Performance in enduro mountain biking: the influence of training status, recovery, and vibration

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Ph.D

2019

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A thesis submitted in partial fulfilment of the requirements of Edinburgh Napier University, the for the award of Doctor of Philosophy

May 2019

Abstract

Enduro mountain bike racing (enduro) consists of timed downhill race stages linked by non-competitive transition stages and general classification is determined by accumulated race stage time. Limited research is available on the physiological requirements of enduro despite a large population of professional elite riders. For this thesis, nine elite enduro athletes (n=8 male, n=1 female; top 100 world ranking) were recruited. Measures of daily training load (TL) and resting heart rate variability (HRV) were collected between three laboratory based tests throughout a season of training and racing. The demands of an international race event were assessed by heart rate, terrain induced accelerations and vibration exposure. Leukocyte subset (Neutrophils, CD4+ T-cell, CD8+ T-cell, and NK cell) redistribution, cortisol and IL-6 concentration were assessed at each laboratory test (pre, post, 1h-post) and the race event (pre, 1h-post, 19h-post). Main findings were that successful performance in enduro requires a large aerobic capacity (VO_{2peak} = 61.1 ± 5.2 ml.kg.min^{-1}, power VO_{2peak} = 410.9 ± 18.2W) coupled with adequate skill, technique and muscle mass to ensure high velocities can be sustained over differing types of terrain. Elevated TL appears to be a key component of training habit and the upper limit of training volume before negative adaptation was identified (>800 A.U. LuTRIMP). No relationship was found between TL and HRV. No significant changes were observed in leukocyte subset redistribution between laboratory tests. The race event induced significantly larger changes in circulating numbers of certain leukocyte subsets when compared to the laboratory test and the magnitude of redistribution of $CD4^+$ senescent T-cells was partially explained by vibration exposure (ΔR^2 = -0.673, $F_{(3,1)}$ = 12.12, p = 0.04). A subsequent novel assessment of vibration loading in mountain biking revealed potentially damaging levels of vibration exposure that could be associated with long term health implications in enduro athletes ($A_i(8)$ range 5.47 to 6.61ms^{-2}). It was concluded that vibration exposure needs to be considered in future models of physiological loading in this discipline.

Acknowledgements

I would like to take this opportunity to thank all of the people who have helped me generate this thesis in one way or another. However great or small their contribution, I certainly could not have done this alone. Firstly, thanks to Professor Geraint Florida-James for all of his wisdom and guidance as a director of studies but also as a great friend. Thanks for believing in me for all these years, for all of the advice in all walks of life, and all the good times out on the bikes – I hope there will be plenty more. Secondly, thanks to Dr Lesley Ingram for all of your help and assistance over the years in various walks of life. Les, you have shown me how to get the work done but still have a great time – the beer is on me at the bar, as promised! Thank you to Dr Eva Malone for all of the help in creating this thesis and all the advice along the way. Thanks must also go to Dr Mark Taylor for all of the 'Noddy diagrams' and help navigating the world of human vibration.

Thanks also to my friends, family and colleagues. In particular, a huge thank you to all of the PGR students at Edinburgh Napier that have helped me through this process with copious amounts of advice, support and most importantly laughter, particularly in 1.B.29. Every one of you has helped in some way. There are too many of you to mention, but a particular thanks has to go to Dave Lawson for always being there to talk about absolutely anything, anytime. I suppose thanks also has to go to Stu for the constant stream of abuse – I still don't like you, but I might miss you. Paul, thanks for the funny stories and to Bruce for all of the old man wisdom.

Of course, I wouldn't have been able to generate this thesis without the willingness of the individuals that participated in the studies. I am eternally grateful for your time and dedication to providing such valuable information, so thank you again.

Amanda, we might have only figured it out towards the end, but I could not have managed these final months without you. Thanks for distracting me with pizza, sticky toffee pudding, running, and cycling. I'm looking forward to plenty more adventures with you in the future!

Lastly, this thesis is dedicated to my mum, Lesley Kirkwood. Thanks for always believing in me, for making be believe I can do anything I set my mind to, and most importantly for your endless love and support. Thanks for always pushing me to do better while still enjoying myself. No son could ever ask for more – think it's about time we got that pint of Trade Winds!

Publications

The following published papers and conference proceedings have been produced from the work contained within this thesis.

Published papers:

- Kirkwood, L., Ingram, L., Cunningham, J., Malone, E. and Florida-James, G. (2017) 'Physiological characteristics and performance in elite vs non-elite enduro mountain biking.', *Journal Of Science & Cycling*, 6(2), pp. 13–21. doi: 10.28985/171231.jsc.10

Peer reviewed unpublished conference proceedings:

- Kirkwood, L., Ingram, L., Malone, E. and Florida-James, G. (2016) 'The physiological characteristics of elite vs non-elite enduro mountain bike cyclists', in *World Conference of Science & Cycling*. Caen, France.
- Kirkwood, L., Ingram, L., Cunningham, J., Malone, E. and Florida-James, G. (2018) 'Immunological responses and energy expediture at an international enduro mountain bike race', in *1st symposium for UK Society for Exercise Immunology*. Loughborough, UK.

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List of abbreviations and symbols

