship between ETL or ITL and sIgA parameters. Further, it was not possible to detect any link between sIgA and URI occurrence which throws into question the potential of using sIgA as a monitoring tool for early detection of illness.

4.2 Introduction

In the case study by Mujika *et al.* (2015) it was shown that paratriathletes produce large TLs in an attempt to maximise beneficial adaptations. However, there is a risk high TLs will increase the likelihood of illness (Foster, 1998), most commonly URI (Derman *et al.*, 2018b). This is supported by research documenting increased URI incidence during, or after, periods of high TLs in a range of AB sports (Cunniffe *et al.*, 2011; Fahlman & Engels, 2005; Gleeson *et al.*, 1999b; Gleeson *et al.*, 2012; Neville *et al.*, 2008) To date, however, this area is understudied in the Paralympic domain (Fagher *et al.*, 2016). URI contraction has the negative consequences of directly impairing performance (Van Tonder *et al.*, 2016) or limiting training availability (Cunniffe *et al.*, 2011). Therefore, it is important for coaches and practitioners, who seek to minimise URI incidence in both AB and Paralympic sports, to have a better understanding of the effect of TL on the URI risk.

One aspect of immune function that has received particular attention in athletes is mucosal immunity. Over 90% of infections involve the mucosa (Brandtzaeg, 2003) and a key antibody in host defence is sIgA. sIgA has been acknowledged as the first line of defence in mucosal immunity and has several important roles in host defence against microbial pathogens (Walsh *et al.*, 2011). Whilst inverse relationships between sIgA measures and URI risk after acute exercise have been documented (Nieman *et al.*, 2002) other research has studied the longitudinal effects of training on mucosal immunity and illness risk. Several authors have shown that over prolonged periods depressions in sIgA increase the likelihood of URI (Fahlman & Engels, 2005; Gleeson *et al.*, 1999b; Neville *et al.*, 2008; Moreira *et al.*, 2014; Orysiak *et al.*, 2017) with suppressions in sIgA proposed to be modulated by high TLs with insufficient recovery (Pyne & Gleeson, 1998).

To date, the majority of research concerning the effects of TL on sIgA and URI incidence has focused on AB athletes. However, there are some who have studied Paralympic populations. Paralympic athletes are a subset that may be at heightened risk of URI due to the propensity for excessive overload, and therefore TLs, caused by movement inefficiencies (Blauwet *et al.*, 2017; Ward & Meyers, 1995). Leicht *et al.* (2012) first presented a negative correlation between TL and sIgA in Paralympic athletes. Following a group of wheelchair rugby players with an SCI over the course of five months, the authors noted the effects of TL on sIgA were similar to those presented in AB athletes. More recently, Edmonds *et al.* (2015) found no significant changes in sIgA concentration over 14 training weeks in a group of Paralympic swimmers with mixed impairments. Discrepancies in the results of Leicht *et al.*

(2012) and Edmonds *et al.* (2015) are further clouded by different impairment populations, representation of results and sampling frequency. As such, there is currently little consensus regarding the effects of TL on mucosal immunity in Paralympic athletes.

Furthermore, there has been a wide variety of methods of TL quantification in the literature for both AB and Paralympic athletes. Methods such as the ETL of distance covered (Gleeson *et al.*, 1999b; Edmonds *et al.*, 2015) or varying representations of ITL from sRPE (Cunniffe *et al.*, 2011), metabolic equivalents estimated via questionnaire (Gleeson *et al.*, 2012) or arbitrary assessment of training difficulty (Orysiak *et al.*, 2017), which likely all have differing degrees of association to mucosal immunity, have been used. It is noteworthy that there is a paucity of studies utilising objective measures of ITL, such as HR, with many studies relying upon athlete, coach or researcher subjective evaluation of TL.

As research into mucosal immunity in Paralympic sport has been limited to wheelchair rugby (Leicht *et al.*, 2012) and swimming (Edmonds *et al.*, 2015), little is known about the effects of TL on paratriathletes' mucosal immunity. As paratriathletes are likely undertaking large TLs (Mujika *et al.*, 2015), and display impairments that result in movement inefficiencies (Blauwet *et al.*, 2017; Ward & Meyers, 1995), they may be vulnerable to excessive overload and/or mucosal immunosuppression resulting in URI incidence, impaired performance or training unavailability. Therefore, it is important for those working within the sport to better understand how athletes respond to TL for future management of URI risk. As such, the aims of this present study were to elucidate the effects of TL, quantified using objective measures, on sIgA and resultant URI incidence in paratriathletes.

4.3 Methods

4.3.1 Participants

Ten elite paratriathletes volunteered to participate in this study, however due to insufficient sampling (<10 samples) or incomplete training log provision, three athletes were excluded from the final analysis. Of the remaining seven athletes, the group consisted of six males and one female (age 30 ± 10 y, body mass 69.5 ± 6.5 kg, cycling $\dot{V}O_{2\text{peak}}$ 4.06 ± 0.61 l·min⁻¹) with mixed impairments (SCI $n=1$, unilateral transfemoral amputation $n=1$, hemiplegia CP $n=1$, transradial amputation $n=3$ and lower leg impairment $n=1$). All provided written informed consent and the procedures were approved by the Loughborough University Ethical Advisory Committee. All participants regularly competed at an international level with six participants

competing at the 2016 Paralympic Games. All reported being free from illness prior to the commencement of the study.

4.3.2 Study design

A prospective longitudinal study design was employed to collect saliva samples from participants over 23 consecutive weeks (February – July) and a further 11 consecutive weeks (November - January) whilst athletes undertook their normal training and competition regimes. During the study period athletes visited the laboratory three times (February, July and November) (Figure 4.1) for physiological testing of parameters used in TL quantification. Due to the variable nature of athletes' racing and training schedules it was not possible to collect samples from every athlete each week (11 to 31 samples per person).

February	March	April	May	June	July	August	September	October	November	December	January
Specific preparatory phase			Competition phase			Off-season			General preparatory phase		
Weekly saliva sampling									Weekly saliva sampling		
Lab testing									Lab testing		

Figure 4.1: Timeline of training macrocycle, saliva sampling and physiological laboratory testing during the study period

4.3.3 Saliva collection

Collection of samples was performed on the same day (06:00 – 08:00 h) every week before training and standardised to the morning after a recovery day, 10 minutes after last fluid intake, whilst fasted and before brushing teeth. These measures were taken to limit any confounding effects of circadian rhythm, residual fatigue from prior exercise, hydration status and salivary stimulating effects of food (Neville *et al.*, 2008). Participants were asked to provide a passive, unstimulated saliva sample over a period of three minutes into a pre-weighed sterile plastic container with minimal orofacial movement. After sample collection, containers were re-weighed with sample volume estimated assuming a saliva density of 1.00 g·ml⁻¹ (Gleeson *et al.*, 2012). Saliva flow rate was calculated from sample volume and collection time. Whole mixed saliva samples were centrifuged for two minutes at 13,400 revolution·min⁻¹ with the supernatant stored at -80°C until later analysis.

Upon provision of saliva samples, participants completed a retrospective illness questionnaire to quantify URI presence, as explained in Chapter 3.4. Participants also reported if training availability was affected by URI symptoms.

4.3.4 Saliva analysis

Sample sIgA concentration was determined through a sandwich ELISA, described in Chapter 3.3. sIgA SR was determined as the product of saliva flow rate and sIgA concentration. Individuals' healthy median sIgA concentration was calculated as the median of an

individual's concentrations when URI was not present (Neville *et al.*, 2008).

4.3.5 Laboratory testing

Participants performed both a submaximal GXT for the determination of AeLT and AnLT and a maximal GXT for the determination of MAP and $\dot{V}O_{2\text{peak}}$ during both cycling and running/racing wheelchair. See Chapter 3.1 for details on cycling GXT protocols.

As with the submaximal cycling GXT, the submaximal run and racing wheelchair GXT began with a self-selected warm-up on the treadmill for ambulant athletes (Saturn®, h/p/cosmos Sports & Medical GmbH, Nußdorf, Germany) or wheelchair ergometer (VPHandisport-25, Tecmachine, France) for the athlete with an SCI. The test then began at a velocity deemed to be below the participants' AeLT (mean starting velocity = 9.5 ± 0.8 km·h⁻¹ for ambulant athletes and 18.7 ± 0.6 km·h⁻¹ for the athlete with an SCI) based on training history or previous testing data. The velocity then increased 1 km·h⁻¹ every three minutes. A 20 µl earlobe capillary blood sample was taken at the end of every three-minute stage for determination of BLa (Chapter 3.1). The test was terminated when participants' BLa exceeded 4.0 mmol·l⁻¹. HR and $\dot{V}O_2$ were recorded throughout. AeLT and AnLT were determined as described previously (Chapter 3.1). After 15-20 minutes active or passive recovery, self-selected by the participant, and provided BLa was <2.0 mmol·l⁻¹, the maximal GXT began. Participants ran/pushed at their velocity of AeLT for two minutes, thereafter velocity increased 0.5 km·h⁻¹ every 30 s until volitional exhaustion. $\dot{V}O_{2\text{peak}}$ was defined as the highest $\dot{V}O_2$ value recorded over a 30 s period whilst maximum aerobic velocity was defined as the average velocity for the last 60 s of the test.

4.3.6 Weekly training load quantification

During the study period, participants undertook their normal training routine, as prescribed by coaches. All training data were uploaded onto an online system (TrainingPeaks, Boulder, Colorado, USA) before later analysis. TL was calculated by several methods to assess which representation may relate best to immune defence. Firstly, training was represented simply as total weekly training duration in hours for swim, bike and run training. This was subsequently relativised as a percentage of the greatest training duration recorded for each individual athlete during the study period. Any resistance training was not included due to its small contribution to total weekly training.

Further, an ETL accounting for differences in the relative stress of triathlon modalities was calculated using the methods of Cejuela-Anta and Esteve-Lanao (2011) (Equation 4.1).

An ITL score was also calculated from a modification of the methods of Cejuela-Anta and Esteve-Lanao (2011) utilising the time spent in pre-determined zones (Equation 4.2), based on the HR associated with lactate thresholds derived from laboratory testing (Lucía *et al.*, 2003). During cycling training, athletes' PO at their lactate thresholds was translated from the lab to the field via the use of Garmin Vector power meters (Garmin, Kansas City, Kansas, USA) whilst accounting for the -8 W offset compared to the Cyclus 2 ergometer (Appendix A). Due to the inability to record HR during swim training this was represented in the equation solely by swim duration. Again, ETL and ITL were relativised to the highest recorded value during the study period.

Equation 4.1

$$ETL = 0.75(\text{swim duration}) + 0.5(\text{cycling duration}) + (\text{run duration})$$

Equation 4.2

$$ITL = [0.75(\text{swim duration})] + [0.5(TIZ1_C + 2[TIZ2_C] + 3[TIZ3_C])] + [(TIZ1_R + 2[TIZ2_R] + 3[TIZ3_R])]$$

TIZ_{nC} – weekly time (min) spent in zone *n* during cycling. TIZ_{nR} – weekly time (min) spent in zone *n* during running. Zone 1 – below aerobic lactate threshold. Zone 2 – above aerobic lactate threshold, below anaerobic lactate threshold. Zone 3 – above anaerobic lactate threshold.

4.3.7 Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics 23.0 software (IBM, New York, USA) and statistical significance was set at $p < 0.05$. Means \pm standard deviations (SD) were produced for normally distributed variables, as assessed via Kolmogorov-Smirnov or Shapiro-Wilk tests, whilst medians and quartiles were computed for all other variables. CVs were calculated for sIgA concentration and SR.

Each salivary variable was matched to the participant's individual TL or training duration for the preceding seven days. A logarithmic transformation was applied to salivary variables to weight increases in the variable by a certain factor the same as decreases by the same factor (Leicht *et al.*, 2012). Slopes of linear regression lines between log-transformed salivary and training variables were calculated for each participant and compared, as a group, to a fixed zero with a Wilcoxon statistic. This was repeated to assess any relationship between salivary flow rate and sIgA measures.

Salivary sIgA concentration, sIgA SR and flow rate were represented as relative change from individuals' median value and analysed, via paired-samples t-test (parametric) or Mann-

Whitney U test (non-parametric), to elucidate any relationship to URI occurrence within two weeks of sample provision. Similarly, salivary variables when URI were present were compared to samples when healthy (>2 weeks to/from URI).

4.4 Results

In total, 132 saliva samples were collected. The between- and within-individual variability in sIgA concentration was 70% and 40%, respectively, whilst in sIgA SR variability was 88% and 46%, respectively. Participants' average sIgA concentration and SR were $162 \pm 127 \mu\text{g}\cdot\text{ml}^{-1}$ and $78 \pm 76 \mu\text{g}\cdot\text{min}^{-1}$, respectively (Figure 4.2). 100% of sIgA concentration and SR values for participant G (female) fell within the 10th and 90th percentile for the group, as such her data was included for analysis.

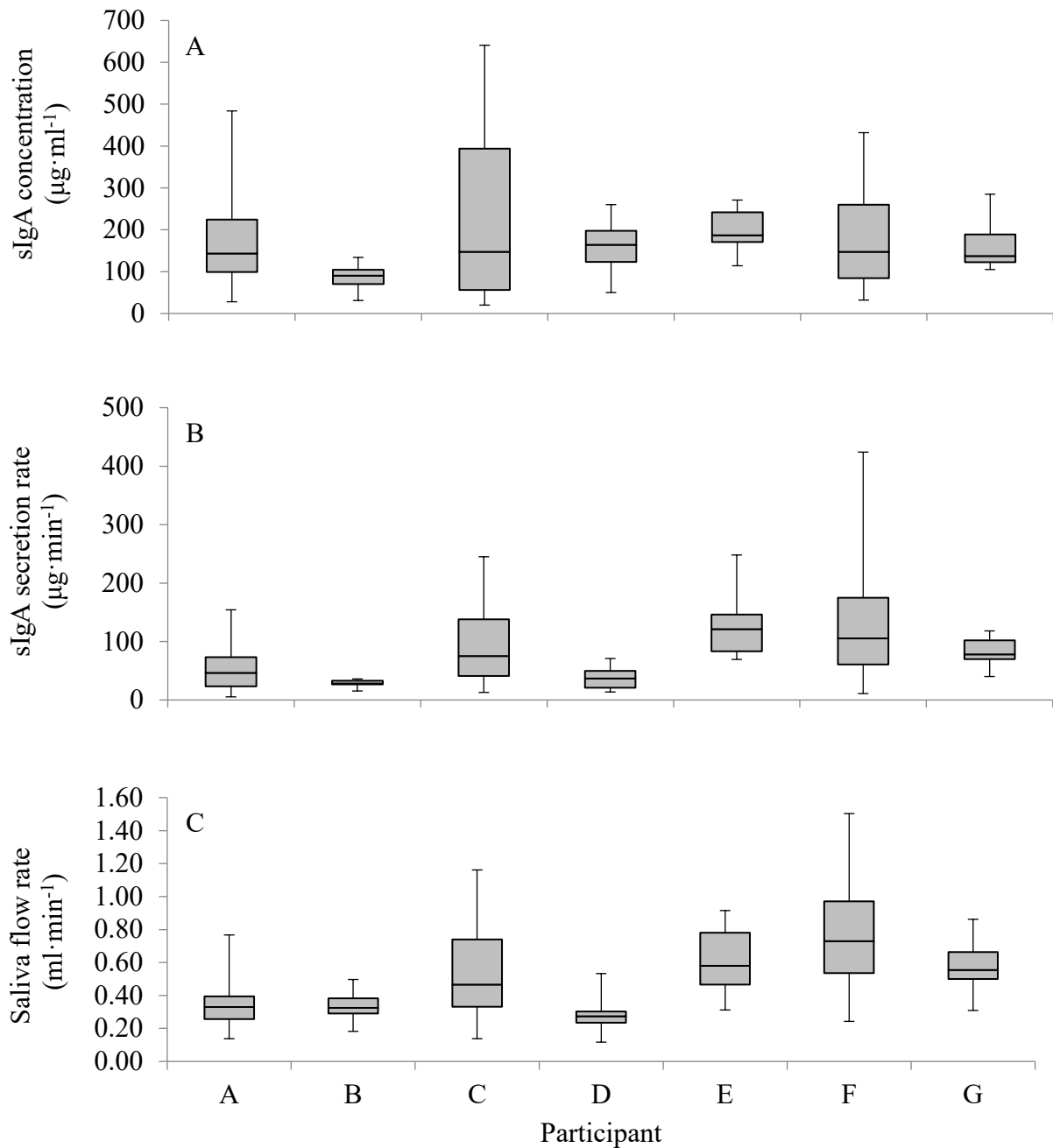


Figure 4.2: Individual athletes' salivary secretory immunoglobulin A (sIgA) concentration (A), sIgA secretion rate (B) and saliva flow rate (C). Values are box plots displaying median, quartiles, and maximum/minimum.

There was a significant inverse relationship between athletes' total training duration and sIgA SR with the slope of individual regression lines disparate from zero ($p=0.028$) (Figure 4.3). The amount of variance in sIgA SR explained by changes in training duration was 12.7% (quartiles 0.2%, 19.2%). There was, however, no significant relationship between ETL ($p\geq 0.398$) or ITL ($p\geq 0.104$) and sIgA SR or concentration. There was a significant inverse

relationship between athletes' individual salivary flow rate and sIgA concentration with slopes of individual regression lines significantly different to zero ($p=0.018$). There was no significant relationship between salivary flow rate and sIgA SR ($p=0.398$).

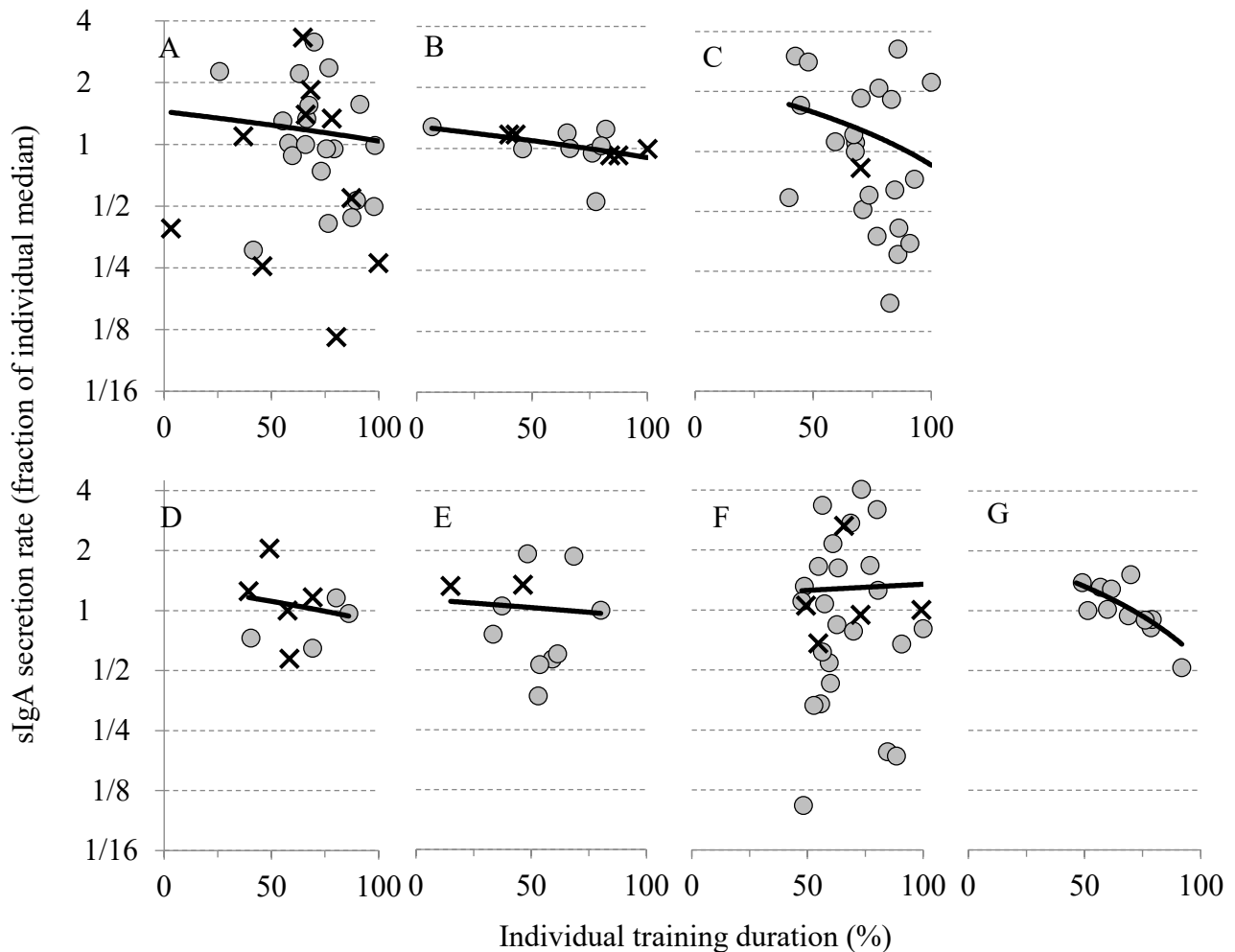


Figure 4.3: Individual athletes' salivary secretory immunoglobulin A (sIgA) secretion rate plotted against individual training duration with linear regression lines. × Samples when upper respiratory tract illness was present. A-G are participant codes.

During the study period, six participants reported at least one URI occurrence with a total of 22 separate URI episodes. On average, athletes presented with URI every seven weeks. During 50% of URI episodes, athletes had to reduce or suspend training. There was no significant difference in relative deviation from individual median sIgA concentration, SR or saliva flow rate between weeks with URI and when healthy ($p \geq 0.228$) (Table 4.1). Similarly, there was no significant difference in relative deviation from individual median sIgA concentration, SR or saliva flow rate between salivary samples with URI within two weeks and samples without URI within two weeks ($p \geq 0.120$) (Table 4.1).

Table 4.1: Relationship between upper respiratory tract illness (URI) state or URI occurrence within two weeks of sampling date and individual deviation of saliva data. Data is median (quartiles).

	sIgA concentration (% indiv. median)	sIgA SR (% indiv. median)	Saliva flow rate (% indiv. median)
URI state			
URI	101 (94, 117)	113 (98, 128)	107 (87, 131)
Healthy	118 (95, 139)	98 (85, 127)	94 (77, 117)
URI within two weeks?			
Yes	83 (54, 151)	76 (49, 142)	100 (84, 105)
No	125 (107, 147)	111 (96, 141)	95 (74, 113)

URI – Upper respiratory tract illness. sIgA – Salivary secretory immunoglobulin A. SR – Secretion rate.

4.5 Discussion

The findings from the present study indicate that, in the sport of paratriathlon, total weekly training duration displays a inverse relationship with sIgA SR, yet this is not the case when related to a quantified ETL or ITL. Further, there was no significant relationship between salivary variables and URI occurrence. On an individual basis, changes in sIgA concentration were related to saliva flow rate.

It was identified that there was a significant inverse relationship between training duration and sIgA SR. On a group level, training duration explained 12.7% of the variance in sIgA SR, though it is worth noting the large inter-individual variability. The variability is likely due to the individualised nature of which training affects just one of myriad immunological variables (Walsh *et al.*, 2011). There was, however, no significant relationship between ETL or ITL and sIgA parameters. Relationships between TL and sIgA have been shown elsewhere in AB sport (Fahlman & Engels, 2005; Guilhem *et al.*, 2015; Moreira *et al.*, 2014; Neville *et al.*, 2008), yet, Leicht *et al.* (2012) are the only researchers to note this interaction in a Paralympic population. The study of Leicht *et al.* (2012), however, was conducted in a ball sport typified by repeated high intensity efforts (Rhodes *et al.*, 2017) and in a group solely

consisting of athletes with an SCI, thus, was quite disparate from the multi-impairment endurance sport of paratriathlon. Furthermore, the aforementioned study relied upon subjective measures of TL quantification rather than objective parameters such as HR, as was used in the present study.

Saliva data was found to be highly variable on an individual and group level. This is in concordance with the findings of Cunniffe *et al.* (2011) and Leicht *et al.* (2012) who found comparable CV values in groups of AB rugby union players and wheelchair rugby players with an SCI, respectively. This is, though, the first study to show the variability of parameters in the sport of triathlon over an extended period, with previous research restricted to a study period <7 d (Libicz *et al.*, 2006). Nonetheless, as this is a common finding, it may be postulated that variation was not related to participants' impairments but rather it was natural variation common in salivary parameters. As previously mentioned, the amount of variance in sIgA SR explained by training duration was highly individual and may also be attributable to the multi-factorial nature of mucosal immunity. As such, a large proportion of the changes in sIgA may be due to other influences such as genetical, nutritional and psychological factors (Moreira *et al.*, 2009; Moreira *et al.*, 2014; Mortatti *et al.*, 2012).

Upper respiratory tract complaints are the most common type of illness reported in athletes (Derman *et al.*, 2018b) and have the capability to negatively impact upon training and competitive performance (Cunniffe *et al.*, 2011; Van Tonder *et al.*, 2016). Over the current study period, athletes averaged one URI episode every seven weeks, which is significantly greater than previously reported values of four URI episodes annually in athletes (Gleeson *et al.*, 1999b) and clinical practice (Gleeson *et al.*, 1999a). However, this may be related to the inability to distinguish between actual infection and other possible causes of URI such as inflammation or allergen triggered events (Pyne & Gleeson, 1998). As the present study did not employ clinical confirmation of pathogenic causes of symptoms, the presence of infection could not be established with certainty. Therefore, the term URI and not 'upper respiratory tract infection' was used. Alternatively, the high URI incidence may be due to Paralympic athletes' vulnerability to illness as a consequence of impaired coughing efficiency in athletes with an SCI (Brown *et al.*, 2006), such as athlete A, or movement inefficiencies increasing the likelihood of excessive overload in athletes with CP or an amputation (Blauwet *et al.*, 2017; Ward & Meyers, 1995). Nonetheless, in 50% of URI incidences athletes stated that their training had been impaired. This highlights the desirability for tracking immune to identify

athletes at risk of illness prior to decrements in training or competitive performance (Gleeson *et al.*, 1999b).

The present study, however, noted no relationship between participants' salivary variables and URI incidence. There was no difference in salivary parameters when URI was present compared to when healthy. Likewise, there was no significant difference between variables when URI occurred within two weeks of a sample to when URI did not occur. Again, the lack of relationship between sIgA measures and URI incidence has been shown elsewhere (Cunniffe *et al.*, 2011; Leicht *et al.*, 2012). This, nevertheless, opposes the reported negative relationship between sIgA and illness incidence (Mackinnon *et al.*, 1987; Nieman *et al.*, 2002). It is likely that this once again relates to the multifactorial nature of illness and immunity. Although sIgA plays a major role in mucosal immunity and defence against microbial pathogens in the upper respiratory tract, there are many mechanisms responsible for host defence. These mechanisms concern both the innate and acquired immune systems and insufficiencies in any, not merely sIgA, are likely to heighten the risk of illness (Neville *et al.*, 2008). Here, the only measure of immune function was sIgA, this limited the scope to fully attribute the cause of illnesses. Analysis of other anti-microbial proteins implicated in mucosal immunity, such as lysozyme and lactoferrin (Gleeson *et al.*, 2012; West *et al.*, 2010), may have further elucidated the interaction between TL and mucosal immunity.

Training load was represented as both ETL and ITL in the present study. Furthermore, total weekly training duration was also included. However, only total weekly training duration displayed a significant relationship to saliva parameters. This may signify a failing of the methods of Cejuela-Anta and Esteve-Lanao (2011) to adequately quantify TL in its relation to illness incidence. As such, it is likely this method of TL quantification does not truly represent the stress imposed on the mucosal immune system from each triathlon modality. Nonetheless, it was surprising that ITL did not relate to sIgA as this is likely to better represented the physical stress imposed by training than external measures. It is worth noting, however, that due to the inability to record HR during swim training, that this modality was only represented by weekly swim duration.

A limitation to the current study was, as previously mentioned, the lack of mechanistic data to further elucidate the relationship between training duration or TL and sIgA. Measurement of cortisol, which has been suggested as a modulating factor in sIgA suppression (Cunniffe *et al.*, 2011; Walsh *et al.*, 2011), or markers of sympathetic activation such as α -

amylase (Edmonds *et al.*, 2015; Leicht *et al.*, 2012), may have provided further insight into the causes of variation of sIgA. Cortisol and α -amylase may also act as markers of psychological stress, another factor that has been proposed to influence mucosal immunity (Moreira *et al.*, 2014; Mortatti *et al.*, 2012). Also, the inclusion of an age and sex matched control group would have improved confidence in the relationship between training and sIgA and to minimise any confounding effect of seasonal variation. Thus, future research should look to address these limitations for a better understanding of training, sIgA and URI interactions. Finally, a significant limitation is the small and heterogeneous sample size which may have inhibited the ability to identify significant relationships. However, due to the small target population and the elite status of the participants this was unavoidable.

4.6 Conclusions

Paratriathletes' weekly training duration has an inverse relationship with sIgA SR, yet there is no significant relationship between ETL or ITL and sIgA parameters. Additionally, it was not possible to detect any link between sIgA and URI occurrence which limits the ability to use the antibody as a monitoring tool for early detection of illness. Whilst it is now elucidated that weekly training duration, but not TL, relate to immune function and that this is unrelated to URI over a prolonged period, it is still not known how paratriathletes may respond to acute changes in TL during periods of IT. Specifically, as athletes deliberately undertake short periods of training overload for positive adaptation, there may be a risk of entering an overreached state.

5

Non-invasive markers of overreaching in response to intensified training in paratriathletes

This chapter has been accepted for publication in a slightly modified form as:

Stephenson, B. T., Leicht, C. A., Tolfrey, K., & Goosey-Tolfrey, V. L. (2018). A multi-factorial assessment of elite paratriathletes' response to two weeks of intensified training. *International Journal of Sports Physiology and Performance*. doi:10.1123/ijsp.2018-0838. [Epub ahead of print].

5.1 Abstract

Purpose: To assess the changes of hormonal, immunological, physiological and psychological parameters in response to IT due to their potential relationship to overreaching and subsequent sporting performance decrements.

Methods: Ten elite paratriathletes were studied for five weeks around a 14-day training camp whereby TL was 137% of pre-camp levels. Athletes provided: six saliva samples for cortisol, testosterone and immunoglobulin A (1 pre-IT, 4 during IT, 1 post-IT); weekly psychological questionnaires (POMS and RESTQ-S); daily RHR and daily subjective wellness measures including sleep quality and quantity.

Results: There was no significant change in salivary measures during IT ($p \geq 0.090$). There was no change in RHR or subjective wellness measures ($p \geq 0.079$). Subjective sleep quality and quantity increased during IT ($p \leq 0.003$). There was no effect on any POMS subscale other than lower anger ($p = 0.049$) whilst there was greater general recovery and lower sport and general stress from RESTQ-S ($p \leq 0.015$).

Conclusions: There was little to no change in parameters commonly associated with the overreached state. This may relate to the training camp setting minimising external life stresses and the careful management of TLs from coaches. This is the first evidence of such responses in Paralympic athletes.

5.2 Introduction

It is commonplace for coaches to deliberately plan overload into athletes' training in an attempt to maximise beneficial adaptations. Athletes may undergo short periods of IT, commonly in the form of training camps, deliberately designed to provide an overload stimulus whereby significant acute increases in TL are observed. Whilst periods of IT may result in improved performance, there is the possibility athletes may be at risk of OR (Bresciani *et al.*, 2011).

In their consensus statement, Meeusen *et al.* (2013) define OR as an accumulation of training and/or non-training stress resulting in decrement in performance capacity. These decrements could be with or without related physiological and psychological signs and symptoms of maladaptation (e.g., fatigue, mood disturbance, sleep disruption) in which restoration of performance capacity may take from several days (functional OR) to several weeks (non-functional OR). Whilst some coaches deem functional OR part of the normal training process due to the potential for improved performance after a taper period (Coutts *et al.*, 2007a), if IT were to continue for athletes displaying functional OR there is a greater possibility of non-functional OR genesis. Thus, it is desirable to identify athletes at risk of OR before any significant performance decline. However, the only way to confirm the presence of OR is via a decrement in maximal, sport-specific performance (Meeusen *et al.*, 2013). Testing for impaired performance in athletes potentially suffering from OR offers inherent problems due to the effect a further fatiguing task may have, also there is potential for disruption to the normal training regime (Le Meur *et al.*, 2013). Consequently, less fatiguing and disruptive methods of detecting OR after periods of IT have been sought to identify athletes at risk prior to any major performance impairment.

Resting levels of cortisol and testosterone are the most commonly studied hormones with respect to OR due to the effect of IT on the autonomic nervous system, specifically hypothalamic axes (Bosquet *et al.*, 2008), and the ease with which both may be analysed, especially in saliva (Papacosta & Nassis, 2011). It is believed that IT results in increases in biologically active, free cortisol with a concomitant decrease in free testosterone, thus a decrease in cortisol:testosterone ratio (sC:T) (Lehmann *et al.*, 1993), representing a greater catabolic state in the body. Studies have supported this, displaying increases in sC (Papacosta *et al.*, 2013; Svendsen *et al.*, 2016) or decreases in sT (Bresciani *et al.*, 2011; Hough *et al.*, 2015) as a result of IT. Whilst cortisol and testosterone are commonly measured in saliva, Coutts *et al.* (2007b) proposed that sIgA may also be a sensitive marker in response to IT. Longitudinal prospective studies have evidenced athletes experiencing depressions in sIgA

during periods of high TL (Fahlman & Engels, 2005; Leicht *et al.*, 2012; Moreira *et al.*, 2014; Neville *et al.*, 2008) or training duration (Chapter 4). However, this suggestion has yet to be supported in studies addressing immunological responses to IT.

Although the responses to IT of other potential markers of OR, such as RHR or sleep quality/duration, have yet to show uniformity (Bosquet *et al.*, 2008; Hausswirth *et al.*, 2014; Killer *et al.*, 2017), subjective psychological states do seem to produce consistent results (Coumts *et al.*, 2007a). The effect of IT on athletes' psychological state has been commonly assessed via the POMS or the RESTQ-S. POMS is a questionnaire capable of profiling total mood disturbances or specific subscales (vigour, depression, confusion, anger, fatigue and tension); whereas RESTQ-S details general or sport-specific recovery or stressing activities (Bresciani *et al.*, 2011). Subjective psychological measures have regularly been suggested as being sensitive enough to detect the early manifestation of OR in response to IT (Bresciani *et al.*, 2011; Coumts *et al.*, 2007a; Hough *et al.*, 2013; Papacosta *et al.*, 2013).

Although the effects of IT on markers of OR have been studied in many types of athletes, triathletes have received particular attention (Coumts *et al.*, 2007a; Coumts *et al.*, 2007b; Hausswirth *et al.*, 2014; Hough *et al.*, 2015; Le Meur *et al.*, 2013; Robson-Ansley *et al.*, 2006); this is partly due to their habitually high TLs, which place them at risk of OR (Le Meur *et al.*, 2013). Despite the extensive research focusing on AB triathletes, little is known about how paratriathletes respond to IT. As the case study of Mujika *et al.* (2015) and Chapter 4 of this thesis allude to, paratriathletes are likely to be undertaking high TLs, placing them at risk of OR. Furthermore, although the training habits of athletes in other Paralympic sports have been presented (Fulton *et al.*, 2010), there is no published literature regarding any hormonal, immunological, physiological or psychological effects of IT. Thus, it is not evident how Paralympic athletes may differ to AB athletes regarding potential markers of OR. This topic is of particular relevance as Paralympic athletes may be at greater risk of OR due to physical impairments causing movement inefficiencies (Blauwet *et al.*, 2017; Ward & Meyers, 1995), thus heightening the internal load of movement, with impairments increasing the stresses of daily life (Webborn & Van De Vliet, 2012).

To form the first basis on which to support Paralympic athletes and minimise the likelihood of performance decrements after IT, the aim of the present study was to elucidate how paratriathletes respond to IT in the form of a 14-day overseas training camp.

5.3 Methods

5.3.1 Participants

Ten elite paratriathletes (7 male, 3 female, age 30 ± 8 y, body mass 66.1 ± 7.6 kg, cycling $\dot{V}O_{2\text{peak}}$ 57.6 ± 6.4 ml·kg⁻¹·min⁻¹) of mixed impairments (thoracic SCI $n=1$, hemiplegia CP $n=1$, bilateral transfemoral amputation $n=1$, unilateral transfemoral amputation $n=1$, unilateral transradial amputation $n=4$, lower leg impairment $n=1$, VI $n=1$), volunteered to participate in this study. All provided written informed consent and the procedures were approved by the Loughborough University Ethical Advisory Committee. All participants regularly competed at an international level with nine athletes competing in the 2016 Paralympic Games.

5.3.2 Study design

This study sought to elucidate the effects of an IT period, in the form of an overseas training camp, on common markers of OR. Athletes were studied over the course of five weeks which consisted of one-week pre-IT, two weeks IT and two weeks post-IT. During this time period, athletes provided: six saliva samples for determination of sC, sT and sIgA; weekly psychological questionnaires (POMS and RESTQ-S); daily monitoring of RHR and subjective wellness measures (Figure 5.1). IT took place during the months January-February in Lanzarote, Spain (mean daily temperature $18.7 \pm 0.9^\circ\text{C}$). During the IT period average weekly training volume increased an average of 37% from pre-IT levels.

Day 1-7	Normal Training	
		*
Day 8-14	Intensified Training	
		*
Day 15-21	Intensified Training	
		*
Day 22-28	Normal Training	
		*
Day 29-35	Normal Training	
		*

Figure 5.1: Schematic of data collection. Grey blocks represent days on which saliva was collected for assessment of cortisol, testosterone and secretory immunoglobulin A. * represents days on which participants completed POMS and RESTQ-S. On all days, participants provided their resting heart rate and subjective ratings of sleep quality and quantity, motivation, muscle soreness and energy levels.

5.3.3 Training load

Changes in TL during the study period were as prescribed by participants' coaches. All followed a similar periodised plan with deliberate overload intended during the IT phase. To assess the changes in TL, training was quantified by the methods of Cejuela-Anta and Esteve-Lanao (2011) whereby total training duration for swim, bike and run were provided intensity factors of 0.75, 0.5 and 1, respectively, and summated.

5.3.4 Saliva analysis

Participants provided saliva samples on days 2, 9, 12, 16, 19 and 30. Each sample was collected in the morning (06:00-08:00) before training, ten minutes after last fluid intake and whilst in a fasted state. These measures were taken to limit any confounding effects of circadian rhythm, hydration status and salivary stimulating effects of food (Neville *et al.*, 2008). A passive unstimulated saliva sample was collected over a period of three minutes into a pre-weighed sterile plastic container with minimal orofacial movement. After sample collection, containers were re-weighed with sample volume estimated assuming a saliva density of 1.00 g·ml⁻¹. Saliva flow rate was calculated from sample volume and collection time. sC and sT concentrations were determined in duplicate using commercially available ELISA kits (Salimetrics Europe Ltd, Newmarket, UK). Mean intra-assay CVs were 1.5% for sC and 2.0% for sT. sIgA was analysed using the ELISA technique described in Chapter 3.3. On days where participants provided saliva samples, a questionnaire of illness symptoms was also

completed, as described in Chapter 3.4, for determination of URI incidence before, during and after IT.

5.3.5 Psychological questionnaires

Participants completed POMS and RESTQ-S on five occasions (days 5, 12, 19, 26 and 33); they were asked to answer POMS questions with respect to how they have felt in the last seven days/nights. Responses from the 65-item POMS questionnaire were used to calculate a total mood disturbance score by summation of negative scales (fatigue, depression, tension, anger, confusion) and subtraction of the positive vigour scale. Further, scales were analysed individually to see any effect of IT on specific mood states. When completing the 76-item RESTQ-S, participants rated how often they experienced general and specific stress or recovery orientated activities in the last three days or nights. RESTQ-S responses were used in the calculation of total stress score via summation of stress-related scales. Likewise, a total recovery score was calculated in the same manner using recover-related scales. Further, general stress, sport-specific stress, general recovery and sport-specific recovery scores were produced using the appropriate scales.

5.3.6 Daily wellness measures

Upon waking every morning, participants were asked to provide several wellness measures. Similar to the questionnaire designed by McLean *et al.* (2010), and used by Buchheit *et al.* (2013), on a six-point Likert, scale participants subjectively rated their energy levels, motivation, muscle soreness and sleep quality whilst providing sleep duration in hours. Additionally, participants recorded their RHR using their personal HR monitor whilst supine for at least five minutes. Participants' daily RHR and wellness measures were averaged over five discrete periods: day 1-7, 8-14, 15-21, 22-28 and 29-35.

5.3.7 Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics 23.0 software (IBM, New York, USA). Statistical significance was set at $p < 0.05$. Data were checked for normal distribution using the Shapiro-Wilk test and homogeneity of variance using Levene's test. Where sphericity could not be assumed, the Greenhouse-Geisser correction was used. Changes in TL, sC, sT, sC:T, sIgA, POMS and RESTQ-S scales and daily wellness measures over time were assessed via one-way within-measures analysis of variance (ANOVA) (parametric) or Friedman's test (nonparametric). The Bonferroni post-hoc test was used to evaluate pairwise comparisons of time points.

5.4 Results

5.4.1 Training load

There was a significant difference in TL over time and post-hoc analyses revealed TL was higher during days 8-14 than all other time points ($p \leq 0.034$) and was higher during days 15-21 than days 1-7 ($p = 0.014$) (Figure 5.2).

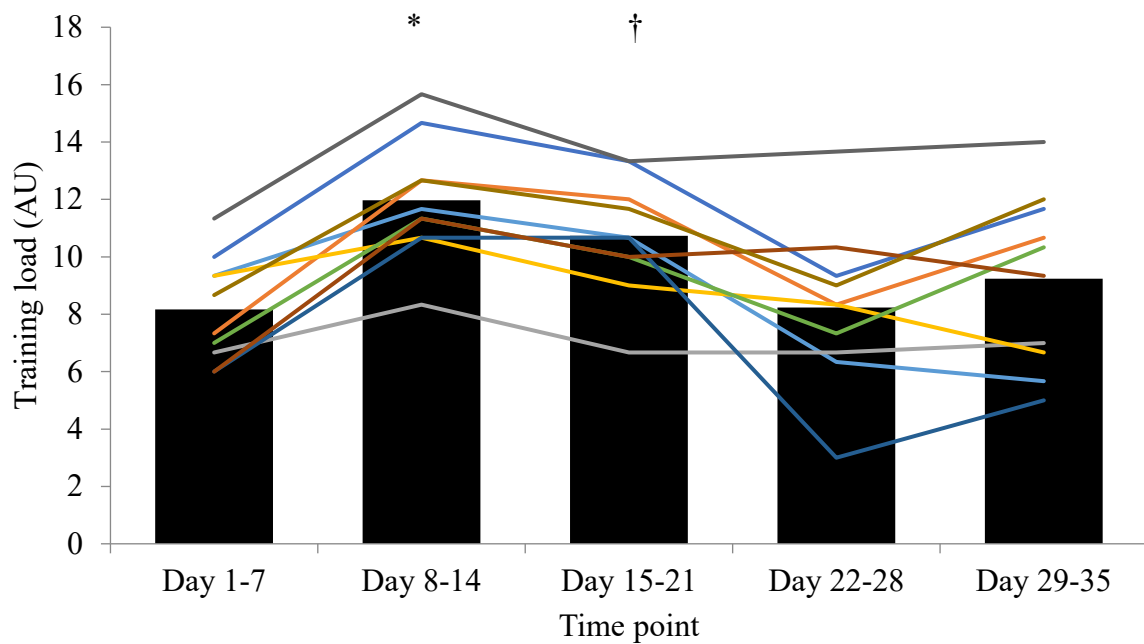


Figure 5.2: Training load before, during and after intensified training. Bars are group mean; lines are individual values. *Significantly greater than all other time points ($p \leq 0.034$). †Significantly greater than day 1-7 ($p = 0.014$).

5.4.2 Salivary cortisol, testosterone and secretory immunoglobulin A

Salivary cortisol displayed significant changes over time as post-hoc analyses revealed a difference between day 2 and day 30 ($p = 0.046$; Figure 5.3). There was no significant change in sT, sC:T or sIgA ($p \geq 0.090$) (Figure 5.3).

5.4.3 Illness incidence

Analysis of illness symptom questionnaires revealed that four participants reported at least one URI during the study. The URI incidence ranged from one to two participants reporting URI per time point (Figure 5.4). In 43% of cases, ability to train was impaired such that was training modified or cancelled.

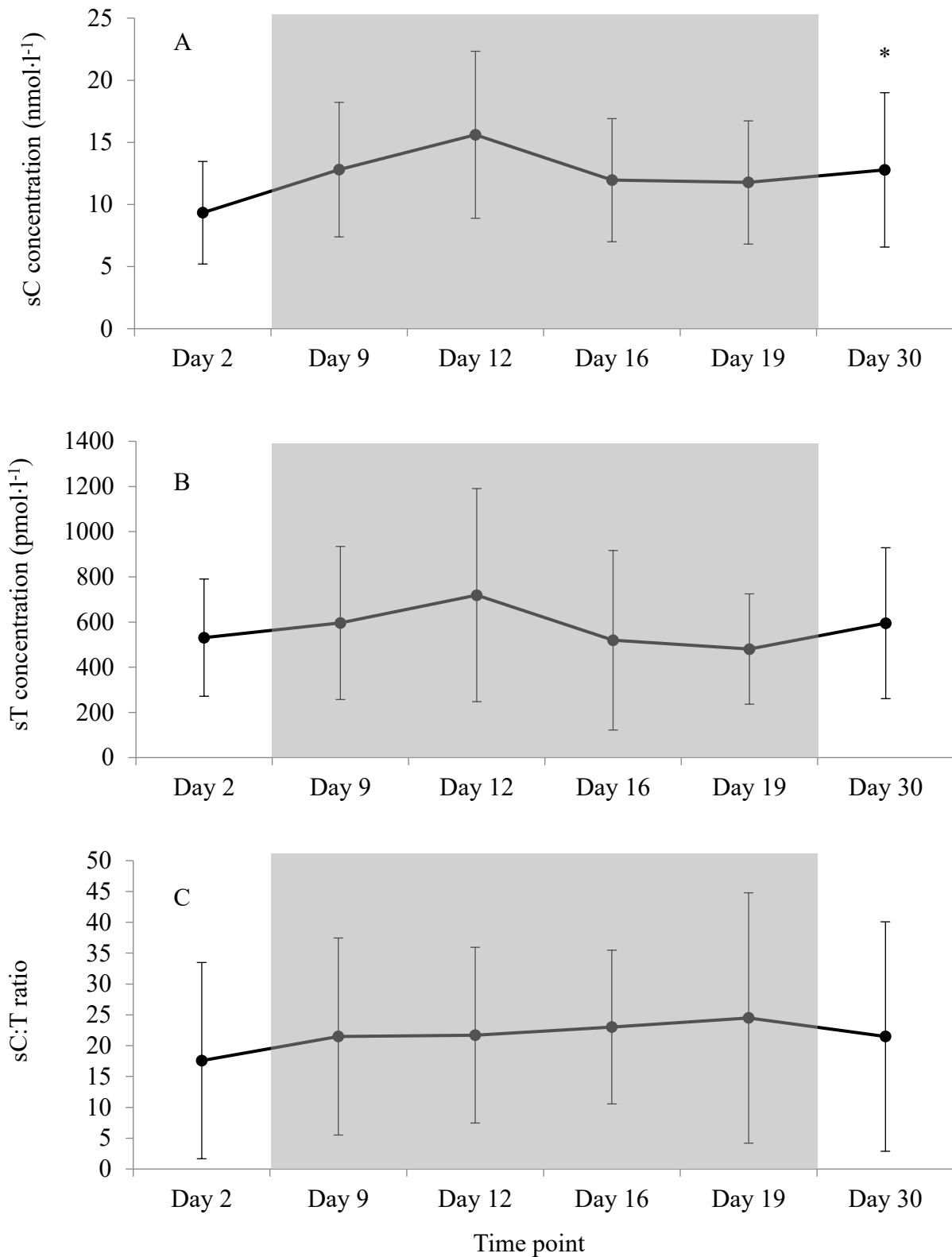


Figure 5.3: Salivary cortisol concentration (A), testosterone concentration (B) and cortisol:testosterone ratio (C) (mean \pm SD). Shaded area signifies intensified training period. *Significantly greater than day 2 ($p=0.048$).

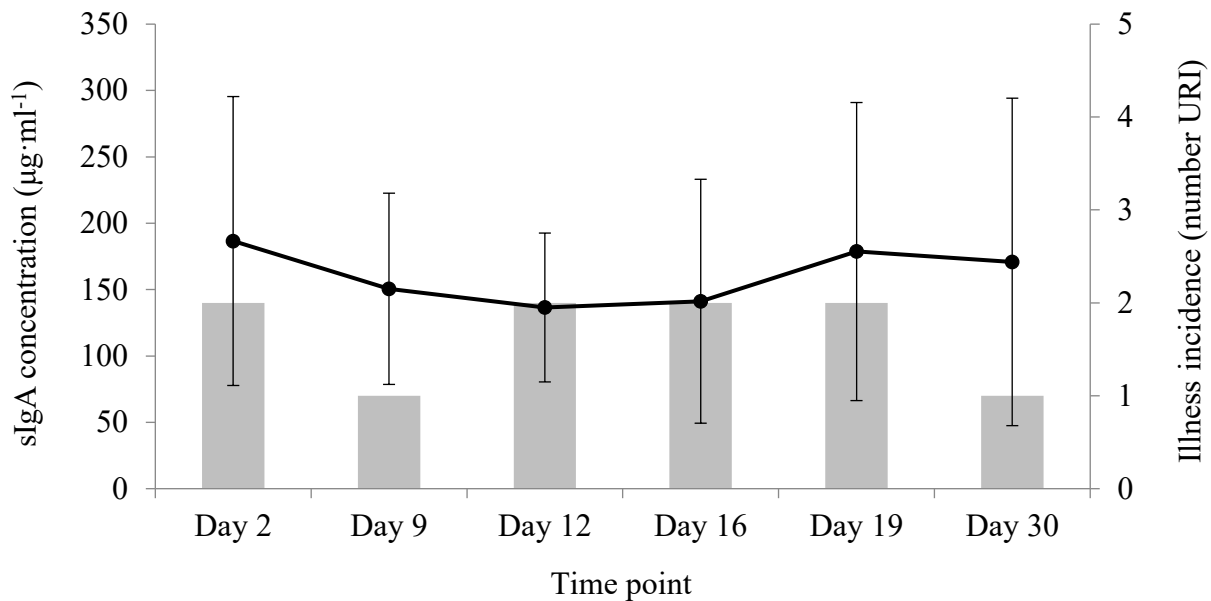


Figure 5.4: Salivary secretory immunoglobulin A concentration changes (dots) and upper respiratory tract illness incidence (bars) before, during and after intensified training (mean \pm SD).

5.3.4 Daily wellness measures

There was no significant difference over time in subjective ratings of motivation, muscle soreness and energy status, nor so self-reported RHR ($p \geq 0.079$). However, there were significant differences in subjective sleep duration and quality; specifically, reported sleep duration was higher in days 8-14 and 22-28 than days 1-7 and 29-35 ($p \leq 0.024$) and was also higher in days 8-14 than days 15-21 ($p = 0.023$) (Table 5.1). Sleep quality was significantly greater in days 22-28 than days 1-7, 8-14 and 15-21 ($p \leq 0.043$) whilst was lower in days 15-21 than 8-14 ($p = 0.023$) (Table 5.1).

Table 5.1: Subjective ratings of energy levels, motivation, muscle soreness, sleep quality and sleep duration with resting heart rate (mean \pm SD). *Significantly different to days 1-7 and 29-35 ($p \leq 0.024$). †Significantly different to days 15-21 ($p \leq 0.023$). §Significantly different to days 1-7, 8-14 and 15-21 ($p \leq 0.043$).

	Day 1-7	Day 8-14	Day 15-21	Day 22-28	Day 29-35
Energy levels (AU)	3.8 \pm 0.7	4.1 \pm 0.4	3.7 \pm 0.6	3.6 \pm 0.7	3.5 \pm 0.7
Motivation (AU)	3.2 \pm 0.5	3.2 \pm 0.4	2.9 \pm 0.6	3.0 \pm 0.5	3.0 \pm 0.4
Muscle soreness (AU)	2.6 \pm 0.8	3.0 \pm 1.1	3.0 \pm 1.2	2.6 \pm 1.0	2.7 \pm 1.0
Sleep quality (AU)	3.2 \pm 0.5	3.1 \pm 0.7†	2.8 \pm 0.7	3.5 \pm 0.6§	3.1 \pm 0.8
Sleep duration (min)	432 \pm 53	487 \pm 53*†	460 \pm 42	481 \pm 49*	460 \pm 57
RHR (beat·min ⁻¹)	50 \pm 6	50 \pm 5	49 \pm 6	49 \pm 5	49 \pm 6

RHR – Resting heart rate.

5.4.5 Psychological questionnaires

There was a significant change in the POMS anger scale as scores were higher on day 5 than days 12 and 19 ($p \leq 0.044$), whilst anger was also significantly higher on day 33 than day 19 ($p = 0.049$). There was no significant difference in any other scale or total mood disturbance ($p \geq 0.079$) (Table 5.2). There were significant differences in RESTQ-S scales for total stress, general stress, sport stress and general recovery; specifically, total stress and general stress were higher on day 5 than days 12, 19 and 26 ($p \leq 0.019$) whilst sport stress was higher on day 5 than days 12, 19 and 33 ($p \leq 0.023$). General recovery was higher on days 12 and 19 than all other time points ($p \leq 0.025$) (Table 5.3).

Table 5.2: Results from POMS before, during and after intensified training (mean \pm SD).

*Significantly different to day 5 ($p \leq 0.044$). †Significantly different to day 33 ($p = 0.049$).

	Day 5	Day 12	Day 19	Day 26	Day 33
Anger	10 \pm 9	5 \pm 4*	5 \pm 3*†	10 \pm 9	11 \pm 10
Depression	13 \pm 13	8 \pm 11	9 \pm 11	16 \pm 15	18 \pm 12
Tension	10 \pm 6	8 \pm 4	8 \pm 5	12 \pm 9	13 \pm 7
Vigour	15 \pm 7	16 \pm 7	15 \pm 7	14 \pm 5	13 \pm 6
Fatigue	9 \pm 5	9 \pm 5	10 \pm 5	11 \pm 5	11 \pm 5
Confusion	7 \pm 5	5 \pm 4	6 \pm 4	9 \pm 7	11 \pm 5
TMD	34 \pm 39	19 \pm 26	23 \pm 28	45 \pm 41	51 \pm 39

TMD – Total mood disturbance.

Table 5.3: Results from RESTQ-S before, during and after intensified training (mean \pm SD).

*Significantly different to day 5 ($p \leq 0.023$). †Significantly different to days 5, 26 and 33 ($p \leq 0.025$).

	Day 5	Day 12	Day 19	Day 26	Day 33
Total stress	2.3 \pm 0.9	1.5 \pm 0.8*	1.7 \pm 0.7*	2.0 \pm 1.0*	2.0 \pm 1.0
Total recovery	2.2 \pm 1.0	2.8 \pm 0.9	2.7 \pm 1.3	2.1 \pm 0.6	1.9 \pm 1.0
General stress	2.3 \pm 1.0	1.5 \pm 0.8*	1.7 \pm 0.7*	2.0 \pm 1.1*	2.2 \pm 1.2
General recovery	1.9 \pm 0.9	2.6 \pm 0.9†	2.6 \pm 1.2†	1.8 \pm 0.5	1.6 \pm 1.0
Sport stress	2.1 \pm 0.9	1.6 \pm 0.9*	1.8 \pm 1.1*	2.1 \pm 0.9	1.5 \pm 1.1*
Sport recovery	2.6 \pm 1.2	3.1 \pm 1.1	2.8 \pm 1.5	2.6 \pm 0.9	2.4 \pm 1.1

5.5 Discussion

The present study is the first to assess the hormonal, immunological, physiological and psychological responses to a period of naturally occurring IT period in a group of elite

paratriathletes. IT resulted in no significant change to sC, sT or sIgA whilst lowering measures of stress and anger and increasing self-reported sleep parameters and perceived recovery.

Although TL during IT was 137% of normal training, similar to previously published studies reporting OR (Hauswirth *et al.*, 2014; Hough *et al.*, 2013; Le Meur *et al.*, 2013), it appears, based on hormonal, immunological, physiological and psychological parameters, that athletes were not showing signs or symptoms of OR. As no maximal, sport-specific performance test was employed, the absence of OR cannot be proven. Others have also noted a lack of OR signs during periods of IT. In their study of Australian Rules footballers, Buchheit *et al.* (2013) reported that a two-week training camp, similar to the present study, resulted in no evidence of impaired performance or OR. In fact, the participants improved their performance during an intermittent running protocol. The authors propose this beneficial adaptation was due to the participants' high-level training background and careful planning of training by the coaches to minimise the risk of OR and any performance decrement (Buchheit *et al.*, 2013). Similarly, Slivka *et al.* (2010) reported no effect of a three-week cycling race, whereby exercise volume increased 418%, on any markers of OR. Specifically, 60 min cycling time trial performance was not impaired nor was performance in a GXT. Furthermore, there was no effect on RHR, sC, sT or sIgA with only minimal influence on the POMS vigour scale (Slivka *et al.*, 2010). The lack of OR was proposed to be due to a minimisation of external life stresses (Slivka *et al.*, 2010).

Salivary cortisol and testosterone have previously been suggested as useful markers of OR after periods of IT due to their ease of analysis (Papacosta & Nassis, 2011) and their potential relationship to overreached states (Lehmann *et al.*, 1993). Although in the current study there was an increase in sC from pre- to post-IT, indicative of cumulative physical or psychological stress, there was little change in sC during the 14-day IT period. Furthermore, sT and sC:T were unchanged, suggesting the catabolic:anabolic hormonal balance was not significantly perturbed. The responses of sC and sT to periods of IT have commonly been studied in AB athletes. However, there appears to be little support for the hypothesised increase in the catabolic milieu. For example, whilst some have shown increases in sC (Svendsen *et al.*, 2016), most have reported no significant changes (Bresciani *et al.*, 2011; Hough *et al.*, 2015; Slivka *et al.*, 2010). Similarly, studies have reported negative effects of IT on sT (Jürimäe *et al.*, 2004) but others have found no change (Bresciani *et al.*, 2011; Slivka *et al.*, 2010). Nonetheless, this is the first study to investigate these responses in Paralympic athletes. It

appears, based on the current findings, that the effects of IT on salivary hormones are not significantly disparate to AB athletes.