µl: Microliter

$A_i(8)$: Partial vibration exposure

ANOVA: Analysis of variance

ANS: Autonomic nervous system

bpm: Beats per minute

bTRIMP: Banister's training impulse

CHO: Carbohydrate

CMV: Cytomegalovirus

dBW: Decibel watt

DH: Downhill

EAV: Exposure activation value

EDTA: Ethylenediaminetetraacetic

EdTRIMP: Edward's training impulse

ELISA: Enzyme-linked immunosorbent assay

ELV: Exposure limit value

EWS: Enduro World Series

FBLC: Fixed blood lactate concentration

FSC: Forward scatter

g: units of acceleration due to gravity

GEE: General estimating equation

GPS: Global positioning system

h: Hours

HAV: Hand-arm vibration

HPA: Hypothalamic-pituitary axis

HR: Heart rate

$HR_{exercise}$: Exercising heart rate

HR_{max}: Maximum heart rate

HRV: Heart rate variability

Hz: Hertz

IL: Interleukin

ISAK: International Society for the Advancement of Kinanthropometry

iTRIMP: Individualised training impulse

$km.h^{-1}$: Kilometres per hour

km: kilometre

km: kilometres

LnRMSSD: Log normal root mean square of successive differences

LuTRIMP: Lucia's training impulse

MHC: Major histocompatibility complex

ml: Millilitres

mmol.L^{-1}: millimoles per litre

MTB: Mountain bike

NfOR: Non-functional overreaching

NK: Natural killer

P: Power

PBMC: Peripheral blood mononuclear cell

P_{max}: Maximum power

psi: Pounds per square inch

r.m.q. : Root mean quad

r.m.s. : Root mean square

RER: Respiratory exchange ratio

RHR: Resting heart rate

RPE: Rating of perceived exertion

SSC: Side scatter

TCR: T-cell receptor

TRIMP: Training impulse

URTI: Upper respiratory tract infection

VDV: Vibration dose value

VO_2: Oxygen consumption

VO_{2peak}: Peak oxygen consumption

VT: Ventilatory threshold

W: Watts

WBV: Whole body vibration

XCM: Cross-country marathon

XCO: Olympic format cross-country

β_2: Beta 2

Δ: delta; change

Chapter 1: General Introduction

Enduro mountain bike racing is a relatively new cycling discipline that was recognised as an international discipline for the first time when the Enduro World Series (EWS) was formed in 2013. Enduro racing comprises of primarily downhill race stages and non-competitive transition stages that must be completed within the provided time limit but do not contribute to overall race result. Accumulated time to complete the race stages forms the general classification (GC) for the race event, however time penalties can be applied in the event of a rule breach such as a missed time check (Enduro World Series, 2018). EWS race events are formed by one or two consecutive days of racing normally preceded by at least one day of practise to allow the athletes to see the race course prior to racing. In the event of no practice days, athletes complete the tracks for a sighting run immediately prior to racing on the same day. During the first year of competition in 2013, almost all athletes had transferred to enduro from the traditional mountain bike disciplines of Olympic format cross country (XCO) and downhill (DH). Since 2013, the industry has driven a rapid progression of the discipline resulting in growing numbers of professional enduro athletes and the development of enduro specific athletes. Despite a large population of professional enduro athletes, limited research concerning the physiological demands of enduro mountain bike racing or the physiological characteristics of elite enduro athletes is available. Only two studies have previously detailed the physiological requirements of enduro racing, both of which concentrated only on the demands of the race stages and omitted the transition stages, the findings of which will be discussed fully in chapter 2 (Hassenfratz, Ravier and Grappe, 2012; Kirkwood *et al.*, 2017).

At present, no information is available regarding the training characteristics completed by elite enduro athletes to meet the physiological requirements of competition. Conversely, a plethora of research concerning the training characteristics of elite athletes in other endurance sports is available (for example: Mujika, 2014; Tnønessen *et al.*, 2014; Solli, Tønnessen and Sandbakk, 2017) to guide the training programming of aspiring coaches and athletes. Quantifying training load allows athletes and coaches to prescribe training stimulus sufficient to promote adaptation while allowing adequate recovery to facilitate positive physiological adaptation. Excessive training load

coupled with insufficient recovery time can lead to negative physiological adaptation and reduction in performance, hence accurate measures of training load and recovery are essential to successful training programming (Meeusen *et al.*, 2013). Quantification of training load in road cycling is well established (Lucía *et al.*, 2003; Sanders *et al.*, 2017). However, the demands of road cycling and mountain biking are not the same (Novak and Dascombe, 2014; Macdermid, Fink and Stannard, 2015), hence further research is required in off-road cycling populations such as enduro.

In addition to accurately monitoring training load it is of benefit to assess the physiological response to training in order to maximise positive adaptation and minimise the risk of prolonged negative adaptation. Several studies have suggested a link between changes in cardiac autonomic nervous system (ANS) activity assessed by heart rate parameters and adaptation to changes in training load (Plews, Laursen and Buchheit, 2016; Bellenger *et al.*, 2017; Nakamura *et al.*, 2018). In light of these findings, several elite athletes and their respective coaches use phone applications to monitor resting heart rate parameters as an estimate of ANS response to training. However, aside from laboratory based studies of reliability and validity (Esco, Flatt and Nakamura, 2016; Giles, Draper and Neil, 2016) the methods widely employed in the field have not been assessed in a field setting. Therefore, more research is required to examine the efficacy of these methods to detect changes in cardiac ANS in response to training stimulus over longer durations.

Alongside alterations in ANS activity, the immune system is also highly responsive to acute exercise, the magnitude of this response dependant on a variety of factors including exercise duration, intensity and frequency (Nieman *et al.*, 1993), training status (Kendall *et al.*, 1990; Schaller *et al.*, 1999), psychological stress (Ingram *et al.*, 2015; Edwards *et al.*, 2018) and environmental factors (Walsh and Oliver, 2016). The immune response to acute exercise was initially thought to increase the risk of infection to the host (Pedersen and Ullum, 1994), however this theory has been refuted recently (Campbell and Turner, 2018); a debate which will be detailed fully in chapter 2. Ultimately, maintaining immunity in elite athletes reduces illness and thus

reduces disruption to training schedules, in turn leading to improved performance in competition (Mårtensson, Nordebo and Malm, 2014; Hellard *et al.*, 2015). Elite athletes appear to be at reduced risk of illness when compared to non-elite but highly active counterparts (Malm, 2006; Hellard *et al.*, 2015), hence investigation of the immune response to acute exercise throughout training and competition in elite athletes is warranted.

The immune system and cardiac ANS have also both been shown to respond to acute vibration exposure (Noguchi and Ando, 2002; Jiao *et al.*, 2004). Interestingly, excessive vibration exposure has also been linked to the development of pathologies such as osteoarthritis (OA) in working populations (Bovenzi, 1998) and further, cycling performance is reduced upon the addition of vibration (Sperlich *et al.*, 2009). Excessive exposure to vibration has been reported during road cycling but no comparable studies are available in mountain biking (Duc, Puel and Bertucci, 2016; Taylor, Edgar and Raine, 2018). Accelerations transferred to the athlete are larger during mountain biking than road cycling (Hurst *et al.*, 2013; Macdermid, Fink and Stannard, 2015), suggesting vibration exposure may be even greater in mountain biking populations. Current training load measures do not account for the influence of vibration, however evidence suggests athletes may be exposed to excessive vibration and further that vibration may contribute to training load and perturbations in ANS activity and immune response. Together, these findings suggest further investigation of vibration exposure in mountain biking is warranted.

The studies comprising this thesis therefore aimed to investigate the physiological requirements of elite enduro mountain bike racing with a particular focus on parameters of the immune response to exercise over the course of a season. The studies were completed in order to gain a greater understanding of the demands of training and racing which then informed the novel investigation of the vibration exposure associated with elite enduro mountain bike racing. Eight chapters are included within this thesis, as detailed below:

- Chapter 2: This chapter provides a review of the current literature for the studies described in this thesis
- Chapter 3: This chapter described the general material and methods used for data collection in this thesis
- Chapter 4: This chapter reports data from the first study focussing on detailing the physiological demands of elite enduro racing and the physiological characteristics of elite enduro athletes.
- Chapter 5: This chapter describes data from the first study concerning the training characteristics of elite enduro mountain bike athletes and further investigates the ability of resting heart rate measures to reflect changes in training load
- Chapter 6: This chapter presents data from the first study focusing on parameters of the immune response to exercise in the laboratory during training and competition. Parameters of the immune response to international competition and the influence of vibration are also investigated.
- Chapter 7: This chapter presents data from the second study investigating the hand-arm vibration exposure associated with elite enduro mountain bike racing.
- Chapter 8: This chapter presents the main findings from this thesis, the limitations of this thesis and potential future directions.

Chapter 2: Literature review

This review will initially focus on the physiological characteristics of mountain biking athletes and the demands of competitive mountain biking events, including the influence of vibration and the assessment of training load in endurance sport. The effect of stress on the autonomic nervous system (ANS) and immune system will then be considered with a particular focus on the influence of endurance training, vibration, and life stress. Road cycling is only discussed in context of vibration exposure due to lack of comparable research in mountain biking. Though a comprehensive overview of the immune system is beyond the scope of this review, the reader is directed to relevant sources in the text where appropriate.

2.1. Physiological requirements of mountain biking

Previous mountain biking research has focussed on Olympic format cross-country (XCO), downhill (DH), and to a lesser extent cross-country marathon (XCM) disciplines. XCO racing is a mass start event requiring competitors to complete multiple consecutive laps of a 4-6km circuit with a total duration between 1h20min and 1h40min for elite competitors, as detailed in table 2.1. In DH racing, individual riders compete against the clock on a course no longer than 3.5km designed primarily to test the technical ability of the rider with a duration of 2-5min. XCM events feature a mass start and must cover 60-160km in distance over 1-3 laps of a course (Union Cycliste Internationale, 2017). Enduro mountain bike racing (enduro) racing compromises of timed downhill sections of trail and non-competitive transition sections that must be completed within the provided time limit but do not contribute to overall race result. The current international governing body of enduro is the Enduro World Series (EWS), which organise races at an international level, and feature a minimum of four stages per event, a maximum elevation gain of 2000m for a single day event and 3200m for a two-day event (Enduro World Series, 2018). Athletes ascend by pedalling or use of mechanical uplift to meet their time checks at the start of each race stage and overall time spent riding is between 3 and 9 hours per day. According to EWS guidelines race stages should comprise of a maximum of 10% ascending terrain. The remaining 90% will feature terrain similar to that of downhill (DH) racing designed to test the riders'

technical ability. The winner of the general classification (GC) is the rider with the lowest combined time to complete all race stages. GC time must exceed 20 minutes per day of competition for the fastest rider overall, averaging 41min 39s per event for the winner in 2018 with a mean total ride time of approximately 5h 30min per day of competition (Enduro World Series, 2018).

Table 2.1: Duration and distance for cycling disciplines specified for enduro and XCO, DH and XCM (Enduro World Series, 2018; Union Cycliste Internationale, 2018).

Discipline	Duration per day (h:min)	Distance (km)
Enduro	3:00-9:00 overall time >0:20 general classification	Not specified
XCO	1:20-1:40	4-6 per lap
DH	0:02 – 0:05	<3.5
XCM	Not specified	60-160km

Previous research has demonstrated that elite cyclists have specific characteristics suited to the demands of their chosen discipline. For example, XCO athletes have a larger aerobic capacity and produce greater power across bouts ≥15s when compared to DH athletes who produce greater power over durations <15s reflecting the longer duration of XCO (~1.5h) compared to that of DH (~4mins) (Baron, 2001; Stapelfeldt *et al.*, 2004; Novak and Dascombe, 2014). Despite a plethora of research focused on other off road cycling disciplines there is very little research available on enduro thus leaving a gap in the literature. Accordingly, investigating the physiological requirements of will be a key focus of this thesis.

2.1.1. Workload in enduro mountain biking

The demands of enduro mountain biking include prolonged durations (3-9h), time constrained uphill transitions (≥2.5h) and average heart rate (HR) of >90% maximum during race stages (Hassenfratz, Ravier and Grappe, 2012; Kirkwood *et al.*, 2016) suggesting a large contribution of aerobic energy systems. One elite rider produced <50W for 37-56% of the duration of timed

stages though average HR was maintained at around 90% of maximum throughout thus suggesting considerable demand placed on the anaerobic energy system (Hassenfratz, Ravier and Grappe, 2012). Similar distributions of workload intensity are found in XCO racing (Stapelfeldt *et al.*, 2004) whereby shorter bouts of power output which surpass the aerobic capacity are interspersed by periods of reduced power output within the capacity of the aerobic system. However, though power output is reduced during some periods of XCO racing, elevated HR is maintained at 90% maximum – suggesting that the energy demands of piloting the bicycle are considerable. Hurst *et al.* (2012) showed considerable physiological work associated with controlling the bicycle in terrain associated with both DH and XCO racing, providing reasoning for the maintenance of elevated HR in XCO racing during descending sections (Miller *et al.*, 2016). Accordingly. physiological workload intensity is often beyond the capacity of the aerobic system during the predominantly downhill enduro race stages (Hassenfratz, Ravier and Grappe, 2012). This finding, coupled with the prolonged overall duration of the races, dictates that the aerobic system is still an important factor in energy production and this was supported by findings of increased $VO2_{peak}$ in elite vs non-elite enduro riders (Kirkwood *et al.*, 2017). GPS accelerometer units placed on the bicycle and the rider's torso also showed increased accelerations measured at the bicycle while accelerations at the torso remained relatively constant during technical terrain vs non-technical terrain. This is proposed to increase the energy demands placed on the rider during technical vs non-technical terrain as a result of increased dynamic muscular contractions, concurring with previous findings in downhill (Hurst *et al.*, 2013; Kirkwood *et al.*, 2017).