As shown in Chapter 4, sIgA displays an inverse relationship to weekly training duration in paratriathletes. However, during IT there was no significant change in sIgA concentration. Similarly, URI incidence was unchanged by IT indicating there was likely no depression of mucosal immunity. Coutts *et al.* (2007b) had suggested that sIgA was a sensitive tool in the detection of OR. This is due to the proposed relationship between high TL, sIgA and URI incidence (Meeusen *et al.*, 2013; Walsh *et al.*, 2011). However, the studies of Halson *et al.* (2003) and Papacosta *et al.* (2013), in which participants were deliberately overreached via a period of IT, showed no significant changes in sIgA. Also, Slivka *et al.* (2010) noted no change in sIgA in a group who showed no signs of OR after IT. Moreover, Born *et al.* (2017) recently stated that the mucosal immune system actually positively adapts to IT by increasing IgA SR. Here, the first evidence is provided in Paralympic athletes.

Participants in the current study perceived their sleep quality and duration to be higher during IT. However, the use of subjective sleep parameters has been questioned. Hausswirth *et al.* (2014) note that in a group of overreached triathletes, sleep quality was degraded as measured via actigraphy, yet perceived sleep quality was unchanged. Furthermore, the authors state that changes in sleep variables are small and thus require extensive monitoring for the detection of OR (Hausswirth *et al.*, 2014). As such, due to the limited objective information gathered on participants' sleep, it is not possible to confirm the increase in sleep quality and quantity, possibly as a reduction of external life stresses, or if participants' subjective reports were inaccurate. Further research should seek to further investigate the link between sleep and stress/recovery because, as stated in a recent review, sleep quality is typically impaired during training camps (Gupta *et al.*, 2017).

Resting HR in the present study was unchanged by IT; however, this is not an unexpected finding as previous studies have also reported RHR to be unrelated to IT. For example, Killer *et al.* (2017) reported no change in morning RHR after a nine-day IT period in trained cyclists, despite evidence of impaired performance. This is despite proposals that HR may be altered by IT due to a negative adaptation of the autonomic nervous system (Bosquet *et al.*, 2008). One reason for the lack of relationship between RHR and IT may be due to the low signal:noise ratio reported by ten Haaf *et al.* (2017) in their study of overreached cyclists.

Specifically, variation in self-recorded RHR, as a result of insufficient measurement control, may have masked any changes in response to IT (ten Haaf *et al.*, 2017).

It has previously been proposed that psychological measures provide the best option for early detection of OR (Bresciani *et al.*, 2011; Coutts *et al.*, 2007a; Hough *et al.*, 2013; Papacosta *et al.*, 2013). Accordingly, in the current study where OR was likely not present, psychological measures showed either little change or slight improvements. There was no significant change in athletes' self-reported motivation, muscle soreness or energy status. The aforementioned measures have been used previously by Buchheit *et al.* (2013) and have been linked to athletes' TL, albeit in a group that was also unlikely to have been overreached. Similarly, there was very little change in athletes' POMS profile over the study period. In fact, there was a decrease in the anger subscale during IT. This again adds support to the lack of OR as previous studies have found a relationship between increases in POMS negative subscale scores and OR (Bresciani *et al.*, 2011; Halson *et al.*, 2003; Killer *et al.*, 2017). Finally, responses to the RESTQ-S indicate that during IT there was a decrease in total, general and sport-specific stress with a concomitant increase in general recovery. The results for the RESTQ-S are particularly pertinent as it supports the notion that during IT, external life stresses were minimised despite the increase in TL. Hough *et al.* (2015) also employed the RESTQ-S to assess the responses of AB triathletes undergoing a 10-day training camp and noted no change in the subscales. The authors proposed that the triathletes were able to cope with the increased TL which is also likely the case in the present study due to coaches' careful structuring of training and minimisation of life stresses. Whilst psychological measures may be the most sensitive parameter in response to IT, it is worth noting that the time commitment to complete large item questionnaires such as the POMS or RESTQ-S may limit their applicability to elite athletes. As such, more concise but still sensitive measures may be explored.

This is the first study that has reported responses to IT in a group of Paralympic endurance athletes. Whilst the topic has been extensively researched in AB sports (Meeusen *et al.*, 2013) little is known from those with physical impairments. Paralympic athletes may be at particular risk of OR due to factors that increase the likelihood of excessive overload such as movement inefficiencies (Blauwet *et al.*, 2017; Ward & Meyers, 1995), as previously discussed. Although the population group in the current study only included one athlete with an SCI, there has previously been shown to be no significant difference in acute sC and sT responses to exercise between AB athletes and those with an SCI (Leicht *et al.*, 2017), thus there is no reason why this athlete may obscure the results. Additionally, sIgA has been shown

to display similar variance between Paralympic and AB athletes (see Chapter 4 and Leicht *et al.*, 2012). Furthermore, the use of the POMS has been validated in male and female Paralympic athletes of mixed impairments (Horvat *et al.*, 1986) although this is not yet the case for the RESTQ-S. Nonetheless, it can be assumed that all measures used in the present study were applicable to Paralympic athletes and that results were not confounded by participants' impairments.

The present study aimed to report a range of responses to IT, previously linked to OR, in a group of elite paratriathletes. From the results it is unlikely OR was present. Nonetheless, the lack of a maximal sport-specific performance test means this cannot be confirmed as a reduction in performance is the main outcome of OR. A performance test was not included to minimise disruption to athletes' pre-season training schedule. Additionally, the usefulness of performance tests was questioned previously due to the additive effect they can have on residual fatigue. Also, a lack of control group prevented certainty that results were due to IT rather than seasonal variation; as such, this is a consideration for future research.

5.6 Conclusions

Despite increases in TL, similar to previously published studies, the paratriathletes in the current study displayed no signs of OR. There was little to no change in hormonal, immunological or physiological parameters commonly associated with the overreached state. In fact, participants displayed positive psychological changes that may have related to the training camp setting of IT minimising external life stresses and the careful management of TLs from coaches. Nonetheless, it is still not understood how TL can be optimised to prevent performance impairment in challenging environments, such as high ambient temperatures and humidity.

6

Thermoregulatory responses to paratriathlon competition in the heat

6.1 Abstract

Purpose: To describe the thermoregulatory profile of paratriathlon competition in the heat as athletes face a heightened risk for excessive strain due to their physical impairments.

Methods: Core temperature was recorded at 30 s intervals in 28 mixed-impairment paratriathletes during competition in a hot environment (33°C, 35-41% RH), via ingestible temperature sensor (BodyCap e-Celsius). Furthermore, in a subset of 9 athletes, T_{sk} was measured. Athletes' wetsuit use was noted whilst heat illness symptoms were self-reported post-race.

Results: Twenty-two athletes displayed a $T_c \geq 39.5^\circ\text{C}$ with 8 athletes $\geq 40.0^\circ\text{C}$. There were increases across the average T_c for swim, bike and run ($p \leq 0.016$). There was little change in T_{sk} during the race ($p \geq 0.299$). PTVI athletes displayed a significantly greater T_c during the run section than PTWC athletes ($p \leq 0.021$). Athletes wearing a wetsuit had a greater T_c when swimming ($p \leq 0.032$) whilst those reporting heat illness symptoms displayed a greater T_c at various timepoints ($p \leq 0.046$).

Conclusions: Paratriathletes face significant thermal strain during competition in the heat, as evidenced by high T_c , relative to previous research in AB athletes, and a high incidence of self-reported heat illness symptomatology. Differences in the T_c profile exist depending on athletes' race category and wetsuit use.

6.2 Introduction

As mentioned in Chapter 2.5, competitive triathlon and paratriathlon events are commonly held in environments with high ambient temperatures and humidity. However, it has long been understood that endurance exercise performance is impaired in situations with added thermal load (Ely *et al.*, 2009; Friesen *et al.*, 2018; Galloway & Maughan, 1997; Levels *et al.*, 2014; Périard & Racinais, 2016). Endurance events require high relative intensities to be sustained for extended periods of time. This increases the likelihood of athletes developing substantial hypohydration, cardiovascular strain, elevated whole-body (core and skin) temperature, reliance upon carbohydrate metabolism, perception of effort and thermal strain, resulting in fatigue, under-performance or even heat illness (Daanen *et al.*, 2018; Guy *et al.*, 2014; Périard *et al.*, 2015; Périard & Racinais, 2016).

Prolonged exercise in hot environments limits the capacity of the body to dissipate heat via evaporative and non-evaporative pathways (Racinais *et al.*, 2015a; Taylor & Cotter, 2006). As heat gain exceeds heat loss elevations in T_c and T_{sk} occur. Such elevations have been attributed to well-documented performance impairments in the heat through central and peripheral processes (Racinais *et al.*, 2017). Furthermore, elevated body temperatures have been implicated in the development of heat illness (Lausen *et al.*, 2006). Whilst laboratory-based studies have well defined the thermoregulatory strain imposed on AB athletes (Galloway & Maughan, 1997), these studies lack applicability to field-based competitive races (Laursen *et al.*, 2006; Lee *et al.*, 2010; Veltmeijer *et al.*, 2015). Consequently, research has sought to characterise the thermoregulatory responses to outdoor competition in the heat (Baillot & Hue, 2015; Del Coso *et al.*, 2014; Laursen *et al.*, 2006; Lee *et al.*, 2010; Veltmeijer *et al.*, 2015). This is in accordance with the recommendations to characterise the sport- and event-specific thermal strain profiles of international athletes competing in the heat using ingestible temperature sensors (Bergeron *et al.*, 2012).

Several studies have now presented the thermoregulatory responses to AB triathlon races of varying lengths (Baillot & Hue, 2015; Del Coso *et al.*, 2014; Laursen *et al.*, 2006) although no research has sought to examine competitive paratriathlon events. This work has shown that AB triathletes' T_c can reach $38.4 \pm 0.7^\circ\text{C}$ (Baillot & Hue, 2015) or $38.8 \pm 0.7^\circ\text{C}$ (Del Coso *et al.*, 2014) at the end of half-Ironman races in the heat ($27\text{-}29^\circ\text{C}$). Furthermore, by acquiring in-race T_c readings, Baillot and Hue (2015) noted that T_c : rose during swimming because of a limited capacity for heat dissipation in warm water; plateaued on the bike due to significant convective cooling through a high cycling velocity and drafting illegality increasing

air flow and rose again when running as a result of limited convective cooling and a high metabolic rate (Baillot & Hue, 2015). Moreover, a similar trend has been presented in laboratory studies during sprint (Peeling & Landers, 2007) and Olympic distance events (Kerr *et al.*, 1998). Whilst these studies built on research confined to post-race T_c measurement, data are still restricted to infrequent T_c sampling during field-based research. Furthermore, although studies have attempted to determine the correlates to in-race T_c (Baillot & Hue, 2015; Byrne *et al.*, 2006; Laursen *et al.*, 2006; Lee *et al.*, 2010; Veltmeijer *et al.*, 2015), as no study has researched Paralympic athletes, it is not possible to discern any relationship between physical impairment and thermal strain imposed.

The finite literature concerning thermoregulation in Paralympic athletes has centred on those with an SCI due to their high propensity for thermal strain (Griggs *et al.*, 2017). Research has characterised the T_c responses to wheelchair rugby and tennis (Griggs *et al.*, 2017; Veltmeijer *et al.*, 2014) whilst Abel *et al.* (2006) presented a peak rectal temperature of 40.4°C at the end of a 42 km handcycling race in temperate (20.0 to 22.0°C) conditions. However, as noted earlier (Chapter 2.5), other impairment groups are also at risk of thermal strain when competing in the heat. Athletes with amputations, CP or VI face: limited body surface area for heat loss due to missing limbs, skin grafts or wearing prosthetic liners (Crandall & Davis, 2010; Klute *et al.*, 2007; Webborn, 1996); increased metabolic heat production (Maltais *et al.*, 2004; Ward & Meyers, 1995); impaired venous return (Kloyiam *et al.*, 2011) and impaired pace or hydration awareness (Runciman *et al.*, 2016; Taylor *et al.*, 2016; Webborn & Van de Vliet, 2012). Yet, these impairments are severely understudied despite athletes' commonality in Paralympic sports. Thus, it is pertinent to better describe the thermoregulatory strain profiles of paratriathlon using ingestible temperature pills, in line with the recommendations of Bergeron *et al.* (2012). Consequently, the aims of this study were to characterise the T_c and T_{sk} responses to paratriathlon competition in the heat.

6.3 Methods

6.3.1 Participants

Twenty-eight paratriathletes (17 males, 11 females; age 31 ± 8 y) volunteered to participate in the present study. Athletes' paratriathlon world ranking at the time of the study was 1st to 24th place. Athletes were competing in the 2017 Iseo-Franciacorta ITU Paratriathlon World Cup (8th July) or 2018 Iseo-Franciacorta ITU World Paratriathlon Series (30th June) (Iseo, Lombardy, Italy; elevation 185 m). Athletes were asked to provide details of their training for

the preceding four weeks to determine heat acclimatisation or acclimation status. Athletes provided written informed consent and the procedures were approved by the Loughborough University Ethical Advisory Committee whilst permission for data collection was granted by the ITU.

6.3.2 Study design

The race consisted of a single lap, 750 m open-water lake swim, 21 km (3 laps of 7 km) non-drafting cycle and a 4.8 km run (2017: 3 laps of 1.6 km; 2018: 4 laps of 1.2 km). The cycle course was largely flat with an elevation change of ~49 m per lap. The races started between 16:00-16:30 (2017 event) and 17:00-18:30 local time (2018 event), depending on athletes' race category. Environmental conditions during the 2017 race were: 33°C, 41% RH, water temperature 27°C and wind velocity 11 km·h⁻¹ and during the 2018 race were: 33°C, 35% RH, water temperature 25°C and wind velocity 18 km·h⁻¹ (RH390, Extech Instruments, Nashua, New Hampshire, USA). The cloud cover, whilst not measured, was minimal, and no precipitation was recorded during either race. Due to the similar environmental conditions, data are pooled into one group. All athletes wore a swim-cap when swimming. Twelve athletes wore a trisuit for the whole race, with cycling shoes during cycling and trainers when running, whilst sixteen athletes chose to additionally wear a wetsuit during the swim stage.

Athletes were provided with a telemetric T_c pill (e-Celsius, BodyCap, Caen, France) which they were asked to ingest ~6 h pre-race in line with the recommendation of Byrne and Lim (2007). The e-Celsius device is capable of measuring and storing T_c data at pre-defined intervals before subsequent data download (Chapter 3.5). Thus, it provided a greater T_c sampling frequency than previous field-based studies (Byrne *et al.*, 2006), with T_c recorded at 30 s intervals. Athletes were free to warm-up and complete the race as normal. Similarly, athletes were free to consume fluids *ad libitum* during the race, but intake was self-reported post-race. No advice was provided regarding hydration or pacing to avoid interference with athletes' habitual race routines (Del Coso *et al.*, 2014). Immediately post-race, athletes reported to investigators for T_c data download. Athletes were asked to state if they had felt any symptoms of heat illness during the race such as confusion, dizziness, fainting, muscle or abdominal cramps, nausea, vomiting, diarrhoea, heat sensations on the head or neck, chills or stopping sweating (Coris *et al.*, 2006).

Athletes' segment times for swim, cycle, run and transitions were recorded by the race organiser and published online. Further, a group of 15 athletes raced with global positioning

system (GPS) devices (Garmin, Kansas, USA) to allow greater precision of bike or run in-race performance changes. Of the 15 athletes, 14 had GPS data for the bike and 6 had data for the run.

In a subset of 9 athletes, T_{sk} was measured via temperature loggers (DS1922L ThermoChron iButton[®]) placed on the *pectoralis major* and *rectus femoris* muscle belly under waterproof adhesive patches (Tegaderm +Pad, 3M, St. Paul, Minnesota, USA). This permitted the calculation of weighted mean T_{sk} using an adaptation of the methods of Ramanathan (1964) whereby T_{sk} was taken as $0.6(\textit{pectoralis major} \text{ temperature}) + 0.4(\textit{rectus femoris} \text{ temperature})$. These sites were chosen to minimise any distraction to athletes when racing and the risk of loggers coming loose.

6.3.3 Data analyses

Athletes were grouped based on their impairment into one of four groups: PTWC for wheelchair athletes ($n=9$), NEURO for athletes with a neurological impairment ($n=6$ PTS2 and PTS3 athletes), AMP for athletes with an amputation ($n=7$ PTS2-PTS5 athletes) and PTVI for athletes with a VI ($n=6$). They were also grouped based on self-reported heat acclimatisation/acclimation status, wetsuit use and self-reporting of heat illness symptoms.

Athletes' T_c during the race was averaged into modality-specific segments for swim, bike, run and transitions: $SWIM_{av}$, $BIKE_{av}$, RUN_{av} , T1, T2. Further, actual temperatures for immediately pre-race (PRE), the end of the swim ($SWIM_{end}$), the end of each bike lap ($BIKE_1$, $BIKE_2$, $BIKE_3$), midway through the run section (RUN_{mid}) and the end of the run (RUN_{end}) were calculated (assuming evenly paced efforts where GPS data was not present). Absolute changes in T_c were calculated for each race segment: $SWIM_{\Delta}$, $BIKE_{\Delta}$, RUN_{Δ} .

Where GPS data are available, athletes' split times for each bike lap and for each half of the run section were relativised to expected splits from an evenly paced effort (overall bike time $\div 3$, overall run time $\div 2$). Consequently, the relative deviation in segment performance from an even pace was calculated.

6.3.4 Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics 23.0 software (IBM, New York, USA). Statistical significance was set at $p < 0.05$. Data were checked for normal distribution using the Shapiro-Wilk between-group test and for homogeneity of variance using

Levene's test. Where sphericity could not be assumed for within-measures components, the Greenhouse-Geisser correction was used.

Changes in T_c and T_{sk} during the race were assessed as a group via one-way ANOVA. Similarly, to determine any relationship with athletes' impairment group, acclimatisation/acclimation status, wetsuit use and reporting of heat illness symptoms, changes in T_c were assessed via two-way ANOVA with group and time factors. Spearman's correlation coefficient was employed to determine the degree of correlation to peak T_c from: T_c during race time points; changes in T_c ; race performance; fluid intake; body mass and paratriathlon world ranking. To determine the influence of T_c on in-race performance characteristics when GPS data was available, correlational analyses were performed using Spearman's correlation coefficient. Correlations were established between T_c at set time points, the time taken to complete race segments and the relative deviation in segment performance.

6.4 Results

The mean \pm SD time to complete the race was 74.9 ± 11.2 min with a range of 54.6 to 103.9 min. The times for swim, bike and run were 14.3 ± 2.6 min (10.5 to 20.0 min), 36.4 ± 6.0 min (28.8 to 52.9 min) and 21.1 ± 5.1 min (13.1 to 36.2 min), respectively.

As a group, there was a significant change in T_c over time. Specifically: PRE was lower than all other time points ($p < 0.001$); RUN_{mid} was greater than $SWIM_{end}$, T1 and T2 ($p \leq 0.039$); RUN_{end} was greater than all other time points ($p \leq 0.031$); whilst there was a significant increase across PRE, $SWIM_{av}$, $BIKE_{av}$ and RUN_{av} ($p \leq 0.016$) (Figure 6.1). There was an impairment-specific interaction as RUN_{Δ} , changes in T_c T2- RUN_{mid} and RUN_{mid} - RUN_{end} were significantly greater for PTVI than PTWC ($p \leq 0.021$) (Table 6.1). Fourteen of the twenty-eight athletes reported being heat acclimatised/acclimated prior to the study. There was a significant difference between those reporting being heat acclimatised/acclimated or not as those who were, displayed a greater RUN_{end} (39.78 ± 0.55 vs $39.22 \pm 0.41^\circ\text{C}$) and change in T_c RUN_{mid} - RUN_{end} (0.47 ± 0.30 vs $0.16 \pm 0.14^\circ\text{C}$) ($p \leq 0.044$). There was a difference in T_c depending on athletes' wetsuit use as those that wore a wetsuit had significantly greater $SWIM_{end}$ (38.76 ± 0.40 vs $38.31 \pm 0.40^\circ\text{C}$) and $SWIM_{av}$ (38.45 ± 0.34 vs $38.03 \pm 0.35^\circ\text{C}$) ($p \leq 0.032$). Sixteen athletes reported experiencing symptoms of heat illness during the race, of which ten wore a wetsuit. Those that were symptomatic had a significantly greater $SWIM_{end}$, $SWIM_{av}$, $SWIM_{\Delta}$ (0.80 ± 0.50 vs $0.38 \pm 0.37^\circ\text{C}$), T1, $BIKE_1$, $BIKE_2$, $BIKE_{av}$ and RUN_{av} than those asymptomatic ($p \leq 0.046$) (Figure 6.2).

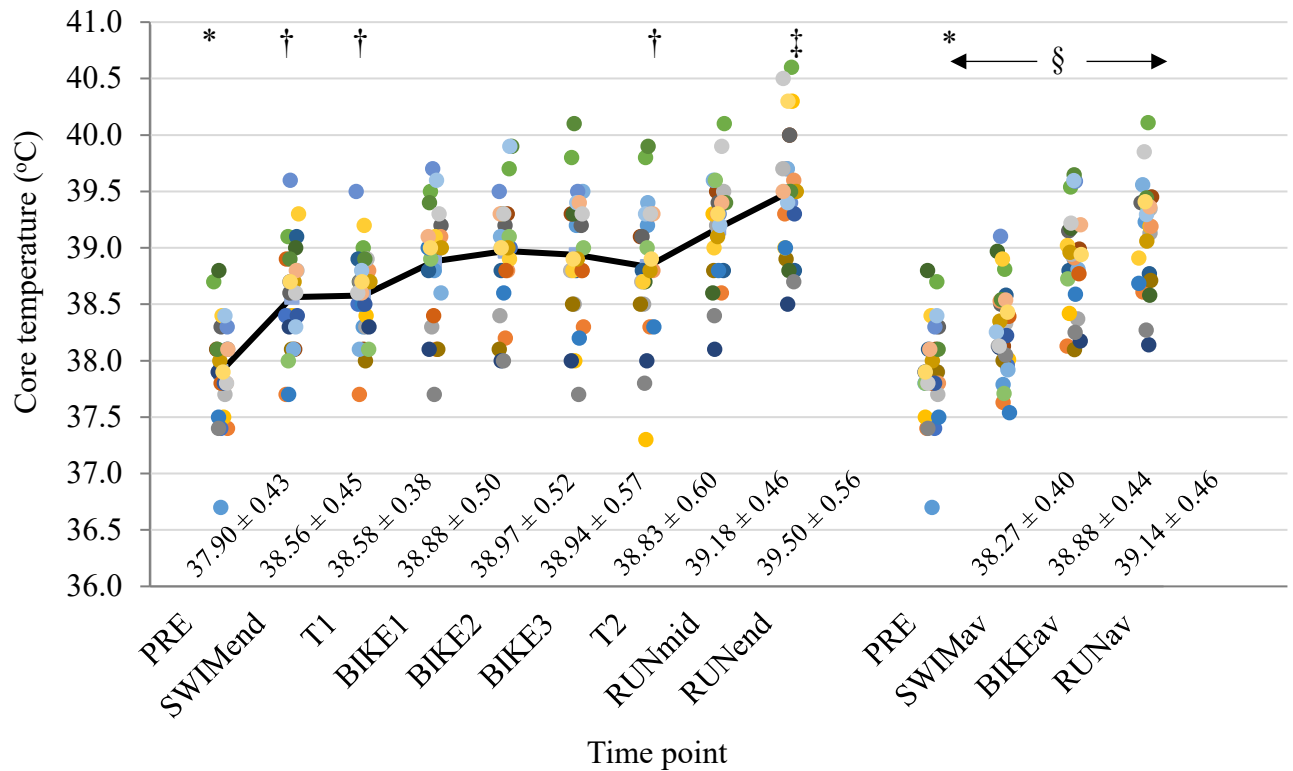


Figure 6.1: Whole-group core temperature changes throughout the race. Dots are individual data; black line indicates group mean. *Significantly lower than all other time points ($p < 0.001$). †Significantly lower than RUN_{mid} ($p \leq 0.039$). ‡Significantly greater than all other time points ($p \leq 0.031$). §Significantly greater at each time point ($p \leq 0.016$).

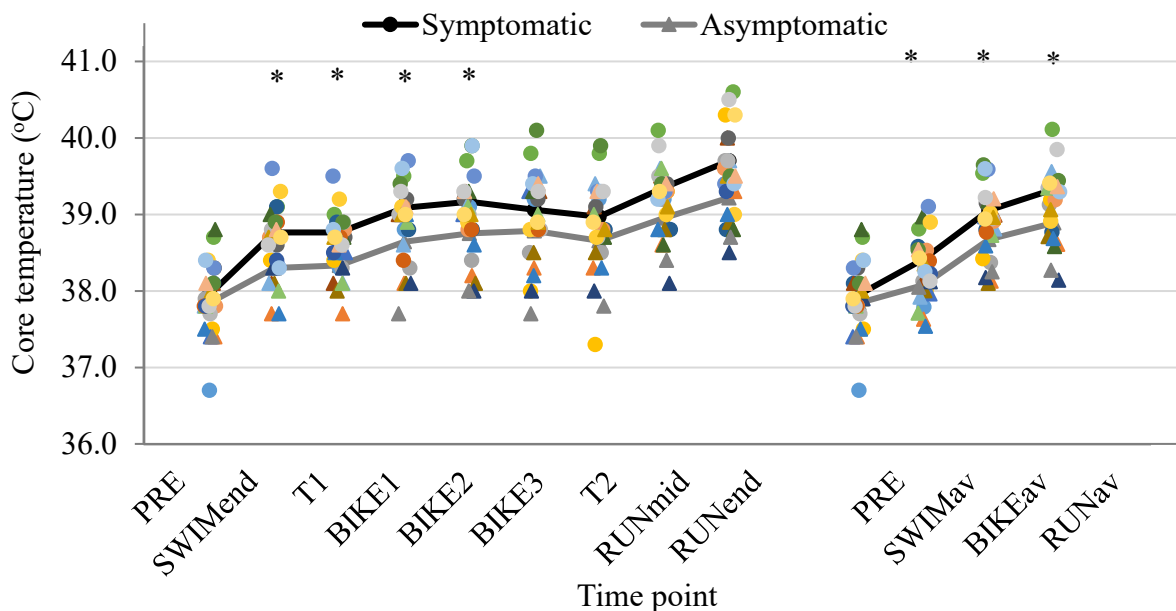


Figure 6.2: Core temperature changes and heat illness symptomatology throughout the race. Circles are symptomatic, triangles are asymptomatic individuals, lines are group means. *Significantly greater in symptomatic group ($p \leq 0.046$).

Table 6.1: Changes in core temperature during the race. Data are mean \pm SD. *Significantly greater in PTVI than PTWC ($p \leq 0.021$).

Impairment	SWIM $_{\Delta}$ (°C)	T1-BIKE $_1$ (°C)	BIKE $_1$ -BIKE $_2$ (°C)	BIKE $_2$ -BIKE $_3$ (°C)	BIKE $_{\Delta}$ (°C)	T2-RUN $_{mid}$ (°C)	RUN $_{mid}$ -RUN $_{end}$ (°C)	RUN $_{\Delta}$ (°C)
PTWC	0.49 \pm 0.59	0.37 \pm 0.33	0.20 \pm 0.20	0.07 \pm 0.30	0.64 \pm 0.51	0.07 \pm 0.15	0.19 \pm 0.11	0.26 \pm 0.15
NEURO	0.83 \pm 0.37	0.15 \pm 0.19	-0.08 \pm 0.17	-0.02 \pm 0.12	0.05 \pm 0.33	0.43 \pm 0.29	0.30 \pm 0.26	0.73 \pm 0.44
AMP	0.45 \pm 0.38	0.43 \pm 0.38	0.00 \pm 0.28	-0.10 \pm 0.22	0.33 \pm 0.61	0.35 \pm 0.26	0.15 \pm 0.26	0.50 \pm 0.36
PTVI	0.60 \pm 0.57	0.18 \pm 0.82	0.15 \pm 0.17	-0.10 \pm 0.14	0.23 \pm 0.92	0.43 \pm 0.16*	0.60 \pm 0.29*	1.03 \pm 0.25*

Analysis of athletes' peak T_c revealed 26 athletes $\geq 39.0^\circ\text{C}$, 22 athletes $\geq 39.5^\circ\text{C}$, 8 athletes $\geq 40.0^\circ\text{C}$ and 2 athletes $\geq 40.5^\circ\text{C}$. There were significant positive correlations between peak T_c and: change in T_c T1-BIKE₁ ($p=0.001$), BIKE₁ ($p<0.001$), BIKE₂ ($p<0.001$), BIKE₃ ($p=0.003$), BIKE_{av} ($p=0.001$), T2 ($p=0.016$), RUN_{mid} ($p<0.001$), RUN_{end} ($p<0.001$), RUN_{av} ($p<0.001$), change in T_c RUN_{mid}-RUN_{end} ($p=0.036$). There was no significant correlation between peak T_c and overall race finishing time, race segment times, finishing position, fluid intake or body mass ($p\geq 0.143$).

From an in-race performance perspective, there were significant inverse correlations between swim performance and BIKE₂ ($p=0.025$; Figure 6.3a) and between the relative performance deviation in the first half of the run and RUN_{mid} ($p=0.030$; Figure 6.3b). There were no other interactions between in-race performance changes and T_c ($p\geq 0.067$).