Intensity beyond the capabilities of the aerobic system results in an increase of energy production via the anaerobic glycolytic pathway (Powers and Howley, 2012). Lactate acts as a mediator between the aerobic (oxidative phosphorylation) and anaerobic (glycolytic) pathways. At rest lactate is utilised from the blood at the rate it is produced, resulting in a constant resting level of around 1.0 $mmol.L^{-1}$ (Brooks, 2018). As exercise intensity increases the concentration of lactate in the blood exceeds the utilisation and blood lactate concentration will rise. It is generally accepted that maximal prolonged

exercise intensity capacity occurs at the point at which blood lactate begins to accumulate (either break point (lactate threshold) or when it exceeds 2.0 or 4.0 mmol.L^{-1} (fixed blood lactate concentration; FBLC2 or FBLC4 respectively; Hall *et al.*, 2016). Identification of lactate threshold can or power output at FBLC can therefore provide understanding of an athlete's 'all day' capacity, particularly informative in enduro where the overall duration of events is typically between 3 and 9 hours. Performance in XCO racing has been linked to power output at FBLC4 in a group of elite XCO athletes of heterogeneous ability (Impellizzeri, Rampinini, *et al.*, 2005), but not in a group of athletes of homogeneous ability (Impellizzeri, Marcora, *et al.*, 2005). Similar findings have been shown in enduro, whereby elite riders exhibit greater relative and absolute power output at FBLC2 and FBLC4 than non-elite counterparts (Kirkwood *et al.*, 2017). This is supported by data of Hassenfratz, Ravier and Grappe (2012) showing that blood lactate concentration in a case study of an Enduro World Series rider rose from a mean of 3.0 mmol.L^{-1} at the start of a race stage to 15.2 mmol.L^{-1} immediately post stage. Together, these findings suggest that the blood lactate response to workload is a key factor in enduro performance.

2.1.2. Contribution of terrain to workload in mountain biking

Enduro race stages are designed primarily to test the riders' technical skills and are thus predominantly comprised of technical terrain occasionally interspersed with non-technical sections (Enduro World Series, 2018). Technical terrain is often based on a narrow track and includes challenging obstacles such as rocks, tree roots, holes, gap jumps, drop-offs, step-ups, banked corners, loose surfaces and adverse cambers (Hurst *et al.*, 2013). Non-technical terrain includes gravel road or smooth sections of trail lacking any challenging obstacles and is often used in enduro as practical means to link technical sections rather than the main focus of the race stage (Enduro World Series, 2018). Technical terrain induces rapid accelerations of the bicycle which are dampened first by the components of the bicycle (Levy and Smith, 2005) and then the limbs of the rider (Macdermid, Fink and Stannard, 2014). Damping accelerations induced by terrain allows the rider to navigate

technical terrain more quickly (Rylands *et al.*, 2016; Lopes and McCormack, 2017) and also serves to protect the central nervous system (Samuelson, Jorfeldt and Ahlborg, 1989a).

Previously reported discrepancy between power and HR may be due to the workload associated with damping accelerations transferred to the rider from the bicycle as a result of rough terrain (Macdermid, Fink and Stannard, 2014). Upper body muscular contractions are shown to contribute to this damping effect with greater magnitude of activity on technical terrain when compared with non-technical terrain (Hurst *et al.*, 2012). Magnitude and frequency of accelerations experienced by the rider are also influenced by suspension (Levy and Smith, 2005; Macdermid, Miller, *et al.*, 2017) wheel size (Hurst *et al.*, 2016), tire size and pressure (Macdermid *et al.*, 2015) thus it is feasible these factors also influence physiological workload. Macdermid, Fink, *et al.* (2017) reported that a full suspension (front and rear suspension) bike reduced vibrations on the downhill section of a cross country course compared to a hardtail bike (front suspension only) but did not report any differences in performance or reductions in physiological workload. These findings are compromised by the use of a metal bar in place of the rear suspension to create a hardtail bike rather than use a purpose built hardtail bike. The resulting detrimental effect on the handling and vibration attenuation properties of the bicycle compared to a purpose built hardtail frame suggest a comparison between full suspension and hardtail bicycles cannot be made with this data. Further research is required to include the specific, likely more technical terrain and specialist equipment used by professionals (e.g. 160-200mm travel bikes) associated with other off road cycling disciplines such as downhill and enduro.

2.1.3. GPS activity profile

GPS devices incorporating accelerometers have been used to create a GPS activity profile in DH mountain biking, and more recently used to describe successful performance in enduro mountain bike racing (Florida-James, Ball and Westbury, 2010; Hurst *et al.*, 2013; Kirkwood *et al.*, 2017). The validity

and reliability of GPS systems to assess activity profile of outdoor sports has been reported extensively (Aughey, 2011). Further, the validity of GPS units incorporating triaxial accelerometers for measuring physical activity has also been reported (Boyd, Ball and Aughey, 2011a) and deemed suitable for use in sports similar to enduro such as DH (Hurst *et al.*, 2013). Rider load was introduced as a term to describe accelerations and accumulated accelerations experienced by the bike rider, detailed previously (Hurst *et al.*, 2013). Rider load refers to magnitude of accelerations and not vibration exposure (Hurst *et al.*, 2013). GPS accelerometer units also allow retrospective timing of split sections, offering a more detailed analysis of performance based on terrain. In enduro racing it was shown that the key section of the race stage could be of varying nature depending on the track, thus requiring the athlete to be as rounded as possible both physically and technically (Kirkwood *et al.*, 2017; Lopes and McCormack, 2017).

Previous work showed that magnitude of rider load was dependant on terrain type, where mean values typically ranged between 3*g* and 5.9*g* when assessed at the torso in elite DH riders (Hurst *et al.*, 2013). However, the body will always try to protect the central nervous system and brain in order to maintain cognitive function (and thus control of the bicycle) by attenuating accelerations from the contact points to the torso/head (Macdermid, Fink and Stannard, 2015; Kirkwood *et al.*, 2017). Reducing the accelerations transferred to the head/torso will also increase control by maintaining contact with the ground, facilitating effective braking and change of direction associated with navigating technical terrain therefore indicating rider skill may influence workload associated with terrain damping (Rylands *et al.*, 2016; Lopes and McCormack, 2017). This issue is compounded by faster riders showing larger mean bicycle load values in enduro racing, suggesting increased workload for faster riders in order to protect the torso/head from excessive accelerations (Macdermid, Fink and Stannard, 2014; Kirkwood *et al.*, 2017).