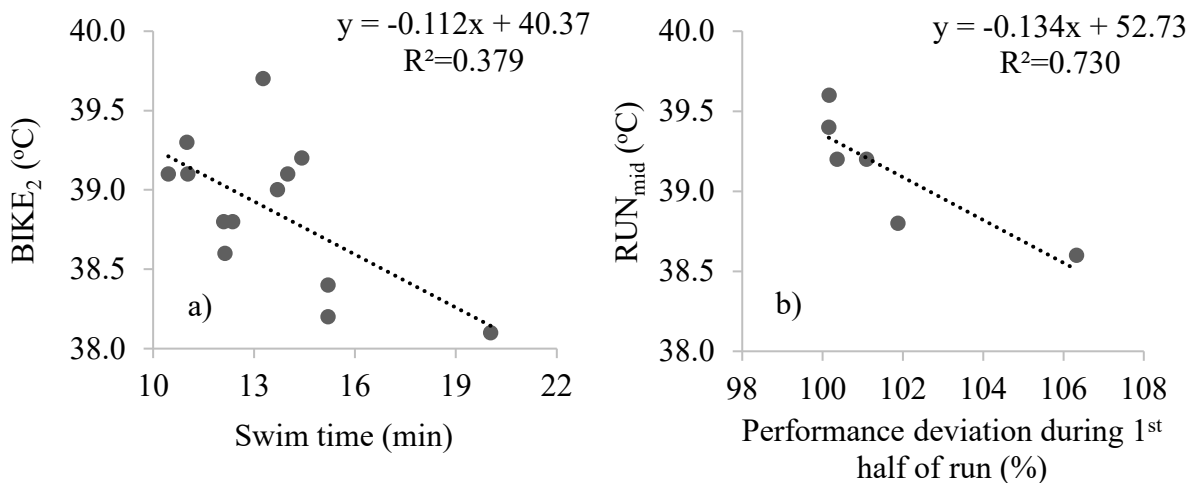


Figure 6.3a: The relationship between swim performance and core temperature after the second bike lap (BIKE₂) ($n=13$). Figure 6.3b: The relationship between the performance deviation during the first half of the run, relative to the whole run, and core temperature midway through the run (RUN_{mid}) ($n=6$).

Whilst there was a significant difference in T_{sk} over time ($p=0.033$), there were no significant pairwise comparisons between timepoints ($p\geq 0.299$) (Figure 6.4). There was a significant difference in changes in T_{sk} across race swim, bike and run as the change was greater during the bike than swim (-1.70 ± 1.15 vs. $-0.02 \pm 0.87^\circ\text{C}$; $p=0.043$).

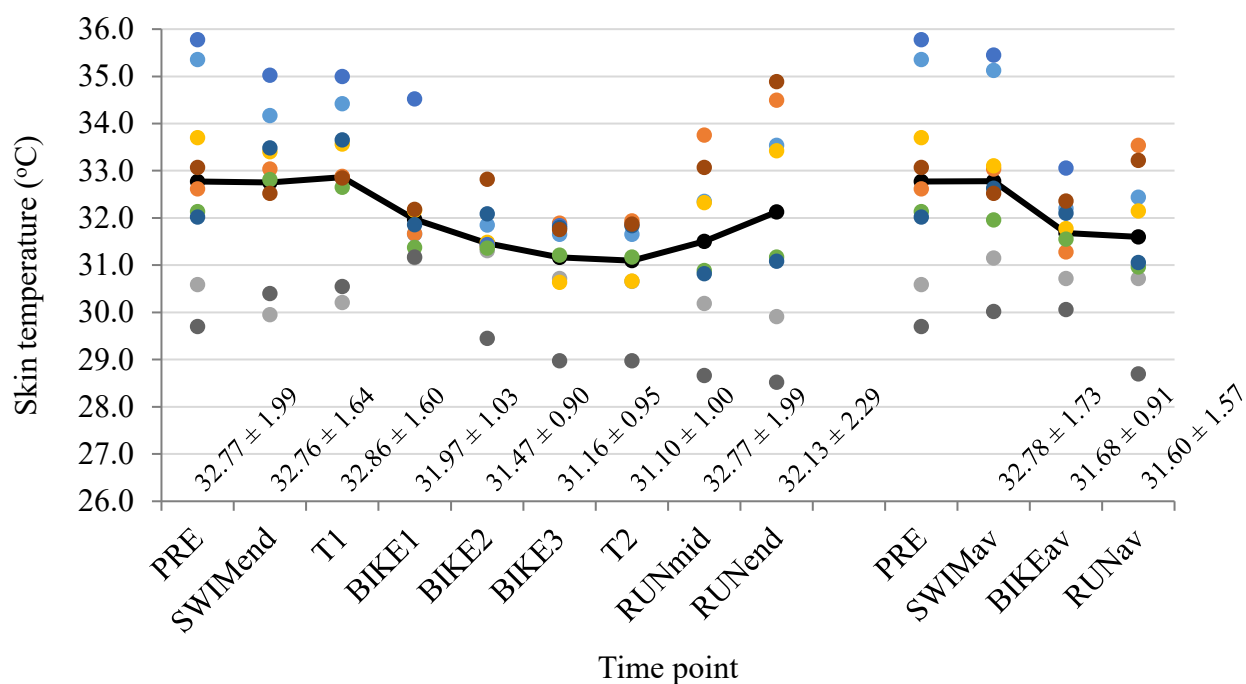


Figure 6.4: Skin temperature changes throughout the race. Dots are individual data; black line indicates group mean.

6.5 Discussion

This is the first study to characterise the thermoregulatory strain imposed by field-based paratriathlon performance in the heat via continuous T_c measurement. Paratriathletes face significant thermoregulatory strain as shown by 22 of the 28 athletes displaying a peak $T_c \geq 39.5^\circ\text{C}$, of which were 8 athletes $\geq 40.0^\circ\text{C}$. Furthermore, a high proportion of athletes experienced self-reported symptoms of heat illness that may be related to wetsuit use which effected the early rise in T_c during the first phase of the race.

During the race, T_c was significantly elevated from pre-race with marked increases during the run segment, as consistent with previous research of AB athletes (Baillot & Hue, 2015), albeit with a greater sampling frequency. Moreover, considering race segment averages, there was a significant rise in T_c throughout the race. Given the impairment types within paratriathlon, it was not surprising to find T_c greater than previously reported in AB half-Ironman events (e.g., peak temperatures of $\sim 38.6^\circ\text{C}$) (Baillot & Hue, 2015; Del Coso *et al.*, 2014). As explained previously (Chapter 2.5), this population may display myriad impairments that diminish thermoregulatory capacity and thus elevate T_c during competition, relative to AB athletes. Moreover, the studies of Baillot & Hue (2015) and Del Coso *et al.* (2014) were conducted in cooler ($27\text{-}29^\circ\text{C}$) but more humid ($73\text{-}80\%$ RH) environments than reported here;

this likely resulted in differing capacities for heat loss. Additionally, due to the significantly greater race durations for half-Ironman events (~320 min) than is typical in paratriathlon races, the relative intensity is markedly lower in longer races, thus representing a discrepant metabolic heat production (Périard *et al.*, 2015). The proportion of athletes with a peak $T_c \geq 39.0^\circ\text{C}$ (93%) was slightly less than in previous studies of AB athletes running a half-marathon in a tropical climate (96-100%) (Byrne *et al.*, 2006; Lee *et al.*, 2010). Furthermore, in these studies there was a greater proportion of individuals with a $T_c \geq 40.0^\circ\text{C}$ (40-56%) which is likely due to the greater oppressiveness of the environment and greater heat production from running for >90 min. However, in the current study, the percentage of athletes $\geq 40.0^\circ\text{C}$ (29%) was still higher than AB individuals running 15 km in a temperate environment (13%) (Veltmeijer *et al.*, 2015) or elite AB cyclists in a recent World Championships in a hot, dry climate (25%) (Racinais *et al.*, 2018). These findings provided context to the level of strain faced by paratriathletes in the heat.

To date, research typifying the thermoregulatory strain of Paralympic sports has been predominantly confined to wheelchair court sports of athletes with an SCI (Abel *et al.*, 2006; Griggs *et al.*, 2017; Veltmeijer *et al.*, 2014). The sole study of a Paralympic endurance sport described a peak T_c of 40.4°C at the end of a 42 km race, in temperature conditions (20.0 to 22.0°C), in a single, male, handcyclist with an SCI (Abel *et al.*, 2006). Previous work has noted that T_c elevations are greater in those with an SCI, relative to non-SCI, during wheelchair rugby or wheelchair tennis (Griggs *et al.*, 2017; Veltmeijer *et al.*, 2014). This study, which now extends scientific understanding by including athletes of mixed impairments, found there were differences in the temperature responses between impairment groups. Specifically, changes in T_c when running were significantly greater for PTVI than PTWC, comprised mostly of athletes with an SCI. Thus, this finding contradicts previous reports. This is presumably due to the considerably disparate race demands across paratriathlon, depending on athletes' race categories. PTWC athletes displayed a greater BIKE_Δ compared to ambulant race categories. This is likely to result from a lower surface area for convective heat loss, a closer proximity to the road surface for radiant heat gain and longer segment durations when handcycling. However, during the run segment, PTWC athletes in a racing wheelchair utilise less active musculature and travel at a greater velocity, thus are exposed to greater air flow for convective heat loss, compared to ambulant runners. Therefore, it is not surprising that changes in T_c were significantly greater for PTVI athletes when running compared to those in PTWC as heat dissipative potential was lower and heat production was higher.

ITU paratriathlon races currently permit wetsuit use up to a water temperature of 28°C. Here, it was shown that wetsuit use resulted in a significantly elevated SWIM_{end} and SWIM_{av} T_c. It is noteworthy that of the 16 athletes reporting symptoms of heat illness, 10 wore a wetsuit. Moreover, those that were symptomatic also displayed a significantly greater SWIM_{end}, SWIM_{av}, SWIM_Δ, T1, BIKE₁, BIKE₂, BIKE_{av} and RUN_{av}. A high T_c rise early in exercise has already been suggested to be implicated in heat illness genesis (Byrne *et al.*, 2006) and has been supported presently. Furthermore, the incidence of heat illness symptomatology was greater in the current study than elsewhere in AB athletes (Baillot & Hue, 2015; Lee *et al.*, 2010; Racinais *et al.*, 2015b). This may relate to paratriathletes' greater susceptibility for excessive thermoregulatory strain due to their physical impairments or lower aerobic fitness (Gardner *et al.*, 1996). Due to the significantly greater T_c at various time points in the heat illness symptomatic group, the negative health consequences of elevated body temperatures during paratriathlon competition are highlighted.

In the present study, there were differences in the T_c responses between those who reported being prior acclimatised or acclimated to the heat. This is the first study to acknowledge this aspect i.e., explore the relationship between acclimatisation/acclimation state and thermoregulation during triathlon competition. Specifically, RUN_{end} T_c and change in T_c RUN_{mid}-RUN_{end} were greater for those with prior chronic heat exposure. Racinais *et al.* (2015b) have previously shown that peak T_c during a 43 km cycling time-trial in the heat was unchanged by heat acclimatisation, albeit with a greater cycling PO than pre-acclimatisation. This may relate to the beneficial thermoregulatory adaptations of chronic heat exposure permitting a greater relative intensity and thus metabolic heat production during racing. However, it is not known how other thermoregulatory variables (e.g., T_{sk}, HR, sweat rate) may have differed between groups in the current study.

Correlates with peak T_c during competition have been assessed previously (Baillot & Hue, 2015; Byrne *et al.*, 2006; Del Coso *et al.*, 2014; Laursen *et al.*, 2006; Lee *et al.*, 2010; Veltmeijer *et al.*, 2015). It has been frequently reported that body mass, finishing time and fluid intake are not related to the highest T_c achieved during races; this is supported by the current findings. Moreover, this study shows that several in-race T_c parameters relate to peak T_c including T_c during the bike and run segments and change in T_c T1-BIKE₁. As peak T_c commonly occurred during the bike and run segments, it is no surprise these time points relate to the peak. Nonetheless, the change in T_c during the first bike lap relating to peak T_c again

highlights the importance of early changes in temperature during competitive events (Byrne *et al.*, 2006).

From athletes' GPS data, there was a significant inverse relationship between athletes' swim performance time and BIKE₂ T_c. This is likely mediated by a greater exercise intensity increasing metabolic heat production. Elsewhere, Laursen *et al.* (2006) note that T_c was greater in quicker swimmers during longer distance triathlon events. Secondly, there was a significant inverse correlation between the relative deviation in the first half of the run segment and RUN_{mid} T_c. Thus, athletes that ran quicker in the first half of the run, relative to the whole run, had a greater T_c. This provides evidence of athlete pacing and relative intensity directly effecting T_c during paratriathlon races. However, overall there were limited correlations between T_c and in-race performance changes, similar to previous work (Ely *et al.*, 2009; Lee *et al.*, 2010; Veltmeijer *et al.*, 2015).

In the subset of athletes in whom T_{sk} was measured, there was a significant change over time, albeit with no pairwise differences. This likely relates to the limited sample size and large variation present. Regardless, a novel feature of this study was to record T_{sk} changes throughout a field-based competitive triathlon. There was a significant difference in the T_{sk} changes across the race segments; specifically, the change was greater during cycling than swimming. The drop in T_{sk} during the cycling segment is presumably due to greater wind velocity augmenting convective cooling at the periphery (Baillot & Hue, 2015). In a laboratory setting, the study of Peeling and Landers (2007) present T_{sk} of 33-35°C during post-swim cycling at 95% AnLT. It is worth noting, however, that Peeling and Landers (2007) employed a fan producing a wind velocity of 16 km·h⁻¹ whilst average cycling velocity was 33 km·h⁻¹ in the current study. Therefore, convective cooling of the skin would expectedly be greater here.

The current study builds on previous research of thermoregulation during competitive sporting events by investigating the sport of paratriathlon whilst utilising regular sampling frequencies. From this, those working with paratriathletes may now understand the T_c time course during racing across impairment groups and choice of wetsuit use. Nonetheless, the small sample sizes limit the ability to determine further impairment-specific responses. Similarly, the restricted number of athletes in whom T_{sk} was measured constrains the likelihood of revealing true changes throughout triathlon races. Lastly, the study relied upon participant reports of several parameters (e.g., fluid intake, heat acclimatisation/acclimation state, heat illness symptoms) which were not confirmed objectively. Of note, a mixed-sex sample is

common in prior studies and not anticipated to influence the findings (Del Coso *et al.*, 2014; Griggs *et al.*, 2017; Veltmeijer *et al.*, 2015).

6.6 Conclusions

Paratriathletes face significant thermoregulatory strain during competition in the heat, as evidenced by high T_c and prevalence of self-reported heat illness symptoms, although the effect on T_{sk} is still ambiguous. Athletes' T_c is typically greatest during the run segment whilst those with a VI display significant increases in T_c during this phase. Finally, it appears that the use of a wetsuit substantially elevates T_c and may be linked to the incidence of heat illness, but this requires verification by controlled laboratory studies or further field trials.

7

Mixed active and passive, heart rate-controlled heat acclimation is effective for Paralympic and able-bodied triathletes

7.1 Abstract

Purpose: To explore the effectiveness of mixed, active and passive HA, controlling the relative intensity of exercise by HR in paratriathletes and determine adaptation differences to AB triathletes.

Methods: Seven paratriathletes and thirteen AB triathletes undertook an 8-d HA intervention consisting of five, HR-controlled sessions and three passive heat exposures. On the first and last day of HA, HSTs were conducted whereby thermoregulatory changes were recorded at a fixed, submaximal workload. The AB group undertook 20 km cycling time trials pre- and post-HA with performance compared to an AB control group.

Results: In both groups, HA resulted in lower T_c , BLa, RPE and TS during the HST with concomitant PV expansion ($p \leq 0.047$). In the AB group, a lower T_{sk} and HR with a greater sweat rate was evident post-HA ($p \leq 0.045$) but this was not present for the paratriathlon group ($p \geq 0.177$). The AB group improved their performance by an extent greater than the smallest worthwhile change based on the normal variation present with no HA (4.5 vs. 3.7%).

Conclusions: Paratriathletes are capable of displaying partial HA, albeit not to same extent as AB triathletes. The HA protocol used was effective at stimulating thermoregulatory adaptations with performance changes noted in AB triathletes.

7.2 Introduction

Competitive triathlons and other major sporting events are commonly held in hot and/or humid environments (Chapter 6). Therefore, strategies are commonly sought to attenuate the performance impairment typical of endurance performance in the heat and humidity (Daanen *et al.*, 2018). One such strategy that has been used by athletes is HA, which refers to procedures used to elicit favourable physiological adaptations to heat stress using artificial conditions (Zurawlew *et al.*, 2015). HA can invoke myriad positive physiological adaptations which improve heat tolerance. Commonly reported adaptations include lower: T_c and T_{sk} ; submaximal HR; carbohydrate metabolism; sweat electrolyte content; also, increased sweat rate, PV expansion and perceptual (RPE and TS) alterations with a resultant improved performance in the heat (Corbett *et al.*, 2014).

Heat acclimation typically involves daily, or alternate days, of heat stress over a 5- to 16-day period whereby T_c , T_{sk} and sweat rate is elevated for 1-2 h (Zurawlew *et al.*, 2015). Typically, the absolute exercise intensity had been held constant across HA (Febbraio *et al.*, 1994; Houmard *et al.*, 1990; Lorenzo *et al.*, 2010; Nielsen *et al.*, 1997; Schmit *et al.*, 2018; Wingfield *et al.*, 2016). However, it has been proposed that as participants begin to acclimate, the relative heat stress and stimulus of a set work load diminishes (Taylor *et al.*, 2014). As such, isothermic protocols have recently been employed to produce a constant heat stress across HA (Gibson *et al.*, 2015; Neal *et al.*, 2016; Ruddock *et al.*, 2016). Using this approach, the external workload is manipulated within- and between-HA sessions to maintain a constant T_c of $\sim 38.5^\circ\text{C}$. However, it is worth noting that isothermic protocols require constant measurement of T_c which brings financial burdens or discomfort from measuring rectal or oesophageal temperature. Consequently, controlling HA intensity using HR has been proposed by Périard *et al.* (2015) as a practical method of maintaining a constant cardiovascular stimulus. Based on evidence that HR is unchanged through isothermic HA (Garrett *et al.*, 2012; Magalhães *et al.*, 2010; Zurawlew *et al.*, 2015), this method of controlling the relative intensity would result in a constant thermal load during HA. Initial evidence suggests this approach may be efficacious in invoking HA in football players (Philp *et al.*, 2017).

Furthermore, commonly studied HA protocols may not be appropriate for elite athletes in preparation for competition, especially in a multi-modal sport such as triathlon. This is due to the protocols typically involving consecutive days of exercise in the heat which does not fit with the weekly training distribution of athletes tapering into competition (Mujika, 2011). As

such, passive HA has recently been explored (Stanley *et al.*, 2015; Zurawlew *et al.*, 2015; Zurawlew *et al.*, 2018). Using post-exercise heat exposures, studies have displayed that positive adaptations are achievable without excessive physical stress (Stanley *et al.*, 2015; Zurawlew *et al.*, 2015; Zurawlew *et al.*, 2018). However, whilst passive HA can stimulate positive responses such as PV expansion, lower T_c and lower T_{sk} , it has been questioned whether it can provide full heat adaptation (Périard *et al.*, 2015; Taylor & Cotter, 2006; Tyler *et al.*, 2016). As such, Guy *et al.* (2014) state that for athletes to optimally adapt to the heat, and in a time efficient manner, protocols may best utilise a combination of active and passive HA. Thus, methods have been developed that provide the optimum balance of heat stress and recovery prior to competitive performance (Ruddock *et al.*, 2016). Yet, to date, none have sought to investigate effective active and passive HA strategies for triathletes, hence accommodating the multi-modal nature of the sport.

Moreover, HA has been studied in a range of AB endurance athletes (Garrett *et al.*, 2012; Houmard *et al.*, 1990; James *et al.*, 2018; McCleave *et al.*, 2017; Lorenzo *et al.*, 2010; Racinais *et al.*, 2015b; Stanley *et al.*, 2015; Ruddock *et al.*, 2016). Nonetheless, little attention has been paid to Paralympic athletes. These athletes are likely to be at heightened risk for performance decrements in the heat as a consequence of: a loss of vasomotor control and sweating capability (Freund *et al.*, 1984); reduced venous return (Hopman *et al.*, 1992; Kloyiam *et al.*, 2011); movement inefficiencies increasing metabolic heat production (Blauwet *et al.*, 2017; Ward & Meyers, 1995); impaired pace awareness (Runciman *et al.*, 2016; Taylor *et al.*, 2016); limited surface area for heat loss via missing limbs or skin grafts (Crandall & Davis, 2010; Webborn, 1996); limb insulation from socket liners (Klute *et al.*, 2007) or a propensity for hypohydration (Webborn & Van de Vliet, 2012) or sunburn (Bothwell, 1997).

In the sole published study of elite Paralympic athletes and HA, Castle *et al.* (2013) researched the adaptive potential of target shooters with an SCI prior to competition. The athletes performed a seven-day protocol whereby each session consisted of 20 min moderate intensity arm cranking followed by 40 min passive heat exposure. The athletes displayed lowered aural temperature, end exercise-HR, RPE and TS pre- to post-HA with a small increase ($1.5 \pm 0.6\%$) in PV. Whilst this provided the first evidence of Paralympic athletes' capability to adapt to the heat, even to a relatively modest heat stimulus, it is not known how this response may differ in endurance trained Paralympic athletes. Further, it is not known how disparate adaptations may have been to AB individuals matched for functional capabilities.

7 – Mixed active and passive, heart rate-controlled heat acclimation is effective for Paralympic and able-bodied triathletes

The aims of this study were thus to study the effects of a mixed, active and passive, HA protocol in the sport of paratriathlon. To negate the issue of potential cost and discomfort associated with isothermic protocols, a controlled relative intensity design was utilised, regulating exercise intensity using HR, which may be more applicable for elite athletes, pre-competition. A further aim was to determine how adaptation may differ to AB athletes whilst assessing any direct performance benefit of HR-controlled HA.

7.3 Methods

7.3.1 Participants

Twenty-nine (22 males, 7 females) trained triathletes and paratriathletes were recruited to partake in the present study. From this pool, three separate groups were formed: a group of elite paratriathletes (PARA; $n=7$ (1 wheelchair dependent, 6 ambulant); below elbow amputation $n=3$, incomplete SCI $n=1$, hemiplegia CP $n=1$, lower leg impairment $n=1$; partial VI $n=1$); an AB acclimation group (AB-ACC; $n=13$); an AB control group (AB-CON; $n=9$) (Table 7.1). All trained at least 5 times per week. All provided written informed consent and the procedures were approved by the Loughborough University Ethical Advisory Committee. No participants reported being heat acclimated/acclimatised prior to the start of the study.

Table 7.1: Participant characteristics for the paratriathlon (PARA), able-bodied acclimation (AB-ACC) and able-bodied control (AB-CON) groups. *Significantly greater than AB-CON ($p=0.039$).

Parameter	PARA (4 male, 3 female)	AB-ACC (9 male, 4 female)	AB-CON (8 male)
Age (y)	31 ± 9*	25 ± 7	21 ± 2
Body mass (kg)	67.8 ± 9.0	69.3 ± 9.4	70.0 ± 6.9
$\dot{V}O_{2peak}$ (ml·kg ⁻¹ ·min ⁻¹)	57.7 ± 7.6	61.5 ± 6.4	62.7 ± 8.1
MAP (W)	324 ± 73	340 ± 74	379 ± 45
AeLT (W)	181 ± 48	187 ± 42	192 ± 28
AnLT (W)	237 ± 54	240 ± 50	254 ± 30

MAP – Maximal aerobic power output. AeLT – Aerobic lactate threshold power output. AnLT – Anaerobic lactate threshold power output.

7.3.2 Study design

Both the PARA and AB-ACC group undertook an eight-day, mixed active and passive, HA period, classified as moderate term HA (Guy *et al.*, 2014). Due to the nature of the paratriathletes' pre-competition routine, it was not possible to gain a direct performance measure in this group. However, this was undertaken in the AB-ACC group pre- and post-HA. The AB-CON group solely undertook the performance trials with no HA to determine natural variation in performance (Figure 7.1).

During the study period, all participants were instructed to maintain their usual training routine. All testing was performed in the same geographical location with an average outdoor environmental temperature of $13.6 \pm 6.2^{\circ}\text{C}$ during the study period. Participants were free to drink *ad libitum* during all testing visits, but fluid intake was restricted to water. Participants were instructed to abstain from alcohol for 48 h before every trial whilst standardising food, fluid, sodium and caffeine intake. During every trial in the heat, participants were instructed to

keep clothing consistent, for males this was typically cycling bib shorts, socks and shoes whilst for females it also included a t-shirt and sports bra. All trials in the heat were conducted in an environmental chamber (Weiss Gallenkamp, Loughborough, UK) set to 35°C and 60% RH (actual conditions: $35.1 \pm 0.4^\circ\text{C}$, $63.4 \pm 4.1\%$ RH) with a fan producing an airflow of $2.0 \text{ m}\cdot\text{s}^{-1}$ at the body (5400FW Fire Weather Meter Pro WBGT, Kestrel Meters, Minneapolis, Minnesota, USA), below the velocity of $2.8 \text{ m}\cdot\text{s}^{-1}$ suggested to significantly reduce the thermoregulatory strain of exercise in the heat (Otani *et al.*, 2018). All acclimation and performance trials were scheduled at the same time of day to limit the confounding effect of circadian rhythm variation (Winget *et al.*, 1985).

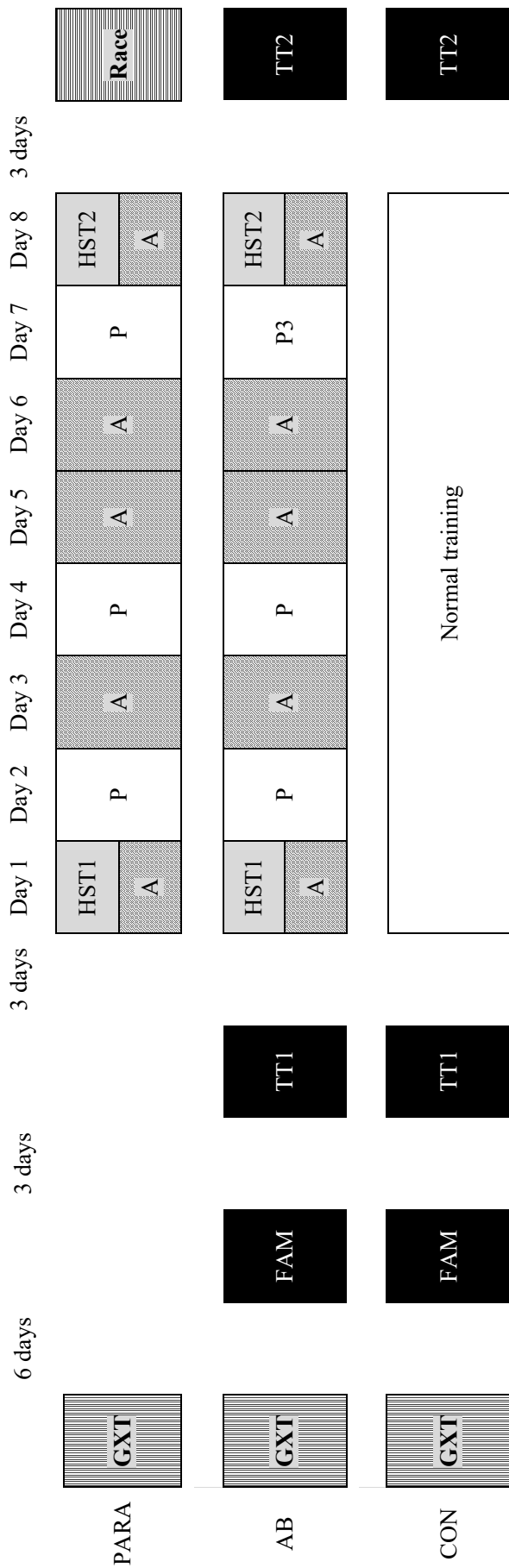


Figure 7.1: Schematic of the study protocol. PARA – Paratriathlon group. AB-ACC – Able-bodied acclimation group. AB-CON – Able-bodied control group. GXT – Graded exercise test. FAM – Familiarisation. TT – Performance trial. HST – Heat stress test. A – Active heat acclimation. P – Passive acclimation.

7.3.3 Graded exercise tests

For all participants, the first trial consisted of a submaximal cycling GXT for the determination of individuals' AeLT and AnLT with a maximal GXT for the determination of maximum HR, MAP and $\dot{V}O_{2\text{peak}}$. This was the only visit conducted in temperate ambient conditions. See Chapter 3.1 for details of the GXT protocols.

7.3.4 Familiarisation and performance trials

The performance trial in the present study consisted of a simulated 20 km time trial in the heat. Six days after the GXTs, participants were familiarised to the performance trial (FAM). During the FAM, pre-acclimation performance trial (TT1), and post-acclimation performance trial (TT2) all testing was performed on the Cyclus 2 ergometer. After arriving in the lab, participants first provided a urine sample for the determination of urine specific gravity (USG) by refractometer (PCE-032, PCE Instruments UK Ltd, Southampton, UK) before nude body mass was recorded via electronic scales (Adam Equipment Co Ltd., Milton Keynes, UK). During all performance and acclimation trials, participants with a USG ≥ 1.020 were advised to increase fluid intake pre-trial. Warm-up for all performance trials was standardised to 10 min cycling at AeLT then 5 min passive recovery. Participants were instructed to perform the test in the shortest amount of time possible with no encouragement given. During all trials, participants were blinded to all measures except cadence and distance covered. HR was recorded continuously (Polar RS400) whilst BLa was assessed pre-trial and every 5 km via the methods described in Chapter 3.1. Similarly, TS (Hardy, 1970) and RPE (Borg, 1998) were collected every 5 km. After the trials, nude body mass was again recorded after towel drying. Fluid intake was estimated from drinks bottle mass changes and sweat loss was estimated from fluid intake and body mass changes. No correction was made for loss of mass associated with the respiratory exchange of O₂ and CO₂ as losses were assumed to be similar between trials due to the comparable durations (Gibson *et al.*, 2015).