2.1.4. Physiological correlates of off road-cycling performance

For several years, researchers have attempted to predict endurance performance using laboratory based physiological tests. This would theoretically allow athletes and coaches to monitor training and performance or identify talent (e.g. team selection criteria) in a controlled environment. Determining the laboratory based physiological characteristics of successful athletes of respective disciplines will also help understand the mechanisms of performance in such events. For example, in a population of mixed ability XCO athletes Impellizzeri, Rampinini, *et al.* (2005) found significant correlations between national level XCO race performance and FBLC4, VO_{2peak} and peak power output (r range from -0.68 to -0.94, $p<0.05$). Correlations were stronger when physiological test values were expressed relative to body mass further highlighting the importance of power-to-mass in off road cycling disciplines. In a homogeneous population of elite XCO athletes, only power at the second ventilatory threshold (VT2) and VO2 at VT2 were moderately correlated with international race time (r range from -0.61 to -0.66; Impellizzeri, Marcora, *et al.*, 2005). These findings indicate aerobic capacity explaining around 80% of variation in race time in the mixed ability group but only 40% in in the elite group. This suggests that whilst large values of VO_{2peak}, FBLC4, and peak power are a prerequisite for performance at elite level, other factors must explain the remaining 60% variance in elite performance times estimated by Impellizzeri *et al.* (2005). The original authors also stated that a proportion of the remaining variance may be attributed to the athlete's technical competence to pilot the bike efficiently over technical terrain, a factor also shown to be important in enduro and DH racing (Hadden and Florida-James, 2011; Chidley *et al.*, 2015; Kirkwood *et al.*, 2017).

It is perhaps not surprising that most laboratory-based tests are not able to entirely predict performance in a homogeneous elite population when the duration of the laboratory test (10-30minutes) is much shorter than typical competition duration (e.g. XCO racing > 1.25 hours). As exercise intensity and duration increases, the inherent challenge to homeostasis is also increased via factors such as accumulation of heat (hyperthermia), depletion of energy stores and changes in cytokine, catecholamine and glucocorticoid production

(Coyle, 1999; Suzuki *et al.*, 2003; Hill *et al.*, 2008; Nieman *et al.*, 2016; Suzuki, 2018). Such physiological responses observed in the field are unlikely to be routinely replicated in a laboratory setting due to the extensive duration of exercise required to induce such responses and the additional confounding stressors, for example competitive anxiety, posed by the field setting (Hanton, Thomas and Maynard, 2004). As such, researchers have moved to physiological assessment of competitive events in the field to investigate the performance requirements of endurance exercise (Hays *et al.*, 2018). Provided the physiological requirements of performance are accurately identified, laboratory testing can offer an insight to relative improvement of the individual athlete in desired physiological parameters (Powers and Howley, 2012). Taken together, laboratory testing offers a useful marker of physiological characteristics and training adaptation in elite athletes throughout a training programme or race season.

2.2. Basic principles of training

The three traditional pillars of exercise training are specificity, overload and reversibility (Mujika and Padilla, 2000; Reilly, Morris and Whyte, 2009; Aubry *et al.*, 2014). Overload refers to stressing the appropriate system or tissues beyond the workload they are accustomed to, both in an acute sense (per training session) and chronic sense (e.g. numerous weeks of intensified training; Powers and Howley, 2012). Specificity dictates that the systems (e.g. muscle fibre type, energy system, and muscle group) to be overloaded are relevant to the desired outcome activity (Pierce *et al.*, 1990; Goutianos, 2016). Reversibility is the principle that the cessation of exercise training will result in physiological changes reverting towards initial baseline values over a period of time, also referred to as detraining (Mujika and Padilla, 2000, 2001).

In the recovery period following exercise, performance is temporarily reduced below baseline and the athlete experiences acute feelings of fatigue due to factors such as depletion of glycogen stores, muscular fatigue, and reduced neuromuscular function (Meeusen *et al.*, 2013). The beneficial adaptation to training stimulus occurs during the recovery from exercise, and thus sufficient rest must also be prescribed in the training regime to ensure that adequate

time is allowed for tasks such as protein synthesis, replenishment of glycogen stores, and mitochondrial biogenesis (Goutianos, 2016). Following sufficient recovery, exercise performance can be improved thus forming the basis of effective training programmes (Hellard *et al.*, 2013; Meeusen *et al.*, 2013). It is considered normal practice to prescribe periods (typically 2-4 weeks) of intensified training load resulting in temporarily reduced performance (e.g. Aubry *et al.*, 2015; Le Meur *et al.*, 2016). This is typically followed by a relative reduction in training load for 1-3 weeks; theoretically inducing improvements in performance (e.g. Mujika *et al.*, 1995, 2004).

2.2.1. Negative adaptation to training stimulus

In the event of excessive training load and/or insufficient periods of recovery, a prolonged detrimental effect on exercise performance may be elicited (Halson and Jeukendrup, 2004; Meeusen *et al.*, 2013). Different terms are given to this situation depending on the severity of maladaptation to training stimulus which were outlined in a position statement recently and are shown in table 2.2 (Meeusen *et al.*, 2013).

Table 2.2: Proposed presentation of stages of positive and negative adaptation to training stimulus (adapted from Meeusen *et al.*, 2013)

Process	**Training**		**Intensified training** →	
Outcome	**Acute fatigue**	**Functional overreaching**	**Non-functional overreaching**	**Overtraining syndrome**
Recovery time	Day(s)	Days-weeks	Weeks-months	Months - years
Performance	Increase	Temporary reduction	Stagnation/ decrease	Decrease