7.3.5 Heat stress tests

After arriving for the heat stress tests (HST), athletes provided a urine sample for the determination of USG and nude body mass was recorded. Participants were then instructed to rest in a seated position for 10 min for the provision of fingertip capillary blood samples, as performed elsewhere (Castle *et al.*, 2013; Otani *et al.*, 2018; Ruddock *et al.*, 2016; Stanley *et al.*, 2015). In duplicate, samples were collected in sodium heparinised haematocrit tubes (Hawksley, Sussex, UK) for the determination of haematocrit whilst 20 μl samples were

collected in capillary tubes (EKF Diagnostics) for the measurement of haemoglobin. Calculation of haemoglobin via capillary samples has been shown to be an accurate and reliable alternative to venous sampling (Hütler *et al.*, 2000). After blood samples were collected, participants were fitted with temperature loggers (DS1922L ThermoChron iButton[®]) using surgical tape to measure T_{sk} at four sites (*pectoralis major* muscle belly, lateral head of *triceps brachii*, *rectus femoris* muscle belly and lateral head of the *gastrocnemius*) (Ramanathan, 1964), on the right side of the body, at a recording rate of 30 s. Subsequently, sweat patches (Tegaderm +Pad) were placed at four sites (forearm, chest, upper back and thigh), after cleaning with deionized water, for the collection of localised sweat on the left side of the body.

The HST consisted of 10 min standardised warm-up at an intensity equating to 80% AeLT before immediately starting 40 min fixed intensity cycling at participants' AeLT. During the HST, T_c was recorded at 5-min intervals via ingestible T_c sensor (CorTemp, HQInc., Palmetto, Florida, USA) taken ~6 h pre-trial (Chapter 3.5). HR was recorded throughout whilst BLA, TS and RPE were collected pre-trial and at 10-min intervals.

After the completion of the HSTs, participants exited the chamber and were instructed to rest in a seated position during which time T_{sk} loggers were removed and sweat patches were cleaned with deionized water and placed in collection tubes (Salivette[®], Sarstedt, Nümbrecht, Germany) for later analysis. Capillary blood samples were then repeated as above. Nude body mass was recorded post-HST and used with fluid intake to estimate sweat rate.

7.3.6 Active heat acclimation

Before all sessions, urine USG, nude body mass and drink bottle mass were recorded. Participants were then permitted a self-selected 5 min (day 1 and day 8) or 15 min (day 3, day 5 and day 6) warm-up. All active HA trials were controlled by HR in a quasi-isothermic manner. Participants were provided with an individualised 5 beat·min⁻¹ HR zone equating to 80% maximum HR, thus providing a constant cardiovascular stimulus during HA. This intensity was chosen, based on preliminary testing and previous data (Gibson *et al.*, 2017), to elicit a T_c of ~38.5°C, a temperature recommended for isothermic HA protocols (Gibson *et al.*, 2015). Active HA sessions lasted for 45 min (day 1 and day 8) or 90 min (day 3, day 5 and day 6). During all sessions, participants were instructed to manipulate their cycling PO, via the Cyclus 2 or cycling power meter (Garmin Vector), to maintain a HR within the predetermined zone. This high thermal loading was intended to emphasise HA more than the training stimulus

to facilitate (PARA) or replicate (AB-ACC) the tapering phase of athletes prior to competition (Garrett *et al.*, 2012; Mujika, 2011). During all sessions, cycling PO was recorded and stored on the Cyclus 2 or a Garmin Edge 500 cycling computer before later export and analysis. HR was recorded continuously whilst BLa, TS and RPE were collected pre-trial and at 15-min intervals.

7.3.7 *Passive heat acclimation*

Passive HA sessions were performed on day 2, day 4 and day 7 and were structured to align with triathletes' typical weekly running frequency when tapering for competition (Mujika, 2011; Mujika, 2014). Participants were instructed to undertake their normal run training, or to run for 30 min at a moderate intensity before entering the chamber. Participants then rested in the heat for 60 min. During passive HA sessions, HR was recorded every 10 min whilst T_{sk} was assessed via an insulated skin thermistor (Squirrel SQ2010 Data Logger, Grant Instruments Ltd, Shepreth, UK) placed at the 7th cervical vertebra (Taylor *et al.*, 2014). Finally, TS was noted pre-trial and at 20-min intervals.

7.3.8 *Analytical methods*

Haematocrit – Haematocrit tubes were centrifuged (Haematospin 1400, Hawksley) at 11,800 revolution·min⁻¹ for 5 min before being assessed via tube reader (Hawksley). As samples were collected in duplicate, the mean value is presented. The CV for duplicate samples was 1.3%.

Haemoglobin – 20 µl blood samples were combined with 5 ml Drabkin's solution with the absorbance of the resultant mixture read via a zeroed spectrophotometer (Cecil series 1000, Cecil Instruments Ltd, Cambridge, UK) at 540 nm. The mean absorbance value of the duplicate samples was subsequently translated into haemoglobin concentration (mean absorbance · 36.77). The CV for duplicate samples was 2.1%.

Plasma volume changes – changes in PV were calculated from haematocrit and haemoglobin using the equation of Dill and Costill (1974).

Sweat composition – Sweat samples at all four sites were first diluted by a 1:200 ratio in deionised water before being analysed for Na⁺ concentration via flame photometry (Model 410c, Sherwood Scientific Ltd., Cambridge, UK). All individuals' samples were analysed in the same batch.

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7.3.9 Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics 23.0 software (IBM, New York, USA). Statistical significance was set at $p < 0.05$. Data were checked for normal distribution using the Shapiro-Wilk test. Where sphericity could not be assumed, the Greenhouse-Geisser correction was used. The Bonferonni (parametric) or Wilcoxon's signed ranks (non-parametric) post-hoc test were used to identify any significant differences where appropriate.

Differences in participants' physical and physiological characteristics between groups were assessed via the Kruskal-Wallis test. Post-hoc analyses were performed using the Mann-Whitney U test. Data from the AB-CON group were used to derive the CV in TT PO without HA. From this, the smallest worthwhile change in PO was calculated as: $(CV + (0.3 \times 0.5 \times CV))$ (Malcata & Hopkins, 2014). PO and HR were averaged over 5 km segments. Changes in PO, HR, BLa, RPE and TS were assessed via two-way ANOVA (parametric) or the Friedman test (non-parametric). Changes in pre-trial USG, fluid intake, sweat rate (TT1 to TT2 and HST1 to HST2) and sweat sodium concentration (HST1 to HST2) were evaluated by paired samples t-test (parametric) or Wilcoxon's signed ranks test (non-parametric). PV changes were assessed against a fixed zero by paired samples t-test. Participants' T_c , T_{sk} and HR during HST were averaged over 5 min intervals. Changes in T_c , T_{sk} , HR, BLa, RPE and TS pre- to post-HA were assessed via two-way ANOVA or the Friedman test.

Due to the differing length of active HA sessions, days 1 and 8 (45 min) were evaluated against each other whilst days 3, 5 and 6 (90 min) were assessed separately. Changes in sweat rate and fluid intake were assessed using paired samples t-test (days 1 and 8) or one-way ANOVA (days 3, 5 and 6). PO and HR were averaged over 5 min intervals and assessed via two-way ANOVA. BLa, RPE, TS were evaluated in the same manner. Changes in HR, T_{sk} and TS during passive HA were all evaluated by two-way ANOVA or Friedman's test.

7.4 Results

7.4.1 Participant characteristics

The PARA group were older than AB-CON ($p = 0.009$) but there were no significant differences in body mass, $\dot{V}O_{2peak}$, MAP, AeLT or AnLT between groups ($p \geq 0.352$) (Table 7.1).

7.4.2 Performance tests

The CV in PO for AB-CON was 3.7% (217 vs. 219 W for TT1 and TT2, respectively) (Figure 7.2); therefore, the smallest worthwhile change in PO for TT2 was 4.3%. The average change in PO for AB was 4.5% (199 vs. 207 W for TT1 and TT2, respectively), indicating a meaningful improvement in average PO during the performance tests post-HA. Eight of the twelve participants in the AB-ACC group experienced an improvement in performance exceeding the smallest worthwhile change (Figure 7.3).

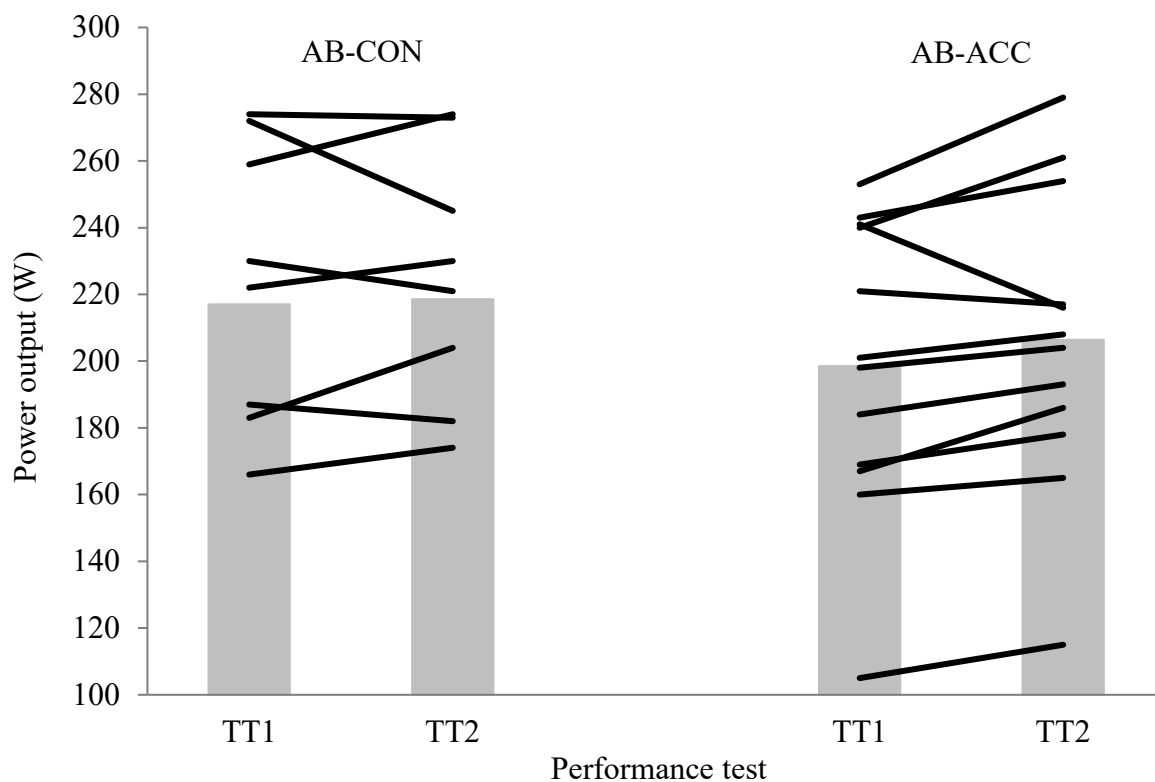


Figure 7.2: Performance trial (20 km cycling time trial in 35°C, 60% relative humidity) average power output for the control group (AB-CON) and able-bodied acclimation group (AB-ACC). Bars are group mean, lines are individual participants.

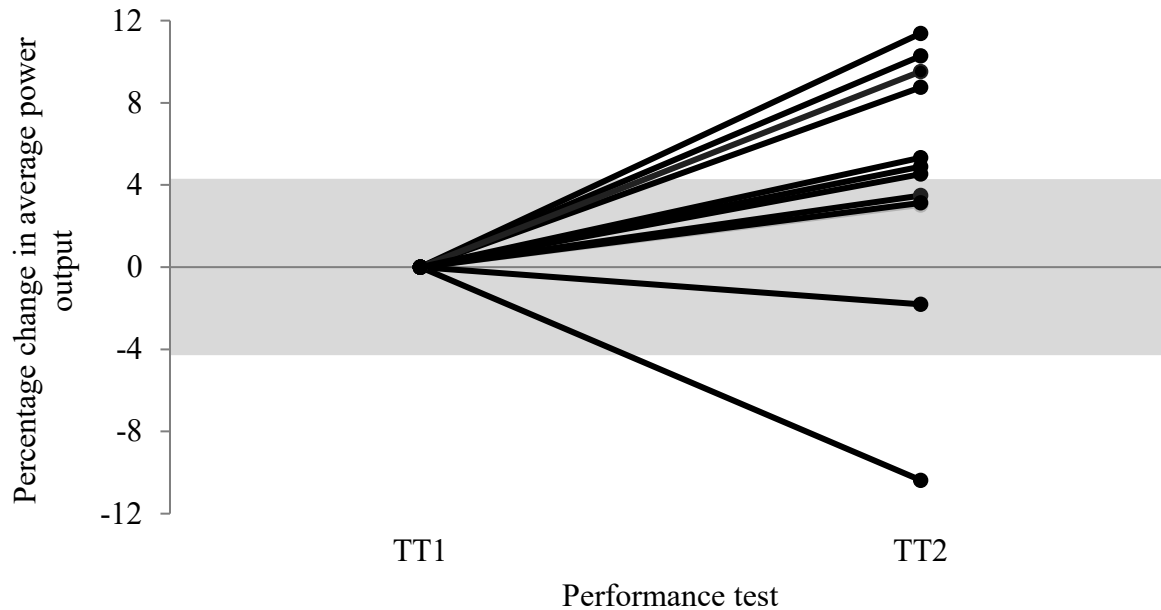


Figure 7.3: Percentage change in average power output post-heat acclimation in the able-bodied acclimation group. Lines are individual participants. Shaded area represents smallest worthwhile change based on the coefficient of variation of control group performance.

There was no significant trial or trial by time effect for PO or HR for either group ($p \geq 0.164$). There was a significant effect of time in both groups for PO and HR ($p \leq 0.030$). Specifically, PO was greater between 0 to 5 km than all other time points ($p \leq 0.043$) and between 15 to 20 km than 10 to 15 km in AB-CON ($p = 0.041$). In AB-ACC, PO was greater between 0 to 5 km and 15 to 20 km than 5 to 10 km and 10 to 15 km ($p \leq 0.047$) (Figure 7.4a). HR was greater at each successive time point in both groups ($p \leq 0.031$) (Figure 7.4b). There was no significant change in BLa for the AB-CON group ($p = 0.370$). For AB-ACC, there was no trial or trial by time effect but there was a significant difference over time ($p = 0.004$) specifically, BLa was greater at 20 km than at 10 km or 15 km ($p \leq 0.030$) (Figure 7.4c).

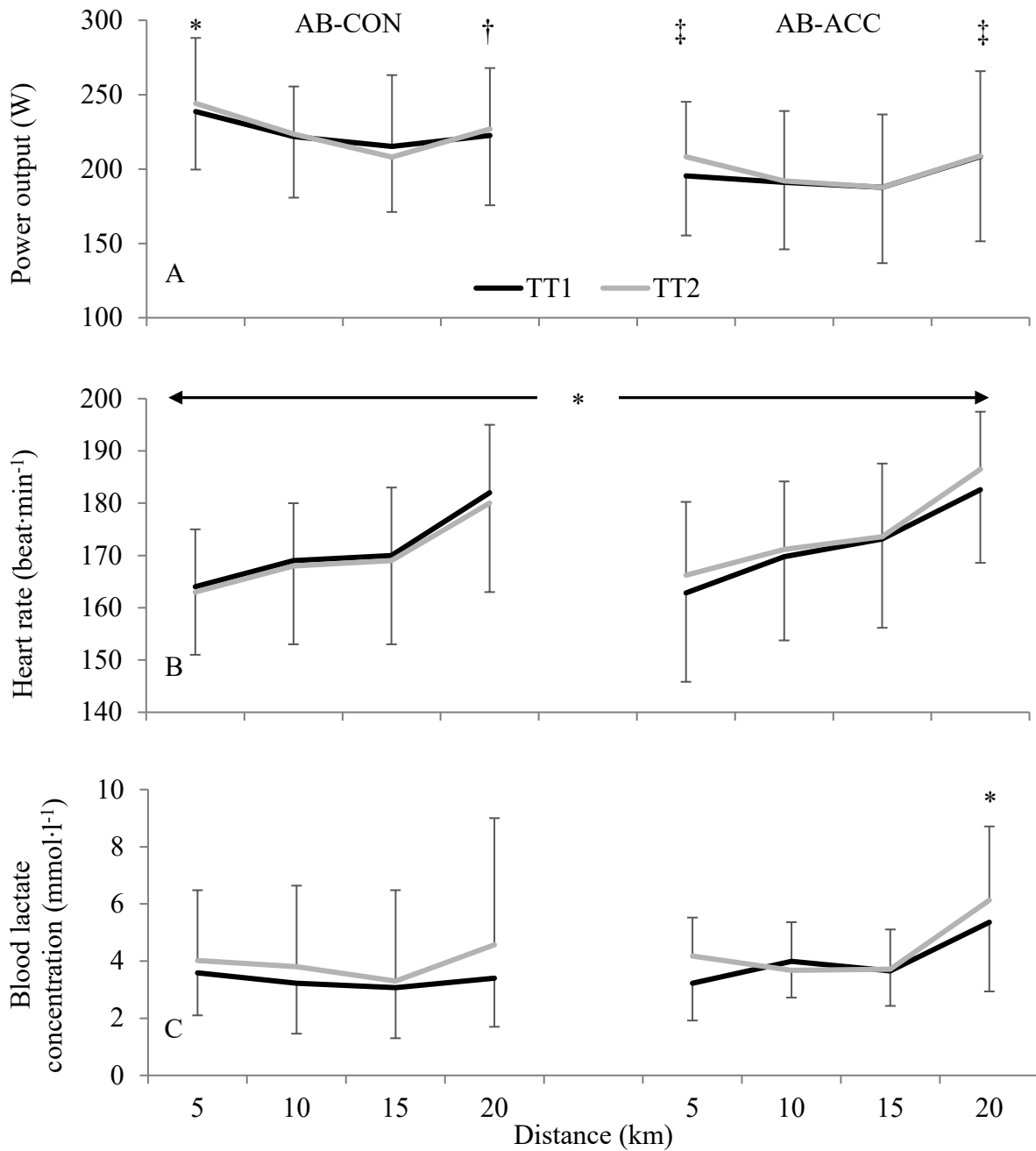


Figure 7.4a: Power output distribution averaged over 5 km sections for control (AB-CON) and able-bodied acclimation (AB-ACC) groups (mean ± SD). *Significantly greater than all other time points ($p \leq 0.043$). †Significantly greater than 10 to 15 km ($p = 0.041$). ‡Significantly greater than 5 to 10 km and 10 to 15 km ($p \leq 0.031$). Figure 7.4b: Heart rate across the performance trials for AB-CON and AB-ACC group (mean ± SD). *Significantly greater over time ($p \leq 0.031$). Figure 7.4c: Blood lactate concentration across the performance trials for AB-CON and AB-ACC group (mean ± SD). *Significantly greater than 10 and 15 km ($p \leq 0.030$).

Thermal sensation and RPE increased at each time point in both AB-CON and AB-ACC ($p \leq 0.007$). Further, TS was significantly lower during TT2 for the AB-ACC group ($p = 0.013$); there was no effect of trial on the TS in the AB-CON group ($p = 0.090$), nor was there an effect of trial on RPE in either group ($p \geq 0.388$).

Fluid intake (TT1: 0.63 ± 0.19 l, TT2: 0.76 ± 0.25 l) and sweat loss (TT1: 1.08 ± 0.25 , TT2: 1.13 ± 0.25) was not significantly different across trials for AB-CON ($p \geq 0.139$). Fluid intake (TT1: 0.44 ± 0.22 l, TT2: 0.56 ± 0.30 l) and sweat loss (TT1: 1.08 ± 0.22 , TT2: 1.21 ± 0.28) were significantly greater during TT2 for AB-ACC ($p \leq 0.031$). There was no significant difference in pre-trial USG for either group (AB-CON; TT1: 1.016 ± 0.010 , TT2: 1.019 ± 0.015 ; $p = 0.604$. AB-ACC; TT1: 1.015 ± 0.009 , TT2: 1.018 ± 0.007 ; $p = 0.266$).

7.4.3 Heat stress tests

There was a significant change in PV pre- to post-HA ($12.7 \pm 10.6\%$; $p = 0.019$) for PARA. However, there was no effect of HA on sweat rate, fluid intake, sweat sodium concentration or pre-trial USG ($p \geq 0.115$) (Table 7.2). For AB-ACC, there was a significant effect of trial on sweat rate and fluid intake ($p \leq 0.045$) and PV change post-HA ($6.2 \pm 7.7\%$; $p = 0.013$) with no significant change in sweat sodium concentration or pre-trial USG ($p \geq 0.678$) (Table 7.2).

Table 7.2: Pre-trial urine specific gravity (USG), fluid intake, sweat rate and sweat sodium (Na^+) concentration during heat stress tests pre- (HST1) and post- (HST2) heat acclimation in paratriathlon (PARA) and able-bodied (AB-ACC) groups (mean \pm SD). *Significantly different to HST1 ($p \leq 0.045$).

	PARA		AB-ACC	
	HST1	HST2	HST1	HST2
Sweat rate ($\text{l}\cdot\text{h}^{-1}$)	1.36 ± 0.73	1.49 ± 0.57	1.35 ± 0.44	$1.52 \pm 0.37^*$
Fluid intake ($\text{l}\cdot\text{h}^{-1}$)	0.88 ± 0.40	0.72 ± 0.16	0.82 ± 0.43	$1.04 \pm 0.55^*$
Average sweat Na^+ ($\text{mmol}\cdot\text{l}^{-1}$)	37.1 ± 7.1	40.7 ± 12.5	49.2 ± 16.1	48.1 ± 20.6
Pre-trial USG	1.019 ± 0.004	1.022 ± 0.007	1.014 ± 0.008	1.015 ± 0.008

For both groups there was a significant effect of trial and time on T_c ($p \leq 0.041$) with values significantly lower in HST2 whilst increasing over time ($p < 0.001$). For PARA there was a significant trial by time interaction ($p = 0.041$) whereby T_c was lower at 40, 45 and 50 min during HST2 compared to HST1 ($p \leq 0.049$), but this was not the case for AB-ACC ($p = 0.473$) (Figure 7.5a). There was no significant effect of trial on T_{sk} for PARA ($p = 0.177$) but T_{sk} was significantly lower during HST2 for AB-ACC ($p < 0.001$). Specifically, post-hoc analyses revealed T_{sk} was significantly lower from 30 min onwards during HST2 for AB-ACC ($p < 0.039$). There was a significant effect of time for both groups as T_{sk} increased up to 35 min for PARA ($p \leq 0.044$) and 45 min for AB-ACC ($p \leq 0.036$) in the HST (Figure 7.5b).

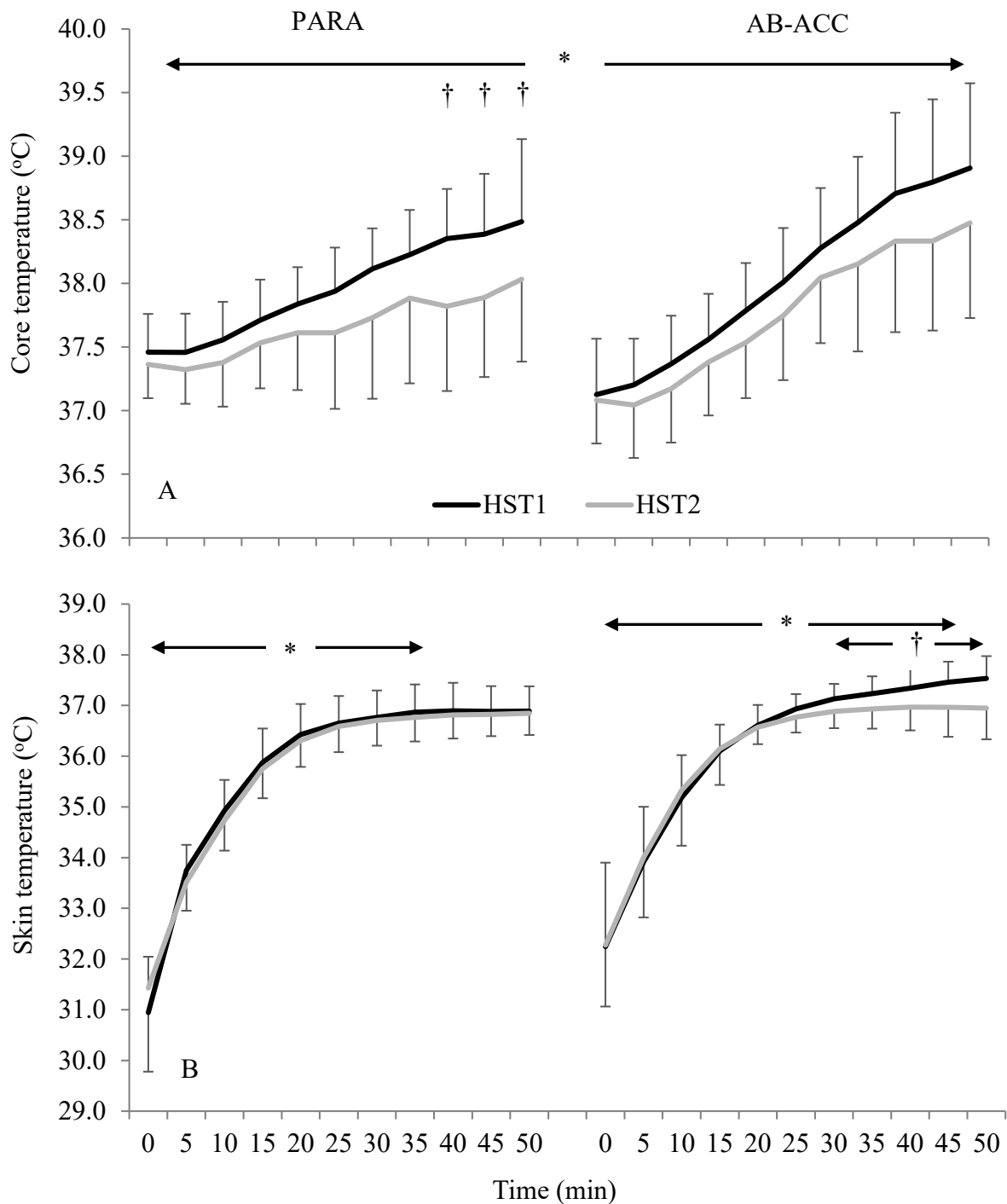


Figure 7.5a: Changes in core temperature across heat stress tests (HST) for the paratriathlon (PARA) and able-bodied (AB-ACC) group (mean \pm SD). *Significantly lower during HST2 and increasing over time ($p < 0.001$). †Significantly lower during HST2 ($p \leq 0.049$). Figure 7.5b: Changes in skin temperature across HST for the PARA and AB-ACC group (mean \pm SD). *Significant increase over time until 35 min (PARA; $p \leq 0.044$) or 45 min (AB-ACC; $p \leq 0.036$). †Significantly lower during HST2 ($p < 0.039$).

There was a significant effect of time for both PARA and AB-ACC as HR increased through both HSTs until 40 min for PARA ($p \leq 0.028$) and 30 min for AB-ACC ($p \leq 0.031$) (Figure 7.6). There was no effect of trial for PARA ($p = 0.878$) whilst for AB-ACC, HR was lower during HST2 ($p = 0.008$). There was no significant time by trial interaction for PARA ($p = 0.968$) but there was for AB-ACC ($p = 0.007$) with HR lower from 15 min onwards during HST2 ($p \leq 0.045$).

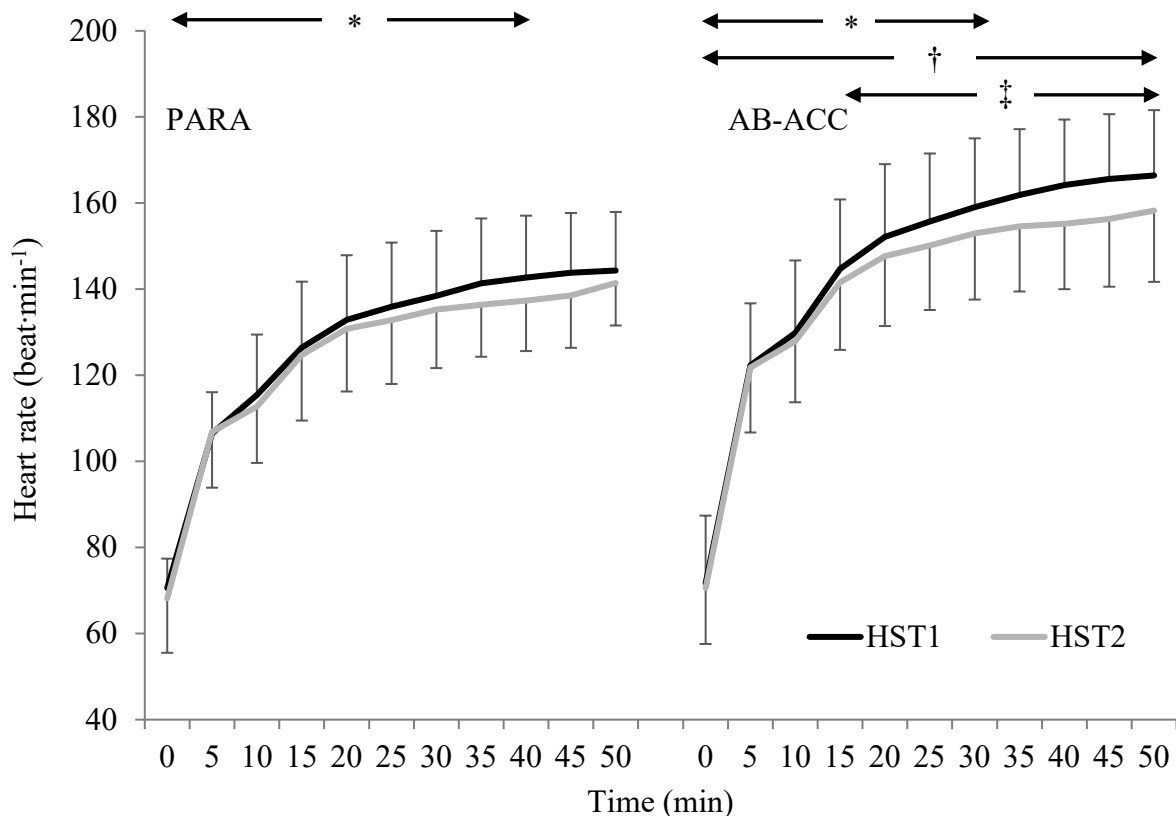


Figure 7.6: Changes in heart rate across heat stress tests (HST) for the paratriathlon (PARA) and able-bodied (AB-ACC) group (mean \pm SD). *Significant increase over time until 40 min (PARA; $p \leq 0.028$) and 30 min (AB-ACC; $p \leq 0.031$). †Significantly lower during HST2 ($p = 0.008$). ‡Significantly lower during HST2 ($p \leq 0.045$).

For both groups, the BLa main effect of trial was significant ($p < 0.001$) with values lower during HST2 (Figure 7.7). Whilst the time effect was not significant for PARA ($p = 0.056$); for AB-ACC, BLa increased over time ($p \leq 0.037$) (Figure 7.7). There was a significant effect on TS and RPE for both groups with values lower for HST2 ($p \leq 0.032$) and increasing over time ($p \leq 0.047$).

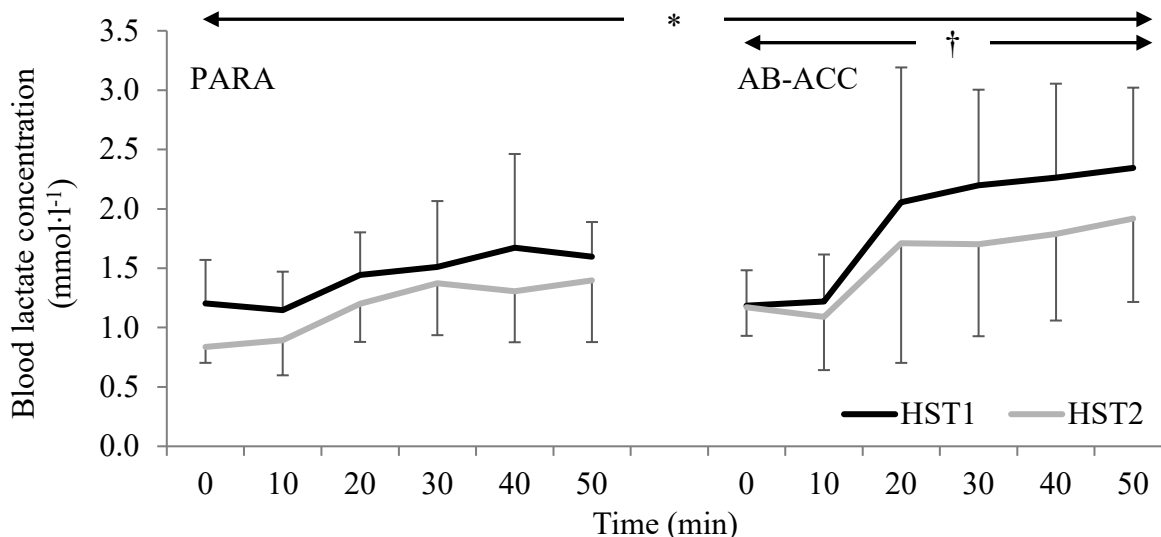


Figure 7.7: Changes in blood lactate concentration across heat stress tests (HST) for the paratriathlon (PARA) and able-bodied (AB-ACC) group (mean \pm SD). *Significantly lower during HST2 ($p < 0.001$). †Significant increase over time ($p \leq 0.037$).

7.4.4 Active heat acclimation

There was no significant difference in PO between days 1 and 8 (45 min) for PARA ($p = 0.522$) but for AB-ACC it was significantly higher on day 8 ($p = 0.048$). There was no change in PO within days 1 and 8 for PARA ($p = 0.646$), but for AB-ACC ($p = 0.010$) it was greater at 20 min than 5 min ($p = 0.020$). Comparing days 3, 5 and 6 (90 min), there was no difference in PO between trials for PARA ($p = 0.483$) however, for AB-ACC post-hoc analyses revealed PO was significantly higher on days 5 and 6 than day 3 ($p \leq 0.021$). There was a significant effect of time for both groups as PO changed within trials ($p < 0.001$). The trial by time interaction for PARA was not significant ($p = 0.596$) but it was for AB-ACC ($p = 0.022$); specifically, PO was greater on day 6 than days 3 and 5 from 65 min onwards ($p \leq 0.044$) (Figure 7.8a). There was no significant difference in HR or BLa between days 1 and 8 ($p \leq 0.068$) or between days 3, 5 and 6 ($p \geq 0.205$) for either group. There was a change within trials with HR increasing up to 20 min ($p \leq 0.040$) (Figure 7.8b) and BLa up to 30 min ($p \leq 0.047$) (Table 7.3). The trial by time interaction effect was not significant ($p \geq 0.518$).

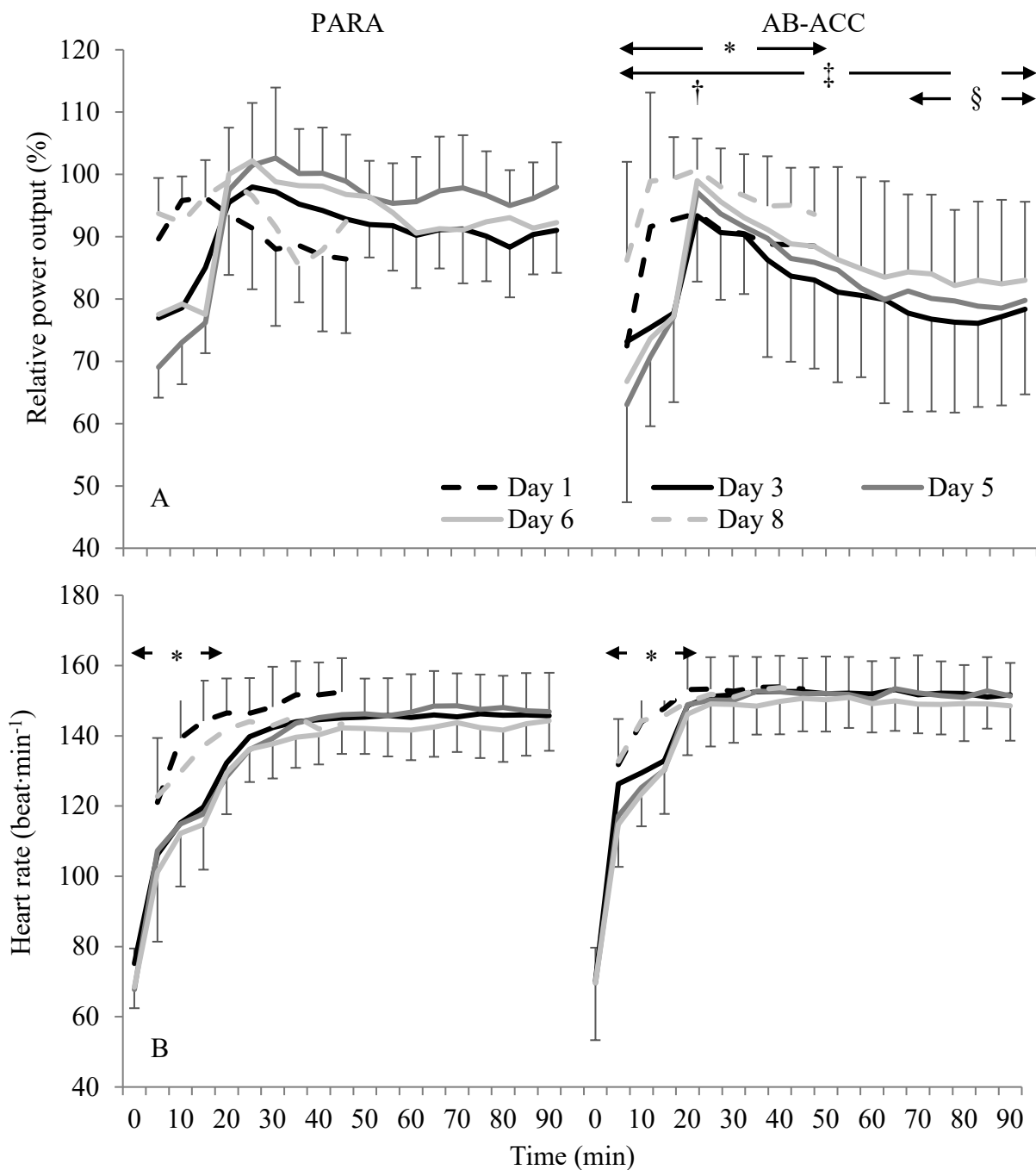


Figure 7.8a: Power output changes between and within active heat acclimation sessions for paratriathlon (PARA) and able-bodied (AB-ACC) groups (mean \pm SD). Data are relativised to individuals' highest power output during day 1 (for day 1 and 8) or day 3 (for day 3, 5 and 6). *Significant difference between day 1 and day 8 ($p=0.048$). †Significantly greater at 20 min than 5 min during day 1 and day 8 ($p=0.010$). ‡Significantly greater than day 3 ($p\leq 0.021$). §Significantly greater on day 6 ($p\leq 0.044$). Figure 7.8b: Heart rate changes between and within active heat acclimation sessions for PARA and AB-ACC groups (mean \pm SD). *Significant increase up to 20 min ($p\leq 0.040$).

Table 7.3: Blood lactate concentration (BLa), thermal sensation (TS), rating of perceived exertion (RPE), sweat rate and fluid intake changes between active heat acclimation sessions for paratriathlon (PARA) and able-bodied (AB-ACC) groups (daily mean \pm SD). TS significantly lower on day 5 than day 3 and day 6 in AB-ACC ($p \leq 0.026$).

	Day 1	Day 3	Day 5	Day 6	Day 8
PARA					
BLa (mmol·l ⁻¹)	1.75 \pm 0.87	1.54 \pm 0.51	1.60 \pm 0.69	1.49 \pm 0.56	1.63 \pm 0.89
TS (AU)	6 \pm 1	5 \pm 2	5 \pm 2	5 \pm 2	6 \pm 1
RPE (AU)	13 \pm 1	12 \pm 2	12 \pm 1	12 \pm 1	13 \pm 1
Sweat rate (l·h ⁻¹)	1.40 \pm 0.50	1.29 \pm 0.52	1.19 \pm 0.47	1.33 \pm 0.52	1.57 \pm 0.45
Fluid intake (l·h ⁻¹)	1.38 \pm 0.90	1.28 \pm 0.62	1.30 \pm 0.50	1.29 \pm 0.56	1.65 \pm 0.51
AB-ACC					
BLa (mmol·l ⁻¹)	1.28 \pm 0.27	1.44 \pm 0.52	1.47 \pm 0.62	1.37 \pm 0.52	1.78 \pm 0.91
TS (AU)	7 \pm 1	6 \pm 2	6 \pm 2	6 \pm 2	6 \pm 2
RPE (AU)	14 \pm 1	13 \pm 2	13 \pm 2	13 \pm 2	13 \pm 2
Sweat rate (l·h ⁻¹)	1.31 \pm 0.45	1.22 \pm 0.40	1.32 \pm 0.36	1.35 \pm 0.39	1.27 \pm 0.81
Fluid intake (l·h ⁻¹)	1.46 \pm 0.77	1.22 \pm 0.54	1.31 \pm 0.57	1.45 \pm 0.64	1.54 \pm 0.48

The time and trial effects on TS and RPE were not significant when comparing days 1 and 8 for either group ($p \geq 0.450$). There was a significant effect on TS and RPE (Table 7.3) for both groups when comparing days 3, 5 and 6 ($p < 0.001$). There was no effect of trial on TS or RPE for PARA ($p \geq 0.664$). For AB-ACC, there was a significant trial effect ($p = 0.017$) as TS was lower on day 5 than days 3 and 6 ($p \leq 0.026$). There was no trial effect in RPE for AB-ACC ($p = 0.163$). TS increased up to 45 min for PARA ($p \leq 0.001$) and up to 60 min for AB-ACC ($p \leq 0.026$) whilst RPE increased up to 75 min for PARA ($p \leq 0.014$) and 60 min for AB-ACC

($p \leq 0.024$). There was no significant change in sweat rate ($p \geq 0.667$) or fluid intake ($p \geq 0.118$) for either group over active HA sessions (Table 7.3).

7.4.5 Passive heat acclimation

The trial, time and trial by time effects on HR and T_{sk} for PARA were not significant ($p \geq 0.224$). For AB-ACC, the trial and interaction outcomes were similarly non-significant, but the effect of time was significant ($p \leq 0.003$) with HR greater at 0 min than all other time points ($p \leq 0.038$) and T_{sk} lower at 0 min than 10 min ($p = 0.032$). For PARA, TS changed across trials and time; specifically, TS was greater on day 2 than days 4 and 7 ($p \leq 0.003$) and was greater at 60 min than 0 and 20 min ($p \leq 0.032$). There were no significant effects on TS in the AB-ACC group ($p = 0.629$) (Table 7.4).

Table 7.4: Changes in heart rate (HR), skin temperature (T_{sk}) and thermal sensation (TS) between passive heat acclimation sessions for paratriathlon (PARA) and able-bodied (AB-ACC) groups (daily mean \pm SD). TS greater on day 2 than days 4 and 7 for PARA ($p \leq 0.003$).

	PARA			AB-ACC		
	Day 2	Day 4	Day 7	Day 2	Day 4	Day 7
HR (beat·min ⁻¹)	74 \pm 8	75 \pm 8	75 \pm 9	78 \pm 13	77 \pm 11	78 \pm 14
T_{sk} (°C)	35.27 \pm	35.21 \pm	35.13 \pm	34.76 \pm	35.00 \pm	35.08 \pm
	1.07	0.88	0.66	1.22	0.87	0.71
TS (AU)	4 \pm 1	3 \pm 1	3 \pm 1	4 \pm 2	4 \pm 2	4 \pm 2

7.5 Discussion

This is the first known study to investigate the efficacy of HA in a multi-impairment, Paralympic endurance sport. Utilising a novel, mixed active and passive HA protocol controlling relative intensity through HR, it has been shown that paratriathletes can display positive thermoregulatory adaptations. These responses were a reduction in exercising T_c , BLA, RPE and TS with concomitant PV expansion. Furthermore, the inclusion of a physiologically matched AB group permitted comparisons to the Paralympic cohort whereby additional adaptations were noted including T_{sk} , HR and sweat rate changes during the submaximal HST. Finally, there is evidence of a direct performance benefit from the employed HA protocol as AB-ACC athletes improved their PO during a 20 km cycling TT in the heat (4.5%) to an extent greater than the variation noted in a non-acclimated AB-CON group (3.7%).

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Due to the 8-day nature of daily heat exposure in the current study, it may be classed as a medium-term HA protocol (Guy *et al.*, 2014). Medium-term HA (8 to 14 days heat exposure) has been commonly studied in the literature with typical adaptations recently documented in the meta-analysis of Tyler *et al.* (2016). This approach has been shown to result in a decrease in exercising: T_c ($0.17 \pm 0.14^\circ\text{C}$); HR ($15 \pm 10 \text{ beat}\cdot\text{min}^{-1}$), T_{sk} ($0.73 \pm 0.14^\circ\text{C}$), BLa ($\sim 0.9 \text{ mmol}\cdot\text{l}^{-1}$; Febbraio *et al.*, 1994), TS and RPE ($\sim 10\%$; Neal *et al.*, 2016); with increased whole-body sweat rates ($30.0 \pm 22.6\%$) and PV expansion ($4.3 \pm 4.7\%$) (Tyler *et al.*, 2016). Thus, this study presents thermoregulatory adaptations for both groups that are within the commonly reported range for similar duration HA whilst the greater T_c ($0.27 \pm 0.32^\circ\text{C}$) and PV ($12.7 \pm 10.6\%$) adaptations here may be a result of the study design ensuring a consistent thermal impulse and relative exercise intensity. Medium-term HA also results in a mean $7 \pm 7\%$ and median 4% improvement in TT performance which was similar to the 4.5% improvement noted in the AB-ACC group. Furthermore, whilst the PARA were older, this was not considered a major concern since previous work has shown age does not affect TT performance changes post-HA, nor so any thermoregulatory variable (Tyler *et al.*, 2016). Regardless, it is likely that a longer HA duration would result in greater performance gains, however this increases the logistical constraints on athletes (Tyler *et al.*, 2016).

As noted earlier, the literature to date concerning HA in Paralympic athletes has been confined to the one study of target shooters with an SCI (Castle *et al.* 2013), despite acknowledgements of the need for greater evidence (Casadio *et al.*, 2017; Price, 2015). The authors stated that the five participants displayed partial HA through a decrease in: resting and exercising aural temperature; end-exercise HR; TS and RPE. These responses were proposed to be caused by a $1.5 \pm 0.6\%$ PV expansion. There was, however, no notable sweat responses which was credited to the nature of athletes' autonomic impairments. In the present study, very similar responses were noted however the extent of PV was greater and more varied. This is most likely attributed to the present study including only one athlete with an SCI, the HA protocol presenting a greater thermal stimulus based on total and individual session durations (8 days, 45 to 90 min vs 7 days, 20 min arm crank ergometry with 40 min passive heat exposure) and the disparate athletic backgrounds. Thus, the present data provides support to the notion that Paralympic athletes are capable of partial heat acclimation although not to the same extent as AB athletes undergoing the same protocol.

Unique features of this work were that differences in HA responses between AB and Paralympic athletes were present using the same protocol. It is worth noting, however, that the limited and heterogeneous sample size in the PARA group may have prevented some statistically significant responses. For example, improvements in HR and whole-body sweat rates during exercise were noted for the PARA group, however these did not reach the predefined threshold of significance. Furthermore, due to the elite nature of the PARA group, and their cardiorespiratory fitness closer to the maximum achievable state when considering physical limiters (Chapter 2.1.5), their adaptive potential may be lower than the AB-ACC group who were not as fit relative to elite AB athletes. This hypothesis is based on proposals that elite endurance athletes already display a partially heat acclimated phenotype (Garrett *et al.*, 2012) which may be similarly true for elite Paralympic endurance athletes. Alternatively, the nature of physical impairments in the PARA group may have restricted any notable adaptation. For instance, the reduced body surface area of those with an amputation, for heat dissipation, may be the limiting factor in any T_{sk} change, rather than PV and capacity for skin blood flow. Further, limited sweating responses as a result of reduced body surface area, skin grafts or disrupted afferent input likely reduces the maximum achievable sweating capacity, thus diminishing the adaptation potential. Nonetheless, improvements in exercising T_c , BLa and perceptual measures demonstrated an enhanced thermoregulatory capacity which was likely mediated by a significant PV expansion post-HA. Whilst not directly assessed, it may be assumed that this would result in a direct improvement in endurance performance in hot environments as athletes display diminished heat storage and reliance upon carbohydrate metabolism. Accordingly, evidence is provided for the feasibility of attenuating paratriathletes' thermoregulatory strain in the heat caused by the varied primary and secondary consequences of their physical impairments.

The use of HR to regulate HA training intensity was based on the recommendations of Périard *et al.* (2015) to provide a constant cardiovascular, and presumed thermoregulatory, stimulus for continued adaptation. This is based on previous work showing a constant HR between HA sessions using isothermic approaches (Garrett *et al.*, 2012; Magalhães *et al.*, 2010; Zurawlew *et al.*, 2015). Whilst isothermic HA protocols have been commonly utilised in the literature to provide a constant thermal stimulus for homeostatic disruption (Tyler *et al.*, 2016), the utility of such approaches has been questioned in the applied sport setting. This is due to the financial burden of measuring T_c via ingestible sensors or participant discomfort from rectal

or oesophageal temperature assessment. Furthermore, rectal temperature measurement poses a risk of autonomic dysreflexia in athletes with an SCI (Price & Campbell, 1999). Therefore, the use of HR provides an easy and feasible means for regulating HA intensity for elite athletes, pre-competition. Athletes in the present study were able to maintain their individualised HR zone of 80% maximum HR, an intensity chosen to elicit a T_c of $\sim 38.5^\circ\text{C}$ based on preliminary testing, within and between HA sessions. Furthermore, due to a lack of significant change in BLA, RPE and TS between active HA, it may be assumed that participants maintained a constant relative intensity each day. Moreover, the AB-ACC group were able to produce a greater external workload throughout HA with PO greater on day 8 than day 1 and on day 5 and 6 than day 3. This suggests that the group displayed thermoregulatory adaptations that permitted a higher workload for a given relative intensity. This is akin to isothermic HA whereby greater workloads are produced for a set T_c (Garrett *et al.*, 2012; Magalhães *et al.*, 2010). However, no significant change in PO was noted for the PARA group. This may be indicative of the greater individual variation in this group with regards to their physical impairments, exercising workload, and capacity for adaptation.

The current study utilised a mixed, active and passive HA protocol in consultation with the paratriathlon coaches. This was chosen to provide an optimal yet time efficient HA stimulus when considering elite athletes' pre-competition training schedules (Casadio *et al.*, 2017; Guy *et al.*, 2014). Whilst several solely passive HA studies have shown support for the use of heat exposure post-exercise via hot water immersion (Zurawlew *et al.*, 2015; Zurawlew *et al.*, 2018) or sauna bathing (Scoon *et al.*, 2007; Stanley *et al.*, 2015) it has been questioned whether this is a suitable substitute for exercising HA (Daanen *et al.*, 2018). Consequently, a combined approach may present a pragmatic solution to overcome the logistical demands of pre-competition schedules whilst potentiating a stimulus for heat adaptation (Ruddock *et al.*, 2016). Nonetheless, few researchers have sought to investigate active and passive HA approaches. However, Ruddock *et al.* (2016) utilised this study design in their case study of a soccer referee. The authors showed that the participant displayed increases in whole-body sweat rate, PV and repeated sprint performance whilst decreasing exercising tympanic temperature, HR and $\dot{V}O_2$. In the present study, passive heat exposure was in the form of post-run training rest in a climatic chamber set to 35°C and 60% RH. This permitted PARA and AB triathletes the capability to maintain running session density and intensity pre-competition whilst continuing daily heat exposure as athletes entered the heat with a presumed prior elevated T_c , T_{sk} and sweat rate.

Whilst T_{sk} did not reach the levels reported by Zurawlew *et al.* (2015) (equilibrating at 40°C water temperature), it was greater than typical resting temperatures, furthermore TS values indicated some thermal strain. Nonetheless, as beneficial adaptations were noted in the present study, for both AB-ACC and PARA, this adds support for the use of mixed active and passive HA for elite athletes, pre-competition. However, it is unknown if a solely active or passive approach would have induced disparate adaptations.

The HR-controlled exercise HA resulted in participants training at a low to moderate intensity in the heat. This has the benefit of allowing athletes' higher intensity sessions to be performed in a temperate environment to thus maintain training quality and prevent diminished training stimuli (Casadio *et al.*, 2017). Further, this prevents the likelihood of athletes developing OR which can occur when HA is performed at a high intensity (Schmit *et al.*, 2018). Moreover, lower HA intensities may invoke beneficial adaptations specific to endurance performance. Wingfield *et al.* (2016) demonstrated that 5 days HA training at 40% MAP for 90 min resulted in a $5.9 \pm 7.0\%$ improvement in 20 km cycling TT in the heat whilst no significant change was noted in a group that trained for 30 min alternating between 40 and 70% MAP. Additionally, Houmard *et al.* (1990) present evidence of no additional adaptation benefit when HA is performed at 75% $\dot{V}O_{2peak}$ for 30 to 35 $\text{min}\cdot\text{d}^{-1}$ compared to when performed at 50% $\dot{V}O_{2peak}$ for 60 $\text{min}\cdot\text{d}^{-1}$. Thus, low to moderate intensity HA permits greater durations of heat exposure for adaptation and may result in adaptation specificity that favours endurance performance.

The timings of this study meant that the PARA group undertook their HA sessions in preparation for a competitive race in the heat. Therefore, it was neither feasible nor appropriate to include a direct performance test for this group. However, this was included in the AB-ACC group with the AB-CON group utilised to determine the natural variation in 20 km cycling performance in the heat without HA. The AB-ACC group displayed an improvement in average PO, the primary performance outcome, greater than the smallest worthwhile change. This provides evidence for a direct performance benefit of the HA protocol. Furthermore, the AB-ACC group displayed physiological responses during TT2 indicative of an enhanced thermoregulatory capacity. Specifically, a greater sweat rate and fluid intake was noted for the AB-ACC group whilst participants produced a greater PO for a similar HR and lower TS whereas no significant changes were noted for the AB-CON group. It can therefore be assumed that these thermoregulatory adaptations permitted the improvement in performance.

Whilst the present study is the first to demonstrate differences in HA adaptations between PARA and AB athletes, the lack of a control group prevented the ability to state that changes in thermoregulatory variables was due solely to HA. Nonetheless, due to the relatively low HA intensity and the high cardiorespiratory fitness levels of both PARA and AB-ACC groups, it is unlikely any non-HA-specific training adaptation occurred (Lorenzo *et al.*, 2010). Furthermore, it is noteworthy that T_c was not recorded across exercise HA. Whilst both groups maintained a constant relative intensity, and is assumed this created a constant thermal stimulus, this was not confirmed, preventing the capacity to determine the absolute heat production which may vary across individuals for a given relative intensity (Gibson *et al.*, 2017).

7.6 Conclusions

A mixed, active and passive HA protocol, controlling exercise intensity by HR, is capable of invoking positive thermoregulatory adaptations in paratriathletes and AB triathletes. Both groups displayed reductions in T_c , BLa, RPE and TS during a submaximal HST with significant PV expansion. Furthermore, the AB-ACC group presented additional adaptations including reduced HR and T_{sk} with an elevated sweat rate. This is the first evidence of differences in thermoregulatory variable changes to the same protocol between physiologically similar Paralympic and AB athletes. The HR-controlled exercise HA resulted in a constant relative intensity between HA sessions with the AB-ACC group capable of producing a greater PO for a set intensity over the study period. Finally, there was evidence of a direct performance benefit as the AB group improved their PO during a 20 km TT in the heat to a greater extent than the natural variation shown in a non-acclimated, matched cohort.

8

General discussion

8.1 Summary of main findings and contribution to scientific understanding

All main findings from the aforementioned experimental studies contribute to the principal aims of this thesis: to better characterise the physiology of paratriathletes, bridging the knowledge gap concerning AB and Paralympic athletes (Figure 8.1), with particular focus on the objectives presented in Chapter 1.2:

- To determine how athletes' mucosal immune system adapts to training load over a prolonged period and how this relates to illness incidence.
- To describe the responses of common, non-invasive, hormonal, immunological, physiological and psychological markers of overreaching to a period of intensified training.
- To characterise the thermoregulatory strain imposed by paratriathlon racing in the heat.
- To analyse the effectiveness of an ecologically valid heat acclimation strategy and assess how adaptations may differ to able-bodied athletes.