Therefore, a balance must be found between overloading the athlete with the largest possible training stimulus to elicit the largest possible positive physiological adaptations whilst ensuring the athlete also has sufficient recovery to reduce the chances of non-functional overreaching (NfOR; Meeusen *et al.*, 2013). One of the key parts of this balance is therefore to

utilise an accurate means of assessing the training load and the individual's response to changes in training load (Bellenger *et al.*, 2017; Sanders *et al.*, 2017). Factors other than exercise can also influence the susceptibility to negative adaptation to training, such as inadequate nutrition, disruption to sleep, psychological and emotional stress (e.g. relationships, work commitments) and illness (Achten *et al.*, 2004; Meeusen *et al.*, 2013; Lastella *et al.*, 2018). Overtraining is considered to be a prevalent issue in elite endurance sports where self-reported data suggests ~60% of elite runners and ~30% of non-elite runners have experienced overtraining syndrome on at least one occasion (Morgan *et al.*, 1987, 1988). More recent data suggests 29% of young English athletes experience symptoms of overtraining further showing those competing at a national or international level at greater risk than club or regional level competitors (Matos, Winsley and Williams, 2011). Despite the clear prevalence of overtraining particularly in elite athletes, evidence has demonstrated that high training volumes can only occur in conjunction with a low number of sick days (Mårtensson, Nordebo and Malm, 2014). Given that every 10% increase in training volume increased the risk of URTI by ~10% in elite swimmers, particular care should be given to ensure a gradual (<10% per week) increase in training volume to reduce the chances of missed training days due to sickness (Hellard *et al.*, 2015). A recent review by Walsh (2018) highlights these findings and further signifies the importance of reducing training volume during periods of increased life stress to reduce the susceptibility to illness in elite athletes. Therefore, while training load is a large contributing factor to development of NfOR or overtraining syndrome, other factors must be taken in to consideration when monitoring overall load placed on athletes (Meeusen *et al.*, 2013). Together, these findings show the importance of accurate training load measures to monitor subsequent physiological and immunological responses in athletic populations.

2.2.2. Models to measure training load

Duration of a session alone is unlikely to offer a sufficiently detailed training load estimate as it does not account for exercise intensity (Banister *et al.*, 1975). Several wearable heart rate monitors, GPS devices and training

analysis software (e.g. Training Peaks) have become an affordable and readily available means of recording training data, little analysis is often carried out. There is a distinct lack of literature surrounding the validity of training load models in mountain bike racing. The initial concept of training impulse (TRIMP) was conceived by Banister in 1975 though it was 1991 before a more succinct equation to estimate training load from a single session was published (Banister, 1991). The formula to calculate Banisters TRIMP (bTRIMP) is as follows:

$$\text{bTRIMP} = \text{Training duration (minutes)} \times \Delta HR \times y$$

Where:

$\Delta HR = (HR_{exercise} - RHR) / (HR_{max} - RHR)$

y = weighting factor (male = $0.64^{1.92}$, female = $0.86^{1.67}$).

The initial TRIMP model proposed (Banister, 1991) provides a TRIMP value for the training period or sub period based on mean heart rate for each period. There is therefore limited application in intermittent activities where periods of very high intensity are interspersed by periods of low intensity, thus not offering a representative TRIMP value. With previous research in enduro showing a mean HR around 75%HR_{max} but peak values of up to 100%HR_{max}, bTRIMP may not suitably reflect fluctuations in HR associated with enduro. The source of the gender specific weighting factors is not provided in the initial model and further does not account for individual differences in the athlete beyond gender. One study has used the more complex model proposed previously by Banister *et al* (1975) to successfully predict performance in two recreational runners (Morton, Fitz-Clarke and Banister, 1990) but little other evidence is shown of a dose-response relationship. As a result, the bTRIMP model has been modified to create summative models by Edwards and Lucia (Banister *et al.*, 1975; Edwards, 1993; Lucía *et al.*, 2000). Edwards (1993) rated exercise intensity by time spent in heart rate zone (zone 1: 50-60%HR_{max}, zone 2: 60-70%HR_{max}, zone 3: 70-80% HR_{max}, zone 4: 80-90% HR_{max}, zone 5: 90-100% HR_{max}). Edwards TRIMP (EdTRIMP) is then calculated by multiplying time (minutes) spent in each heart rate zone multiplied by weighting factor (1-5, i.e. (time in zone 1 x 1) + (time in zone 2 x 2)). EdTRIMP is limited in a similar

fashion to bTRIMP whereby the mean HR of the session is used and will not accurately represent training sessions with fluctuations in HR such as interval training. Additionally, the coefficient system dictates that training in zone 5 (>90% HR_{max}) will induce 5 times greater training adaptation than training in zone 1 (50-60% HR_{max}). Again, this weighting system appears arbitrary and is supported by limited evidence as there has been no published evidence of a dose-response relationship with physiological or performance outcomes. Lucia, Hoyos, & Chicharro (2001) proposed a training load model using heart rate values corresponding to the first and second ventilatory thresholds (VT1 and VT2) determined by laboratory testing. Time spent at HR < VT1 was provided a weighting factor of 1; time at HR between VT1 and VT2 a weighting factor of 2 and time at HR > VT2 a weighting factor of 3. Time spent in each heart rate zone is multiplied by the relevant weighting factor and summated to form Lucia's TRIMP (LuTRIMP). LuTRIMP uses more individualised approach to calculating training load by creating zones based on the first and second ventilatory thresholds (or LT1, LT2; Sanders *et al.*, 2017) of the individual. However, similar to EdTRIMP the coefficient system dictates that time spent above VT2/LT2 will induce 3 times the physiological adaptation compared to that below LT1; an assumption that is not based on any scientific evidence. One benefit of this model over bTRIMP and EdTRIMP is the summation of each time point rather than mean HR value, suggesting fluctuations in workload may be more accurately reflected in LuTRIMP value (Sanders *et al.*, 2017).

The latest TRIMP model was proposed by Manzi et al. (2009) and accounted for the individual blood lactate response in relation to heart rate reserve for the individual athlete based on laboratory testing. Rather than using time in heart rate zones, an iTRIMP value for every heart rate value is summated to generate an accumulated iTRIMP value for the training session (Manzi, Iellamo, *et al.*, 2009). iTRIMP is therefore calculated by the following formula:

$$iTRIMP = \sum \text{Time (mins)} \times \triangle HR \times y_i$$

Where:

$\triangle HR = (HR_{exercise} - RHR/HR_{max} - RHR)$

y_i = individual weighting factor

The iTRIMP model (Manzi, Iellamo, *et al.*, 2009) provided the first fully individualised calculation of TRIMP based on the blood lactate response and heart rate reserve in the individual athlete. As each HR value is assigned a TRIMP value, the model is particularly sensitive to fluctuations in workload intensity such as that found in enduro racing (Hassenfratz, Ravier and Grappe, 2012). Most importantly, a dose response relationship was observed between weekly iTRIMP and running velocity at FBLC2 and FBLC4 in recreational long distance runners (r = 0.87 [95%CI: 0.41 to 0.97], 0.74 [95%CI: 0.07 to 0.95], respectively). A potential issue with this model is the requirement of relatively regular (~4-6 times per year (Manzi, Iellamo, *et al.*, 2009)) physiological testing to ensure the correct exponential is used to calculate load depending on changes in training state.

Until recently, no research was available regarding the validity of these models to predict performance outcomes in an elite cycling population. An eloquent study by Sanders *et al.* (2017) assessed internal (HR, RPE) and external (power) based training load of 15 national-level male road cyclists over a period of 16 weeks. FBLC2 & 4, VO_{2peak} and 8km performance time were assessed pre and post the 16 week training period. Results showed a superior dose-response relationship between weekly average of LuTRIMP and iTRIMP and changes in VO_{2max}, power at FBLC2 and 8km TT performance when compared to EdTRIMP and bTRIMP. Unfortunately, no upper limit of training load was discovered whereby change in performance was reduced or blunted following the retrospective analysis of training period. Indeed, ethical considerations make it difficult to purposefully induce a state of NfOR or overtraining syndrome in participants. In addition, all of the training models presented thus far are designed for use in running or road cycling and have not been validated for use in mountain biking. When considering the application of these training models to mountain biking, it should be noted that all of the training models utilise heart rate to predict physiological workload. It appears that vibration may contribute a significant component of the overall physiological workload in enduro and that physiological workload may be

underestimated when using measurement of heart rate values alone in the presence of vibration exposure (Rønnestad *et al.*, 2018). Together, these findings suggest that further work is required to determine vibration exposure during mountain biking and its potential influence on overall workload (Hurst *et al.*, 2012; Macdermid, Fink and Stannard, 2015; Sanders *et al.*, 2017). Vibration exposure and the associated physiological and immunological responses are discussed in detail in section 2.5 within this review.

2.3. Monitoring autonomic nervous system activity response to training in athletes

As discussed in section 2.2, assessing the individual physiological response to a known training load is a key component of a successful training programme (Manzi, Castagna, *et al.*, 2009; Hellard *et al.*, 2013). However, this theory relies on the accuracy and reliability in measurement of both physiological response and training load input variables. A popular method of assessing physiological response is measurement of resting heart rate variables, which have been shown to relate to mean weekly training load (Manzi, Castagna, *et al.*, 2009). Modulation of the cardiac ANS can be assessed by recording the beat to beat interval of the heart under specific conditions of time, body position and subsequent analysis (Task Force, 1996). It has been proposed by some authors that monitoring ANS activity provides information on the training status of the athlete, specifically positive or negative adaptation to training stimulus with the ultimate aim to avoid a state of overtraining. However, the means of measurement, analysis and interpretation are crucial to correct use of heart rate based assessments of ANS activity. For all measures of cardiac ANS modulation a minimum of 3 daily measures per week must be collected in order to produce weekly average values for analysis as reported previously (Plews, Laursen and Buchheit, 2016).

2.3.1. Heart rate and heart rate variability

Resting heart rate (mean beat to beat interval, beats per minute; bpm) is increased by sympathetic nervous system (SNS) activity and reduced by parasympathetic nervous activity (PNS; Cardiology, 1996). Therefore, many

researchers and practitioners argue that significant increases or decreases in heart rate can be used to identify large changes in ANS activity and potentially provide an indication of negative adaptation or impending illness when interpreted correctly (Buchheit, 2014). Monitoring of resting heart rate is therefore often employed by athletes and coaches due to its low cost, reliability, and ease of measurement and interpretation to aid assessment of training responses (Achten and Jeukendrup, 2003). Heart rate variability (HRV) is the variation in beat to beat interval and can be analysed in a plethora of statistical methods including time and frequency domain methods (Task Force, 1996).

As HRV has shown to be capable of indicating positive or negative adaptation to periods of increased training load, interest in the reliability and accurate analysis of measures in athletic populations has grown (Plews, Laursen, Stanley, *et al.*, 2013). The most commonly used method in longitudinal studies of elite athletic populations is time domain based analysis, in particular the natural logarithm of the root mean square of successive differences (LnRMSSD; Plews *et al.*, 2013; Buchheit, 2014). LnRMSSD is proposed to represent parasympathetic activity, though it should be considered that the sum of sympathetic and parasympathetic activity is dynamic and thus one cannot predict the other (Task Force, 1996). LnRMSSD can be assessed over ultra-short recording periods of as little as 55 seconds using a smart phone application and heart rate strap validated against gold standard 5-lead electrocardiogram (ECG) devices (Flatt and Esco, 2013; Esco, Flatt and Nakamura, 2016). This reduces the chances of participants forgetting equipment, is simple to use, data can be uploaded from any location to a secure cloud based service for viewing by coach or researcher, and the short duration is likely to increase adherence in longitudinal studies (Plews *et al.*, 2014). The ratio between RR interval and LnRMSSD (LnRMSSD:RR) is shown to offer indication of parasympathetic saturation in elite athletes with extensive training history (Plews, Laursen and Buchheit, 2016). Parasympathetic saturation is a reduction in HRV despite maintenance of low resting HR which occurs as a result of sustained parasympathetic control of the sinus node thus reducing respiratory cardiac modulation and in turn

reducing LnRMSSD (Malik and Camm, 1993; Plews, Laursen, Stanley, *et al.*, 2013). An alternative solution is taking the heart rate recording standing up as this may negate the influence of parasympathetic saturation on heart rate variability measures (LnRMSSD) though issues of diminished athlete adherence have been encountered when requesting standing measures (Bellenger *et al.*, 2016).

2.3.4. Effects of acute exercise and HRV

The cardiovascular system facilitates several physiological response during recovery from a single bout of exercise, primarily via thermoregulation and delivery of nutrients and removal of waste products. To meet the increased demand for nutrients/removal of waste cardiac output is increased via reduced cardiac parasympathetic modulation lasting >48h following an acute high intensity exercise bout and <24h following acute low intensity exercise bout (Stanley, Peake and Buchheit, 2013). A return to normal cardiac parasympathetic modulation values as assessed by HRV may therefore offer indications of restoration of homeostasis and possibly coincides with perceived wellness, though further research is required. These observations led to the investigation of HRV guided training whereby high intensity training sessions were only completed on days where HRV values taken in the morning before training were either above normal or within one smallest worthwhile change of normal values (Kiviniemi *et al.*, 2007; Vesterinen *et al.*, 2016; Torres *et al.*, 2018). The HRV-guided method generally presented a trend for greater enhancement in endurance performance than traditional programming across 4-8 week training periods. However, the effect size presented in these studies is small and thus further work is required to investigate to provide conclusive evidence to support HRV guided training practises (Kiviniemi *et al.*, 2007; Vesterinen *et al.*, 2016; Torres *et al.*, 2018). A single reading of HRV can be also influenced by several factors such as heat, plasma volume, altitude and/or psychological stress (Plews, Laursen, Stanley, *et al.*, 2013). As such, any decision about the recovery state or daily training manipulation of an athlete based on one HRV reading must be interpreted alongside information of environmental and psychological factors.