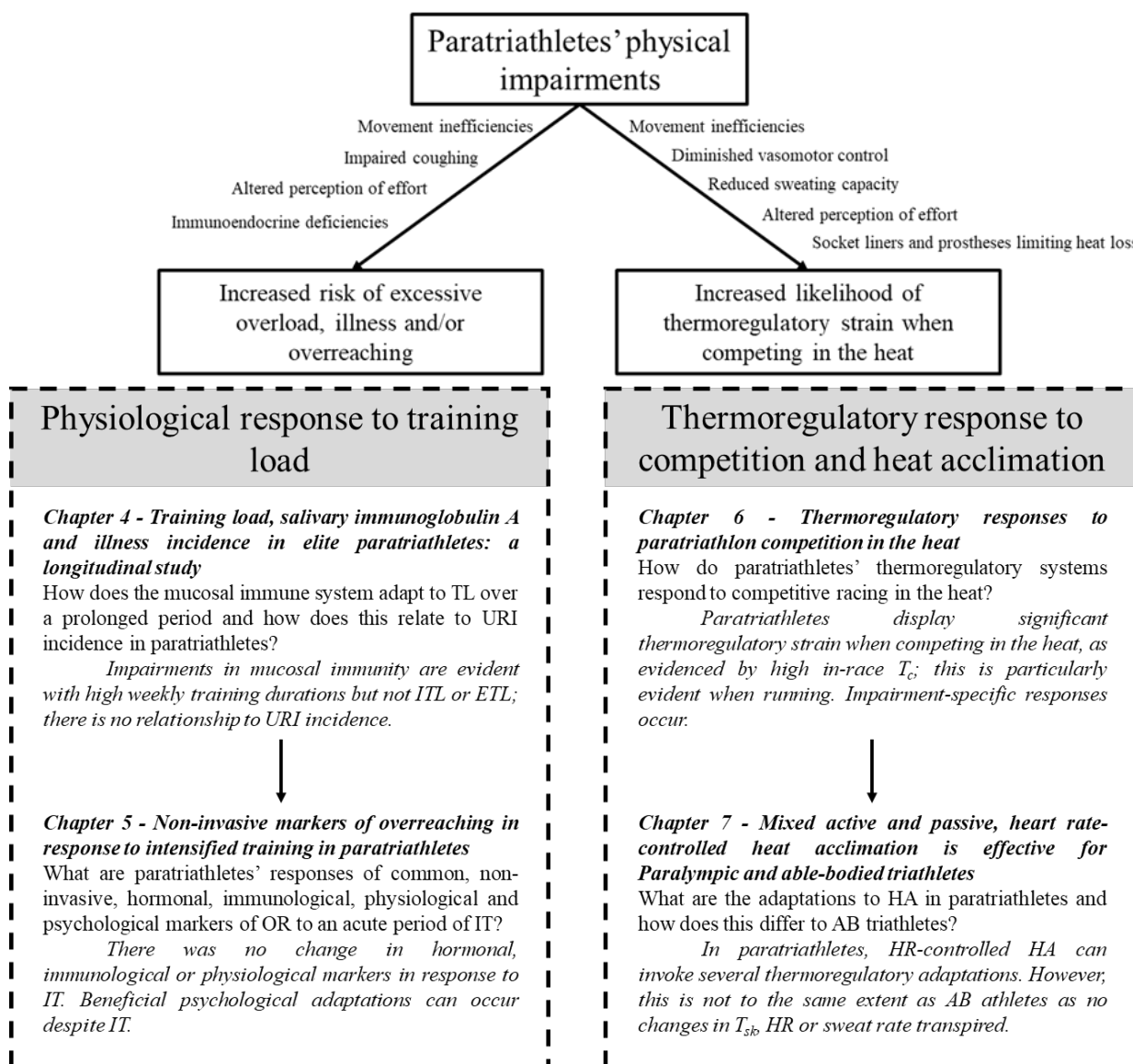


Figure 8.1: Schematic representation of experimental studies of this thesis. TL – Training load. URI – Upper respiratory tract illness. ITL – Internal training load. ETL – External training load. OR – Overreaching. IT – Intensified training. T_c – Core temperature. HA – Heat acclimation. AB – Able-bodied. HR – Heart rate. T_{sk} – Skin temperature.

Chapter four showed that, by longitudinally tracking both paratriathletes’ mucosal immunity (sIgA) and training over 34 weeks, sIgA displayed an inverse relationship with weekly training duration (with changes in training explaining 12.7% of the variance in sIgA SR), although there was no meaningful relationship between sIgA and measures of ITL and ETL. However, despite athletes displaying a high illness incidence, relative to AB athletes and the general population (Gleeson *et al.*, 1999a; Gleeson *et al.*, 1999b) and likely mediated by

impairments increasing URI susceptibility, there was no relationship to mucosal immunity. This is despite URI occurrence limiting athletes' training availability 50% of the time, with this occurring 43% of the time in **Chapter five**. While relationships between sIgA and URI incidence have been shown elsewhere in AB athletes (Fahlman & Engels, 2005; Gleeson *et al.*, 1999b; Gleeson *et al.*, 2017; Moreira *et al.*, 2014; Mortatti *et al.*, 2012; Neville *et al.*, 2008; Orysiak *et al.*, 2017), this is the first research in paratriathletes. Furthermore, this study builds on limitations in previous research by using objective measures of TL, specifically HR, in line with the recommendations of Kellmann *et al.* (2018).

In **Chapter five**, it was noted that the cohort of paratriathletes were robust to IT and displayed no signs of maladaptation, despite increases in TL similar to previous research in AB athletes where OR was present (Hauswirth *et al.*, 2014; Hough *et al.*, 2013; Le Meur *et al.*, 2013). Specifically, improvements in self-reported sleep, recovery and stress parameters were noted with no change in salivary hormonal (sC and sT) and immunological (sIgA) markers, or other wellness measures. Others have also shown a lack of OR signs or symptoms in AB athletes around IT (Buchheit *et al.*, 2013; Slivka *et al.*, 2010). Nonetheless, the absence of OR could not be confirmed due to the lack of a maximal, sport-specific, performance test. Regardless, this is the first study to characterise the response to IT in a group of multi-impairment, Paralympic endurance athletes, despite athletes being at heightened risk for excessive overload.

The thermoregulatory strain of paratriathlon competition in the heat was characterised in **Chapter six** by continuous T_c measurement. Not only was this the first study to note the thermoregulatory strain of competitive sprint distance triathlon racing, but the originality of including athletes with multi-impairments was of great significance. We found that athletes faced significant strain, with T_c and self-reported heat illness symptomatology significantly greater than previous research in AB athletes (Baillot & Hue, 2015; Del Coso *et al.*, 2014; Lee *et al.*, 2010; Racinais *et al.*, 2015b). Furthermore, in-race trends of T_c changes were found for paratriathlon race categories with preliminary evidence regarding T_{sk} , the impact of wetsuit use, and in-race performance changes also presented. Specifically, PTVI athletes display a significantly greater T_c during the run section than those in the PTWC category, which likely relates to disparate race modalities across impairments.

Finally, acknowledging the thermal strain of paratriathlon competition in the heat shown in **Chapter six**, in **Chapter seven** the efficacy of an ecologically valid HA strategy was

explored. It was noted that a mixed, active and passive, 8-d HA intervention, controlling the relative intensity of exercise via HR, was efficacious in stimulating partial adaptation in paratriathletes. However, the breadth of thermoregulatory adaptations were not as extensive as in physiologically matched AB triathletes whereby improvements in T_{sk} , HR and sweat rate were noted during the submaximal HST. Finally, the AB group displayed performance improvements during a 20 km cycling time trial in the heat. Specifically, this group improved their performance to a greater extent than the smallest worthwhile change calculated from the natural variation present in a group with no HA. The findings of this study are comparable to the work of Castle *et al.* (2013), who also noted partial HA in a small group of target shooters with an SCI. The efficacy of mixed, active and passive HA supports recent suggestions that this method may provide the optimum pre-competition strategy for elite athletes, whereby heat stimuli may be potentiated without excessive ITL or disruption to training routines (Casadio *et al.*, 2017; Guy *et al.*, 2014; Ruddock *et al.*, 2016). Moreover, the usefulness of HR to regulate HA intensity substantiates the proposal of Périard *et al.* (2015), that this method is a viable alternative to continuous T_c measurement during isothermic HA. This is due to adaptations being similar to previous studies of a comparable duration (Tyler *et al.*, 2016).

8.2 Practical application of findings

Given the lack of scientific literature concerning paratriathlon, this thesis provides the starting point for research in the sport. From this, coaches and practitioners may now have a better understanding of paratriathletes' physiological and thermoregulatory response to training and competition. Consequently, each experimental study has practical applications for those working within the sport, as detailed below.

8.2.1 Mucosal immunity and illness incidence

In **Chapter four** there was no significant relationship between URI incidence and sIgA, despite a high URI prevalence reported in **Chapters four** and **five**. Subsequently, the use of sIgA for the early, proactive detection of future URI risk is questioned in this population group. However, coaches and practitioners should be aware that during periods of high weekly training duration, although not TL, depressions in paratriathletes' sIgA SR can occur. Nonetheless, there must also be an acknowledgement of other non-training stressors such as psychological or environmental stress and the effort of daily ambulation in Paralympic athletes (Leicht *et al.*, 2012; Webborn & Van de Vliet, 2012). It should be noted, however, that a large inter-individual variability existed regarding the relationship between sIgA and training

duration, thus there may be merit in an individualised examination of the efficacy of sIgA monitoring.

8.2.2 Monitoring for signs and symptoms of overreaching

In **Chapter five**, it appeared that athletes did not show any signs or symptoms of OR, despite their TL increasing by an amount that may have been expected to lead to OR (Hauswirth *et al.*, 2014; Hough *et al.*, 2013; Le Meur *et al.*, 2013). This was hypothesised to be mediated by the training camp environment minimising external life stresses commonly experienced by Paralympic athletes (Webborn & Van de Vliet, 2012). Furthermore, as proposed elsewhere, high physical fitness and the careful management of TL by coaches likely contributed to athletes' toleration of the imposed training (Buchheit *et al.*, 2013). Consequently, those working with paratriathletes may be advised to similarly minimise non-training stresses during IT to reduce the likelihood of OR signs and symptoms, whilst carefully managing the TL with integrated recovery periods. Although, it is likely that tolerance to IT would not be as great and more signs of OR may be evident in paratriathletes with lower physical fitness (more akin to the normative data presented in **Chapter two**).

8.2.3 The thermoregulatory strain of paratriathlon competition in the heat

Although paratriathlon races are commonly held in hot and humid environments, **Chapter six** provided the first evidence of the thermoregulatory strain imposed by such events. From these data, coaches, practitioners and medical staff now have a better understanding of the thermoregulatory strain imposed by paratriathlon competition in the heat. This may subsequently prompt the implementation of strategies to alleviate such strain. For example, athletes may look to utilise HA (**Chapter seven**) or heat acclimatisation strategies due to the potential for improvements in thermoregulatory variables. Furthermore, pre- or per-race cooling strategies (Stevens *et al.*, 2017) may be employed and modified depending on athletes' race category and expected T_c responses (e.g., the use of cooling strategies when handcycling for PTWC athletes and when running for ambulant athletes). Athletes' race and pacing strategies may also be examined based on the **Chapter six** findings. Due to the relationship between relative race intensity and later T_c response, athletes may look to utilise an even-paced effort to minimise the likelihood of a consequential excessive T_c rise (Ely *et al.*, 2008). Finally, athletes' use of a wetsuit in similar environmental conditions to the experimental study may be discouraged due to its potential effect on T_c and heat illness symptomatology and may prompt a change in ITU paratriathlon race legislation.

8.2.4 Ecologically valid heat acclimation

To gain a greater understanding of how to prepare paratriathletes for potential environmental conditions shown in *Chapter six*, *Chapter seven* studied the responses of paratriathletes and AB triathletes to an ecologically valid HA protocol. The findings of this experimental study revealed that differing thermoregulatory adaptations occur between paratriathletes and AB triathletes. Nonetheless, these results do confirm that paratriathletes are capable of partial heat acclimation as improvements in other thermoregulatory parameters were noted. Moreover, the results of this study support the proposal of Périard *et al.* (2015) that HR can be a cost-effective and athlete-friendly alternative to traditional isothermic approaches which necessitate constant T_c measurement. Also, it was noted that in AB athletes the intervention resulted in a performance gain during a 20 km cycling time-trial in the heat. Therefore, although the paratriathlon group did not undertake any performance tests, it may be speculated that the thermoregulatory adaptations invoked by HA would result in performance gains. As such, paratriathletes or AB triathletes may seek to mimic a mixed, active and passive HA protocol, using HR to regulate exercise intensity when preparing for competition in hot environments.

The main findings, contributions to scientific understanding and practical applications of this thesis' experimental studies are summarised in Table 8.1.

Table 8.1: Summary of experimental chapters, their contribution to scientific understanding and practical applications.

Chapter	Measures	<i>n</i>	Setting	Main findings	Contribution to science	Practical applications
Four	The relationship between sIgA from 34 weeks of saliva samples and training duration, ITL, ETL and URI incidence.	7	Training	A significant negative relationship between weekly training duration and sIgA SR; no meaningful relationship to ITL or ETL. No meaningful relationship between sIgA and URI incidence.	Understanding the link between training, sIgA and URI incidence in a multi-impairment endurance sport. Research has previously been confined to AB and a limited number of Paralympic sports.	This study could not support the use of sIgA as an early marker of potential illness. Nonetheless, high weekly training durations may impair mucosal immunity.
Five	sC, sT, sIgA, sleep, wellness and psychological (POMS & RESTQ-S) measures during 14-day IT.	10	Overseas training camp	No meaningful change in salivary or wellness measures during IT. IT increased subjective sleep quality and quantity, lowered POMS anger subscale whilst there was greater general recovery and lower sport and general stress from RESTQ-S.	Report of adaptation to IT in a group of Paralympic, endurance athletes. Responses of salivary parameters appear similar to previous results in AB athletes.	During IT, careful management of TL and a minimisation of external life stresses can reduce the likelihood of negative hormonal, immunological, physiological and psychological adaptations.
Six	T_c and T_{sk} during competition in the heat with wetsuit use noted and self-reporting of heat illness symptomatology.	28	In-race	As a group, T_c increased over time with a significant increase whilst running. T_c increases were augmented for athletes in PTVI when running, compared to those in PTWC. Wetsuit use and heat illness symptomatology resulted in a greater T_c early in the race. There was little change in T_{sk} .	Evidence of thermoregulatory responses to field-based sprint triathlon performance with previous literature confined to long-distance events. Knowledge of impairment-specific responses to paratriathlon racing and the effect of wetsuit use. Novel inclusion of T_{sk} measurement.	The thermoregulatory challenge to paratriathlon racing in the heat is now defined. Strategies can be put in place to alleviate this heat strain based on impairment- and race-phase-specific T_c responses.

sIgA – Salivary secretory immunoglobulin A. ITL – Internal training load. ETL – External training load. URI – Upper respiratory tract illness. SR – secretion rate. AB – able-bodied. IT – Intensified training. sC – Salivary cortisol. sT – Salivary testosterone. POMS – Profile of mood states. RESTQ-S – Recovery-stress questionnaire for sport. TL – Training load. T_c – Core temperature. T_{sk} – Skin temperature.

Table 8.1 continued: Summary of experimental chapters, their contribution to scientific understanding and practical applications.

Chapter	Measures	n	Setting	Main findings	Contribution to science	Practical applications
Seven	Paratriathletes undertook eight days of mixed active and passive, HR-controlled HA with an HST on the first and last day. AB triathletes performed a 20 km cycling time trial in the heat with (n=13), or without (n=9) HA.	29	Lab	HA invoked thermoregulatory adaptations in the paratriathletes, however, this was not as extensive as the AB athletes because paratriathletes displayed no meaningful changes in T_{sk} , HR or sweat rate during the HST. The AB athletes increased their cycling workload for a set HR during HA. HA induced performance improvements as the AB athletes undergoing HA increased their cycling PO to a greater extent than the natural variation of performance found without HA.	Support for the efficacy of controlling HA relative intensity via HR with thermoregulatory and performance adaptations of HA in present. Assessment of HA in a multi-impairment Paralympic sport.	When preparing for competition in a hot environment, controlling HA via HR is an efficacious and alternative to continuous T_c measurement. Moreover, paratriathletes are capable of displaying thermoregulatory adaptations, albeit not to the same extent as AB triathletes.

HR – Heart rate. HST – Heat stress test. AB – Able-bodied. T_{sk} – Skin temperature. PO – Power output. T_c – Core temperature.

8.3 Future directions

This thesis has provided the first reports of paratriathletes' training and competition from a physiological perspective. However, there are several areas for future research that studies may look to address, as discussed below.

8.3.1 Training load, illness risk and overreaching

Firstly, it may be pertinent that future studies seek to explore different methods of quantifying TL. Although the importance of TL quantification has been well acknowledged for endurance sports (Mujika, 2013; Mujika, 2017), there has been little recognition of the complexities when calculating TL in a multi-modal sport whilst still incorporating training intensity. For example, Lucía's TRIMP (Lucía *et al.*, 2003) does not account for the relative strain imposed by triathlon or paratriathlon's constituent sport modalities. Alternatively, although the equation proposed by Cejuela-Anta and Esteve-Lanao (2011) does build on this limitation, there is no inclusion of training intensity factors. Furthermore, there has been little attention paid to the appropriateness of TL measures in the Paralympic athlete population.

Consequently, whilst it was beyond the scope of this thesis to assess the limitations of previous work regarding TL quantification that may be suitable for a multi-impairment, multi-modal, endurance sport, future research should seek to address this issue. For example, studies may assess the appropriateness of the modality weighting factors proposed by Cejuela-Anta and Esteve-Lanao (2011) based on previous research regarding the energy cost of swimming, cycling and running. This may be particularly relevant for paratriathlon whereby the relative cost of each modality will vary depending on the athletes' impairment or race category (e.g., PTWC vs. PTVI). Also, research should consider other methods of ITL quantification beyond that of Lucía *et al.* (2003). For example, incorporating other TRIMP methods such as Banister's TRIMP (Banister, 1991) or Edwards' summated HR zones (Edwards, 1993), whilst remaining cognisant of potential confounding factors when using HR with Paralympic athletes (Paulson & Goosey-Tolfrey, 2017). Similarly, further investigation of the applicability of sRPE (Foster, 1991) is warranted as this may negate the issues of differences in modality stress imposed, whilst again considering this may be limited in athletes with CP (Runciman *et al.*, 2016). Finally, the Training Stress ScoreTM concept, which calculates an ETL or ITL from training time and intensity relative to AnLT, may also be worthy of future study due to its rising popularity within endurance sports (De Groot *et al.*, 2018; Sanders *et al.*, 2017)

In *Chapters four* and *five*, it was noted that although athletes displayed a high URI incidence, this was not related to sIgA. As acknowledged previously, sIgA is merely one of myriad immunological parameters that are likely to be influenced by TL. As such, monitoring other immunological variables would provide further insight into the relationship between TL and URI incidence and may permit the prognostic detection of impending illness. For example, other salivary anti-microbial proteins, such as lactoferrin, lysozyme or α -amylase, have been suggested as alternative non-invasive markers of immune state (Leicht *et al.*, 2012; Walsh *et al.*, 2011). Furthermore, the longitudinal tracking of markers of physical or psychological stress and sympathetic nervous system activation, such as α -amylase, cortisol or chromogranin A would provide greater mechanistic data related to changes in immune variables caused by TL (Leicht *et al.*, 2012).

The results of *Chapter five* suggest paratriathletes of a high training status may be resilient to large, acute changes in TL. However, as noted in *Chapter five*, the absence of OR could not be unequivocally confirmed due to the lack of a maximal, sport-specific performance test. This testing method has been employed in previous studies of AB athletes as the main consequence of OR is a decrement in sport-specific performance (Meeusen *et al.*, 2013). However, the usefulness of this in potentially overreached athletes may be questioned due to the effect a further fatiguing task can have (Le Meur *et al.*, 2013). Nonetheless, future research may investigate alternative approaches for the detection of OR. For example, the use of short, sub-maximal exercise tests has recently been studied. The Lamberts and Lambert Submaximal Cycling Test (Lamberts *et al.*, 2011) and Lamberts Submaximal Running Test (Siegl *et al.*, 2017) are two such examples whereby athletes exercise for a total of 15 min at three, submaximal intensities. During the tests, HR and RPE are collected whilst HR recovery is noted for 60 s post-test. These tests show good potential for the early, non-invasive detection of OR and the associated performance decrements, without excessive physical strain imposed on athletes (Hammes *et al.*, 2017; Lamberts *et al.*, 2011; Siegl *et al.*, 2017). Therefore, future research may look to include short, submaximal exercising protocols to aid the detection of OR in paratriathletes after periods of IT. However, there should still be an understanding of potential issues with utilising HR or RPE for this population, as mentioned previously, whilst also still utilising a multifactorial approach, thus not neglecting the importance of psychological metrics (Siegl *et al.*, 2017).

8.3.2 Acute strategies to maximise performance in the heat

As shown in **Chapter six**, paratriathletes face significant thermoregulatory strain when competing in the heat that results in a high prevalence of heat illness symptomatology. Whilst **Chapter seven** presents a strategy to ameliorate the thermal strain of exercise in the heat (in the form of HA) there is scope for future research to investigate more acute interventions.

In **Chapter six**, it was shown that athletes choosing to wear a wetsuit for the swim section of the race resulted in a significantly elevated T_c early in races. Furthermore, this was related to a higher tendency for heat illness symptomatology. Consequently, future research should consider the appropriateness of the current 28°C upper water temperature limit for volitional wetsuit use in ITU paratriathlon races. A future study could monitor the thermoregulatory responses, particularly T_c and T_{sk} , to a 750 m swim in varying water temperatures with or without the use of a wetsuit. This may provide information on the highest water temperature tolerable for paratriathletes when wearing a wetsuit which does not cause excessive thermoregulatory strain. Such information may inform athletes' decision to wear a wetsuit or facilitate a change in ITU paratriathlon legislation.

As noted in Chapter 8.2.3, a practical application of **Chapter six** is a greater insight into race category-specific T_c trends which may form a basis for targeted pre- or per-race cooling strategies. Whilst recent reviews have been produced regarding the efficacy of such strategies (e.g., ice slurry ingestion or cold-water immersion) (Bongers *et al.*, 2017; Stevens *et al.*, 2017), it is not known how appropriate these methods may be for paratriathlon competition in the heat. Research may wish to investigate how these strategies impact the likelihood of autonomic dysreflexia in athletes with an SCI as cold exposure may invoke a reflexive sympathetic discharge in this population (Hubli *et al.*, 2018). Similarly, research may note how cooling effects hypertonia in athletes with CP as anecdotal reports from athletes suggest cold exposures can significantly elevate athletes' tone and spasticity. Finally, there are reports that pre-cooling strategies diminish athletes' sweat responses (Choo *et al.*, 2018; Stevens *et al.*, 2017). Paratriathletes are a population that may exhibit an already diminished sweating capacity relative to AB athletes, thus research should show if pre-cooling strategies lower sweat responses to exercise and if this exists beyond the swim section of races whereby evaporative heat loss is irrelevant.

From a per-race perspective, the review of Stevens *et al.* (2017) supports the use of cold fluid or ice slurry ingestion, with or without the addition of menthol, and localised cooling of

the neck and face. However, as stated above, there may be contraindications with paratriathletes that limit the efficacy of cold fluid or slurry ingestion. Furthermore, menthol use or head and neck cooling have been shown to be effective at lowering thermal perception when exercising in the heat (Stevens *et al.*, 2017). However, if this is not accompanied by a lowering of body temperatures in athletes with a potentially impaired pace and thermal perception, such as those with CP or a VI, it may be counterproductive and prevent athletes downregulating their intensity to prevent excessive thermal strain. This is particularly pertinent as **Chapter six** provides preliminary evidence that a greater exercise intensity early in paratriathlon races can result in a later increase in T_c . Furthermore, previous research in Paralympic athletes has shown localised cooling can decrease *ad libitum* fluid intake during simulated competition in the heat, although no body temperature measures were made (Goosey-Tolfrey *et al.*, 2008). Nonetheless, this is an avenue for future research as there is no current evidence for these strategies in paratriathletes.

8.3.3 Chronic strategies to maximise performance in the heat

Whilst **Chapter seven** presented one such strategy to alleviate the thermal strain of exercise in the heat, typical of that in **Chapter six**, there are avenues for future research to build on these findings. Firstly, whilst **Chapter seven** showed the efficacy of a mixed HA approach for invoking partial acclimation in paratriathletes, it is not known how adaptations may differ between solely active or passive approaches. Both approaches have shown to be efficacious in AB endurance athletes (Daanen *et al.*, 2018); however, in **Chapter seven** a mixed method was utilised to minimise disruption to athletes' pre-competition training routines whilst potentiating the stimuli for adaptation. Nonetheless, future research may look to establish the adaptive capacity of paratriathletes to just active HA or passive HA of a similar duration.

Another area worthy of research is the scheduling of HA in athletes' race calendar. HA increases the physiological toll of training which may be counterproductive when tapering for competition (Casadio *et al.*, 2017). Although the methods of **Chapter seven** attempted to reduce this strain, further improvements may be made. Specifically, rescheduling HA further before competition and using shorter re-acclimation protocols may be beneficial and is an approach studied recently in two AB sailors (Casadio *et al.*, 2016). The study noted that most thermoregulatory adaptations were retained for two-weeks post-HA, despite training in thermoneutral conditions, and two days of re-acclimation were sufficient to regain full acclimation status (Casadio *et al.*, 2016). Thus, athletes may acquire a thermal 'memory' that permits rapid re-acclimation. Furthermore, other preliminary evidence suggests the addition of

periodic heat exposure every 5 d post-HA may retain HA status up to 25 d (Pryor *et al.*, 2019). Together, this may permit the rescheduling of HA earlier in athletes' calendars whilst using intermittent heat exposures and very short re-acclimation protocols to complement athletes' pre-competition tapering strategies. Nonetheless, as this is a very new area of research, there is scarce literature to support the efficacy of these strategies for either AB or Paralympic athletes. However, this may be an impactful opening for future direction. Specifically, strategies that alleviate the thermoregulatory strain evident in **Chapter six**, whilst minimising the likelihood of paratriathletes experiencing excessive TL (as discussed in **Chapter four** and **Chapter five**) would build on the findings of **Chapter seven** to further aid performance.

8.3.4 Ethical considerations

Due to the nature of the experimental studies in this thesis, there were areas which should be highlighted from an ethical standpoint and may pertain to future research. Firstly, in **Chapter five** athletes may have experienced OR as a consequence of IT. As discussed previously, this did not occur due to several factors such as the careful management of TL and integration of recovery periods. Nonetheless, in future studies athletes may develop OR or even overtraining syndrome if they are of a lower physical fitness or protective mechanisms are not in place. In this scenario, it is imperative that athletes have full prior knowledge of the potential risks of IT, whether it be planned by a coach or researcher, and can withdraw at any point.

Secondly, in **Chapter six** athletes competed in a thermoregulatory challenging environment that resulted in significant thermal strain and prevalence of heat illness symptomatology. As with the above point, it was important athletes understood the risks of intense exercise in the heat and considered their medical contraindications. Nonetheless, athletes were already due to compete in the race setting so inclusion in the study provided no additive risk to their intended routine.

8.4 Closing statement

This thesis provides the first documentation of paratriathletes physiology from a training and competition perspective, utilising a range of study settings. It has been shown that paratriathletes of a high physical fitness, relative to normative values in Paralympic endurance sports, may be resilient to TL fluctuations from an immunological and hormonal standpoint. However, paratriathletes face significant thermoregulatory strain when competing in the heat although preparatory strategies can be efficacious at stimulating beneficial adaptations.

9

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10 - Appendices

Appendix A - Reliability and validity of Garmin Vector cycling power meter compared to the Cyclus 2 ergometer

Abstract

Purpose: To assess the reliability and validity of the Garmin Vector (GV) power meter in comparison to the Cyclus 2 (C2) ergometer over a range of cycling PO.

Methods: Nineteen AB participants twice undertook a maximal cycling GXT and three maximal sprints two to seven days apart to assess GV reliability. During the protocol PO was recorded continuously via both devices to determine validity.

Results: The mean CV for GV PO was 2.1%. There was a strong positive correlation between GV and C2 PO ($p < 0.001$), however GV significantly underestimated PO (systematic error = -8 W, random error = 6W; $p < 0.001$). The 95% limits of agreement were -19 to 3 W. There was no heteroscedasticity or proportional bias in the PO offsets during the GXT. During the sprints, there was a significant difference in PO ($p < 0.001$) whilst offsets were heteroscedastic, and bias was proportional.

Conclusions: Despite a -8 W offset between the two devices, it is possible to transform PO. Further, with the acceptable PO CV, the GV can be a useful tool to regulate and monitor cycling PO, albeit not during maximal sprints.

Introduction

Mobile power meters are becoming a commonly used instrument by cyclists and triathletes to permit field-based cycling PO and cadence measurement (Pinot & Grappe, 2011). This allows: regulation of training intensity, often based on laboratory derived training zones; the capability to track changes in performance; and, the calculation of ETL (Delattre *et al.*, 2006). Several mobile power meters have been tested for reliability and validity of PO measures against criterion values (Paton & Hopkins, 2001). It has been suggested that the Schoberer Rad Meßtechnik (SRM) power meter (SRM GmbH, Jülich, Germany), which measures PO at the crank set with multiple strain gauges, offers the most reliable and valid commercially available mobile power meter (Paton & Hopkins, 2001). Due to the high level of reliability and validity in SRM power meters, it was employed by Reiser *et al.* (2000) to confirm the accuracy of the Cyclus 2 ergometer (C2) (RBM elektronik-automation GmbH, Leipzig, Germany), which displays a <1 W offset in comparison to the criterion SRM. This has again been shown in a recent study, confirming the device's validity against the SRM device at PO <500 W (Rodger *et al.*, 2016). The C2 is an electromagnetically braked ergometer that permits athletes to use their own bike, thus limiting any variation that can be caused using an unfamiliar fixed position (Hopkins *et al.*, 2001). Additionally, the ergometer is designed to permit lateral movement of the bicycle, via elastically constructed axels, to mimic natural field-based cycling (Reiser *et al.*, 2000). Due to its accuracy, and the ability for participants to use their own bike, the C2 ergometer is commonly used during laboratory tests to benchmark athletes' performance (Sanders *et al.*, 2017). Several other devices have since been tested for PO measure reliability and validity against either SRM power meters or other criterion values with varying degrees of acceptability (Bertucci *et al.*, 2005; Bouillod *et al.*, 2017; Duc *et al.*, 2007; Gardner *et al.*, 2004; Millet *et al.*, 2003; Sparks *et al.*, 2015).

However, there has been a recent interest in pedal-based power meters since they are typically lighter than other systems whilst detecting force closer to the site of force production and can be easily transferred between bicycles (Bouillod *et al.*, 2017; Novak & Dascombe, 2016; Sparks *et al.*, 2015). Sparks *et al.* (2015) found a Look Keo (Cadex, France) power meter to display strong agreement compared to an SRM power meter over a range of POs typical during cycling rides (50-350 W), it was therefore deemed a valid measure. It was not, however, shown to be reliable with this effect likely exacerbated by participants riding on an unfamiliar ergometer. Others have studied the new Garmin Vector (GV) (Kansas City, USA) which

measures PO via the combination of strain gauges (eight per pedal) based in the pedal spindle and a magnet activated cadence sensor (Novak & Dascombe, 2016). Several studies all found GV to be valid measures of PO in comparison to SRM devices (Bouillod *et al.*, 2017; Nimmerichter *et al.*, 2017; Novak & Dascombe, 2016), although Novak and Dascombe (2016) did note the requirement to transform PO data obtained from a range of maximal efforts (5 to 600 s). Nonetheless, the aforementioned studies were limited due to small sample sizes (Bouillod *et al.*, 2017; Nimmerichter *et al.*, 2017) limited range of POs (Nimmerichter *et al.*, 2017) or lack of a reliability measure (Novak & Dascombe, 2016). Further, to date, neither GV nor other pedal-based power meters have been assessed for reliability or validity against the C2.

The aim of this study was thus to assess the reliability and validity of PO and cadence measures from a GV device in comparison to previously accepted ergometer, the C2. A testing protocol, of a cycling GXT to exhaustion followed by three maximal sprints, was chosen to replicate POs typical during road riding whilst also containing measures proposed to have the lowest typical error and, therefore, provide the best opportunity to track changes in performance (Paton & Hopkins, 2001).

Methods

Participants

Nineteen physically active, AB participants (fourteen males, five females) volunteered to partake in the study; their characteristics are displayed in Table 1. All procedures were approved by the Loughborough University's Ethics Committee. Participants completed health screen questionnaires and provided informed consent before starting the study.

Table 1: Participant characteristics (n=19). All values are mean \pm SD.

Parameter	Value
Age (y)	25 \pm 6
Body mass (kg)	74.9 \pm 7.6
Stature (m)	1.80 \pm 0.09
$\dot{V}O_{2\text{peak}}$ (ml \cdot kg $^{-1}\cdot$ min $^{-1}$)	50.5 \pm 9.2
MAP (W)	285 \pm 54

$\dot{V}O_{2\text{peak}}$ – Peak rate of oxygen uptake. MAP – Maximum aerobic power.

Study Design

Each participant visited the laboratory on two separate occasions separated by two to seven days. Trials were performed at the same time of day to minimise any circadian rhythm effects; they consisted of two separate test protocols, an GXT to exhaustion plus a maximal sprints protocol, similar to the methods used by Sparks *et al.* (2015).

All trials were performed on a road bike (Viking Race 700c) fitted onto the C2. The bike frame was placed on an electronic brake with the chain over the pinion driving the braking mechanism (Reiser *et al.*, 2000). GV, unused before the start of the study, were fixed onto the bike cranks following the manufacturer's instructions (34 – 40 Nm). The bike geometry was customised to each participant and replicated across both trials. The use of both the C2 and GV permitted simultaneous collection of PO and cadence data from both devices. During all testing procedures, data were obtained from the C2 on a single pedal revolution basis whilst GV data were transmitted to a Garmin Edge 500 cycling computer (Kansas City, USA) at a frequency of 1 Hz for storage before being exported later.

Graded exercise test

During the first part of each trial, participants completed a GXT to volitional exhaustion. The methods for this are described in Chapter 3.1.

Maximal sprints protocol

During the second part of the test, 30 minutes after the completion of the GXT, participants completed a repeated sprint protocol consisting of three maximal ten second efforts separated

by three minutes of active recovery at a PO <100 W. During the test the C2 provided a braking force equivalent to 7.5% body mass when participants' cadence reached 70 revolution·min⁻¹, thus the test commenced from a rolling start. The application of the braking force marked the start of the ten second sprints where participants were asked to produce the greatest PO possible over the full duration, whilst remaining seated. Verbal encouragement was provided throughout all sprints. From the sprints, peak power output (PPO) was defined as the highest PO value recorded by each device whilst mean power output (MPO) was calculated as the average PO achieved during the ten second period.

Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics 22.0 software (IBM, New York, USA) and statistical significance was set at $p < 0.05$. PO and cadence data from the GXT were averaged over the three-minute stage duration for comparisons between C2 and GV.

To assess the reliability of PO and cadence measures, the mean CV for each workload stage were calculated. This was achieved by assessing the variation over both trials at all repeated workloads for each participant. Additionally, reproducibility coefficients were produced in accordance with the methods proposed by Bland and Altman (1999) by multiplying the within-participant SD by $1.96\sqrt{2}$. 95% reproducibility coefficients were compared to 95% limits of agreement to determine how agreement of PO measures may be influenced by devices' reproducibility.

Power output and cadence data from both devices were assessed for normal distribution. Further, heteroscedasticity was evaluated by assessing the heteroscedasticity coefficient between the absolute difference in PO (or cadence) measures by both devices and the mean PO (or cadence) value. Similarly, proportional systematic error (bias) was assessed by calculating the correlation between raw difference in PO (or cadence) measures and mean PO (or cadence). Differences between the two devices' recorded PO and cadence were assessed via paired t-test (parametric) or Wilcoxon's signed rank test (non-parametric) to determine GV validity. 95% CI were calculated for the mean differences between the devices. Spearman's correlation coefficient (r) was used to assess the degree of association between both devices' PO and cadence recorded during the GXT. The 95% limits of agreement were calculated using the systematic and random errors for the PO and cadence data using the methods described by Bland and Altman (1986).

Results

Graded exercise test

Reliability

The mean CV in GV PO for all participants' stages was 2.1%. The reproducibility coefficient for GV PO was 23 W meaning repeat PO measures using GV will be within ± 23 W of the mean 95% of the time. Mean CV for GV cadence was 3.8% whilst mean CV for C2 cadence was 3.6%.

Validity

Analysis of the PO and cadence data sets revealed homoscedastic residuals ($p \geq 0.186$) and the biases were not proportional to the mean ($p \geq 0.311$). A strong positive correlation ($p < 0.001$) existed between the PO measured by the two devices (Figure A1.1). However, during the test, there was a significant difference between the PO measured by the devices ($p < 0.001$) with the GV PO displaying a systematic error of -8 W (95 % CI: -9 to -7 W) and random error of 6 W. The 95% limits of agreement were -19 to 3 W (Figure A1.2), thus falling within the ± 23 W of the mean calculated as the reproducibility coefficient.

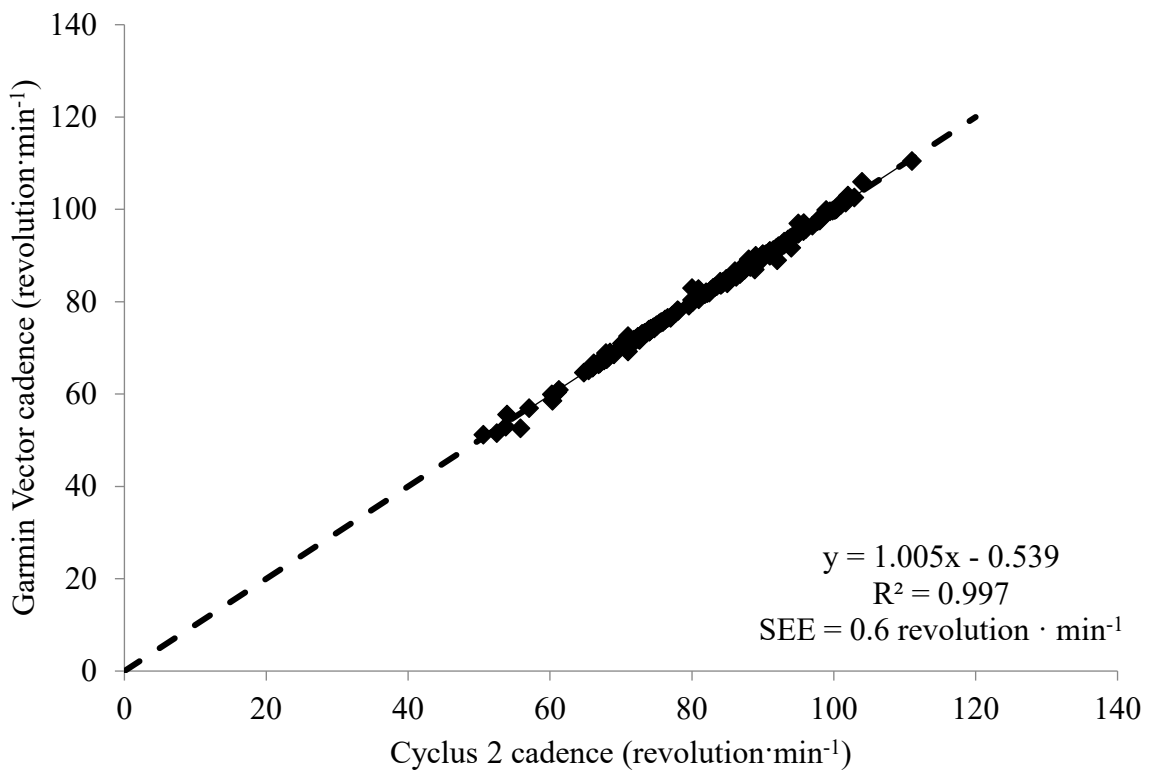
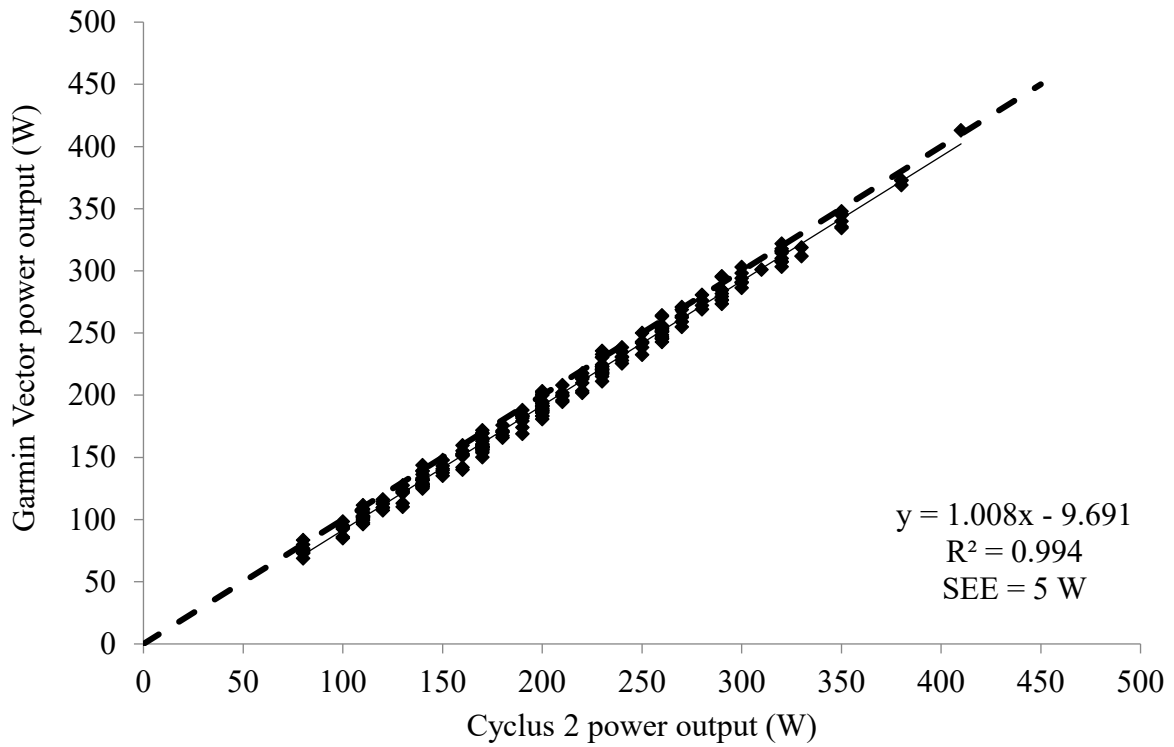


Figure A1.1: Regression analysis between Cyclus 2 and Garmin Vector power output (top) and cadence (bottom). Dashed line represents line of identity.

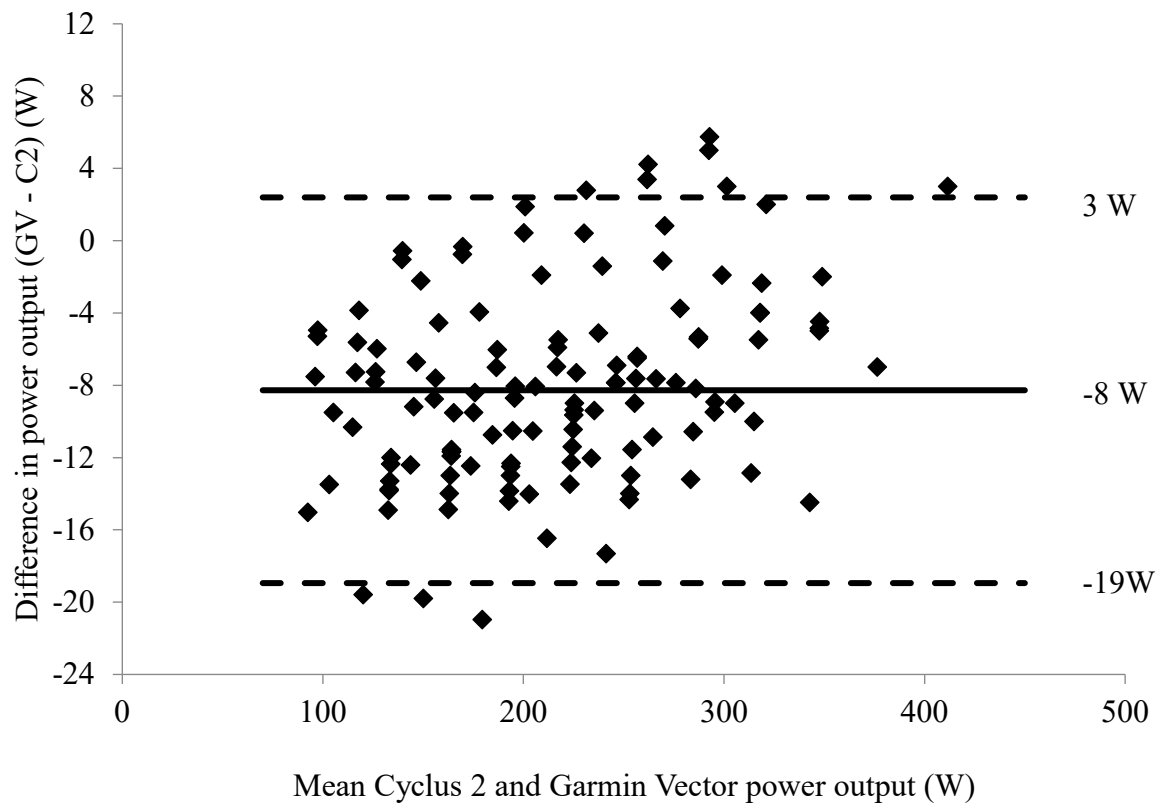


Figure A1.2: Bland-Altman plot of power output differences between Cyclus 2 (C2) and Garmin Vector (GV) for all participants' workloads. Solid line represents mean systematic error (bias) whilst dashed lines show 95% limits of agreement.

There was a strong positive correlation between cadence measures from GV and C2 ($p < 0.001$). There was no significant difference between cadence measures (median difference -0.1 (95% CI: -0.2 to -0.1) $\text{revolution} \cdot \text{min}^{-1}$; $p = 0.102$). The 95% limits of agreement for cadence between GV and C2 were -1.4 to 1.1 $\text{revolution} \cdot \text{min}^{-1}$.

Maximal sprints test

Mean variation of devices' PPO and MPO are displayed in Table 2. The PPO and MPO residuals between GV and C2 were proportional to the mean and heteroscedastic; therefore, all sprint PO data were transformed using a natural logarithm and ratio limits of agreement were calculated. PPO was significantly different between the two devices ($p < 0.001$). The PPO ratio limits of agreement were 0.97 to 1.39 (systematic error = 1.16 (95% CI: 0.94 to 1.38); random error = 1.19) (Figure A1.3). There was a significant difference between MPO recorded by the

two devices ($p < 0.001$). Ratio limits of agreement for MPO measurements were 0.89 to 1.22 (systematic error = 1.04 (95% CI: 0.83 to 1.26) and random error = 1.17) (Figure A1.4).

Table 2: Mean and 95% confidence intervals of devices' coefficient of variation during maximal sprints.

	Garmin Vector		Cyclus 2	
	Mean CV (%)	95% CI (%)	Mean CV (%)	95% CI (%)
PPO (W)	6.6	5.4 to 7.8	2.9	2.1 to 3.8
MPO (W)	5.8	4.8 to 6.8	3.5	2.6 to 4.5

CV – Coefficient of variation. CI – Confidence intervals. PPO – Peak power output. MPO – Mean power output.

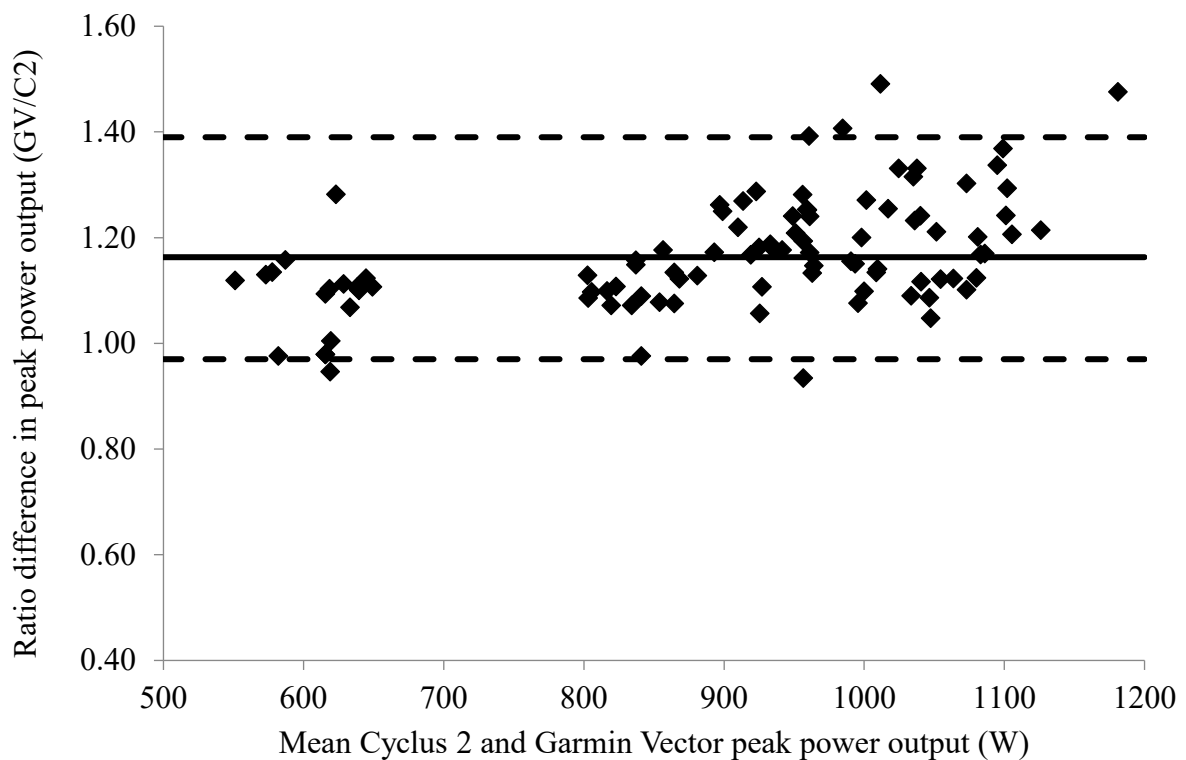


Figure A1.3: Bland-Altman plot of peak power output differences between Cyclus 2 (C2) and Garmin Vector (GV) for all participants' maximal sprints. Solid line represents mean systematic error (bias) whilst dashed lines show 95% ratio limits of agreement.

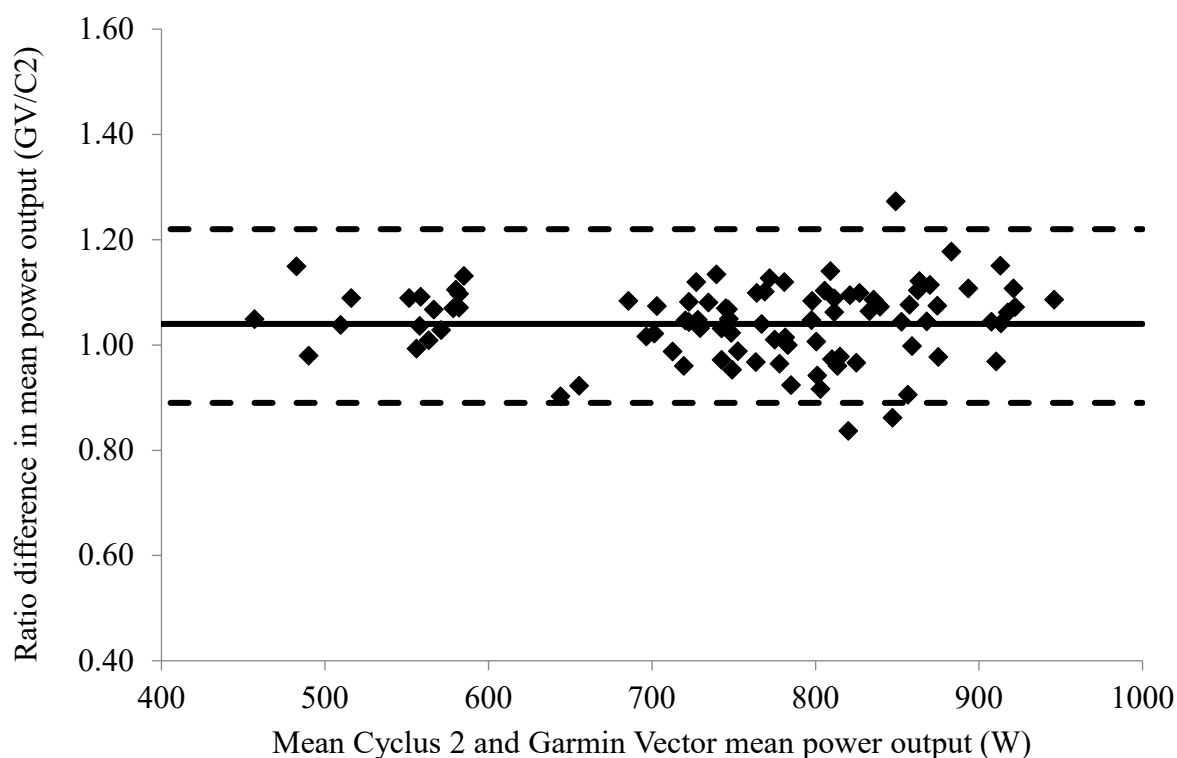


Figure A1.4: Bland-Altman plot of mean power output differences between Cyclus 2 (C2) and Garmin Vector (GV) for all participants' maximal sprints. Solid line represents mean systematic error (bias) whilst dashed lines show 95% ratio limits of agreement.

Discussion

The aim of the present study was to assess the reliability and validity of GV mobile power meters in comparison to a C2 ergometer. Results indicate that although GV demonstrated a systematic bias of -8 W compared with C2, this offset is consistent across a range of POs typical during cycle rides (80 to 400 W). Therefore, a simple modification can be made to translate POs between the two devices. Furthermore, GV was shown to be reliable with coefficients of variation similar to that of previously tested power meters (Bertucci *et al.*, 2005; Bouillod *et al.*, 2017; Duc *et al.*, 2007; Millet *et al.*, 2003; Nimmerichter *et al.*, 2017).

Due to the 8 W offset in PO measures between the two devices, the GV power meter cannot, strictly speaking, be classed as a valid instrument for PO measurement in comparison to the C2. This is unlike other commercially available power meters such as PowerTap (Madison, Wisconsin, USA) 1 or Look Keo (Sparks *et al.*, 2015) which provide comparable measures of PO to the criterion values. However, non-proportional bias and homoscedastic residuals suggest it is possible to transfer PO data between GV and C2 with the simple

addition/subtraction of the 8 W offset. These data support recent evidence that, in comparison to SRM power meters, GV PO measures should be treated with some caution due to the requirement for PO transformation, yet do remain useful (Bouillod *et al.*, 2017; Novak & Dascombe, 2016). However, this opposes the results of Nimmerichter *et al.* (2017) who found no significant difference to an SRM device, albeit over a limited range of POs.

The lack of proportional systematic error (bias) or heteroscedasticity permits a simple translation of any laboratory-based GXT results from the C2 ergometer to the field where athletes use GV to regulate cycling PO. Further, due to the low random error (6 W), and the subsequent 95% limits of agreement (-19 to 3 W) similar to previously accepted devices (Bouillod *et al.*, 2017); the GV can be used with confidence at submaximal intensities. The GV also confers advantages over other power meters in that, being a pedal-based device, it is typically lighter and easier to change between bicycles, all at a lower financial cost. The cause of the GV systematic error found in the current study is unknown. Given the differences in PO measurement location between the two devices (pedal spindle for GV and chain for C2) it could be anticipated that PO would be greater closer to the site of power production, i.e. in the pedal spindle. This trend was shown in the study of Novak and Dascombe (2016) whereby GV overestimated PO in comparison to crank-based SRM power meters. Moreover, as transmission losses in the bicycle chain are typically 2 to 4% (Martin *et al.*, 1998), it is surprising that GV still underestimates PO.

Despite the need to adjust PO data between C2 and GV to account for the systematic underestimation, the GV appears to be reliable under repeat-test situations. It is important for coaches, athletes and sport scientists using power meters to be confident that the device is dependable to detect meaningful changes in performance parameters or ETL (Jobson *et al.*, 2009), thus reliability of PO measurements is vital. The mean CV between tests for GV was 2.1 (95% CI 1.7 to 2.5%) with any differences due to device error and biological variation (Duc *et al.*, 2007). This is similar to other, well-accepted power meters tested previously including the SRM power meter and PowerTap which display CVs of 1.9 to 2.1% (Bertucci *et al.*, 2005; Duc *et al.*, 2007) which are deemed reliable enough to detect meaningful changes (Hopkins *et al.*, 2001). Further, the CV in the current study is lower than that reported by Bouillod *et al.* (2017) ($2.5 \pm 1.3\%$) across a range of cycling conditions in a single cyclist. Consequently, PO data from the GV can be used in confidence when comparing data over repeated tests or field rides to assess training adaptations.

Whilst GV displayed a consistent systematic bias at submaximal intensities, this was not the case during maximal sprints from a rolling start. Relative to C2, GV systematic biases were 16% and 4% for PPO and MPO, respectively. Furthermore, there was evidence of proportional systematic error (bias) and heteroscedasticity as the offset in PO measures was related to PO produced, although log transformation did eradicate this for MPO. As such, we do not recommend using GV for measurement of maximal sprints, supporting previous findings in studies of GV power meters compared to SRM devices (Bouillod *et al.*, 2017; Novak & Dascombe, 2016; Nimmerichter *et al.*, 2017). Several other power meters have also presented issues when detecting PPO (Bertucci *et al.*, 2005; Sparks *et al.*, 2015) with sampling rate a proposed cause of any offset. In the present study, GV recorded PO at a 1 Hz frequency whereas C2 did so every pedal revolution. As such, there is a greater likelihood GV would not be sensitive enough to detect a true PPO. Nonetheless, the lack of validity of GV during maximal sprints will be of less concern than during submaximal intensities as athletes are less likely to use PPO or MPO to guide for training intensities.

In the present study, GV were found to be a valid measure of cadence compared with C2. There was <1 revolution \cdot min⁻¹ offset between GV and C2. This corroborates the findings of Novak and Dascombe (2016) that GV and SRM power meter cadence measures were not significantly different. However, this opposes Nimmerichter *et al.* (2017) in their discovery of a small but significant difference in cadence measure between GV and SRM devices. Nevertheless, the study of Nimmerichter *et al.* (2017) was potentially underpowered by its low sample size. It is worth noting that cadence was more variable than PO with CV of 2.9% and 2.9% for GV and C2, respectively. This may be explained by individual self-selected cadence and, with the sub-elite status of participants, there is greater natural variation due to the unfamiliarity of cycling (Hopkins *et al.*, 2001).

The current study, similar to that of Sparks *et al.* (2015), utilised a design that provided a large number of measurement points over a range of intensities albeit with a larger sample size than the aforementioned study. Likewise, the PO ranges used were similar to those common in tests of power meters and typical cycling rides (Bertucci *et al.*, 2005; Duc *et al.*, 2007; Sparks *et al.*, 2015) to provide a representative assessment of power meter reliability and validity. By producing reliability measures over a range of submaximal POs, utilising a larger sample population, the current study builds on limitations of recent studies (Bouillod *et al.*, 2017; Novak & Dascombe, 2016; Nimmerichter *et al.*, 2017).

A limitation of the current study is that all tests were confined to a laboratory. Whilst this permitted a direct comparison of GV and C2 over a range of controlled intensities common during cycling, it did not allow investigation of the power meter in the field. Outdoor cycling is typified by long duration rides with varying terrain and cycling position. A selection of power meters has been investigated during outdoor cycling (Duc *et al.*, 2007; Millet *et al.*, 2003; Reiser *et al.*, 2000); however, only the studies of Bouillod *et al.* (2017) and Nimmerichter *et al.* (2017) utilised a pedal-based power meter. The authors reported contrasting findings of GV validity, thus future research should look to elucidate the effect of the aforementioned factors including ambient temperature (Gardner *et al.*, 2004).

Conclusions

Although GV power meters underestimate C2 submaximal cycling PO systematically, PO can be corrected easily by adding 8 W. Furthermore, the GV's excellent reliability allows the device to track changes in performance accurately over repeat tests and to use PO to quantify TL. However, caution should be exercised when using GV to measure maximal sprint POs.

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