2.3.5. HRV and endurance performance

A trend for significant reduction of mean cardiac parasympathetic modulation is observed throughout weeks of intensified training with values returning equal to or above baseline when sufficient rest is prescribed (Pichot *et al.*, 2000). It was initially proposed that prolonged reduction in parasympathetic modulation in response to increased training load reflected negative training adaptation in cases of over training syndrome. However, recent findings showed that world championship winning rowers displayed reduced LnRMSSD throughout the 7-week training period prior to the championship winning performance (Plews, Laursen and Buchheit, 2016). The authors suggested this was due to parasympathetic saturation indicated by low correlation between LnRMSSD and RR interval plots compared to two other championship winning rowers who did not display reduced LnRMSSD prior to winning performances (Plews, Laursen and Buchheit, 2016). The same group investigated LnRMSSD and LnRMSSD:RR of two athletes 10 weeks prior to a rowing world championships. One athlete performed optimally (second position, 0.12% behind the leader) whilst one performed poorly (5th place, 1.92% behind the leader despite finishing in the top 3 positions the year prior). The authors argue that reductions in LnRMSSD alongside increases in LnRMSSD:RR were indicative of fatigue or underperformance syndrome in the poorly performing athlete. Alternatively, a pre competition reduction in both LnRMSSD and LnRMSSD:RR was observed in the second place rower in the same event, proposed by the authors to indicate readiness to perform (Plews, Laursen, Stanley, *et al.*, 2013). The physiological rationale for reduced HRV during taper periods preceding world championship winning performances is not fully understood at present. Potential physiological mechanisms influencing the observed reduction in HRV may include psychological stress (discussed later within this section) and reduced training volume during taper periods causing a reduction in plasma volume, though more work is required in this area (Convertino, 1991; Buchheit *et al.*, 2009). While it is beneficial to see data from an elite population, the small differences in performance used to define ‘optimal’ and ‘poor’ performance employed by Plews *et al* (2013) in addition to very small sample size (n=2) mean that more work is required to investigate the use of HRV in determining readiness to perform.

Greater resting parasympathetic activity has been linked to increased VO_{2peak}, maximal aerobic running speed, faster 10km and marathon time in moderately trained runners in laboratory and field settings (Buchheit and Gindre, 2006; Manzi, Castagna, *et al.*, 2009; Buchheit *et al.*, 2010). These findings suggest that higher parasympathetic activity accompanies improved performance and a larger capacity for exercise training (Vesterinen *et al.*, 2013). However, parasympathetic withdrawal signified by a reduction in LnRMSSD during two weeks of tapering approaching a major championship has been viewed as a potentially positive response indicative of 'readiness to perform' in world champion rowers (Plews, Laursen, Stanley, *et al.*, 2013). Such significant alterations in autonomic nervous system activity need to be monitored closely to avoid repeated stressors and insufficient recovery time leading to a state of prolonged sympathetic dominance and potentially over reaching and overtraining if not addressed appropriately (Aubert, Seps and Beckers, 2003; Plews, Laursen and Buchheit, 2016). An additional confounding factor which must be considered when assessing and interpreting daily heart rate variability data is the plethora of psychological factors which can influence day to day readings. This is particularly important when considering the popularity of using heart rate variability smart phone applications to guide day-to-day training decisions. For example, increased pre competition anxiety can reduce heart rate variability independent of subsequent competitive performance (Fortes *et al.*, 2017). Sleep, cognitive tasks (e.g. Stroop test), personal relationships, life stress and trans meridian travel can all alter autonomic nervous system activity and are extremely difficult to account for in a field setting (Delaney and Brodie, 2000; Tateishi and Fujishiro, 2002; Werner *et al.*, 2015). Thus, whilst heart rate variability has been shown to correlate with several aspects of sporting performance, it must be considered in the wider context when utilised to make day to day decisions about training programmes.

2.3.6. HRV and response to training stimulus

Several studies have investigated the ability of baseline heart rate variability to predict responses to exercise training interventions. Initially, it was found

that baseline nocturnal recordings of cardiac parasympathetic modulation accounted for 27% variation in response to a training intervention in a population of healthy but sedentary males (Hautala *et al.*, 2003). Although there is a lack of data in elite populations, pre training resting LnRMSSD was positively correlated with maximal aerobic velocity following a 14 week intense training period in recreational endurance runners (R^2 = 0.32; Vesterinen et al., 2013). Conflicting findings were shown in moderately trained endurance runners where reduced baseline resting LnRMSSD was associated with larger improvements in 10km run time (R^2=0.57; Martin Buchheit et al., 2010). In the same study, Buchheit et al. (2010) retrospectively divided participants in to responder (>0.5% improvement in 10km run time) and non-responder groups (<0.5% reduction in 10km run time). Results showed that non-responders had higher mean baseline values of parasympathetic activity and endurance performance assessed by maximal aerobic speed and 10km run time (Buchheit *et al.*, 2010). Therefore, these findings may be compounded by initial differences in physiological characteristics (non-responders were better trained at baseline) and the utilisation of bTRIMP rather than the more accurate iTRIMP model (Sanders *et al.*, 2017). This could disguise differences in individualised training load between groups, potentially suggesting that the non-responder group did not receive sufficient training stimulus to induce improvements in running performance (Manzi, Castagna, *et al.*, 2009; Sanders *et al.*, 2017). Therefore, although it initially appears that increased cardiac parasympathetic modulation at baseline suggests increased potential for positive endurance training adaptation, evidence is not conclusive and further work is required in elite populations.

Alterations in resting heart rate measures (HR, HRV) in over trained athletes are equivocal, with increases (Hedelin *et al.*, 2000), decreases (Hynynen *et al.*, 2006) and no changes (Bosquet *et al.*, 2003) reported. Comparison of these findings is difficult due to methodological differences in resting heart rate recordings (Bellenger *et al.*, 2016) and difficulty associated with diagnosing the exact status of overtraining (see section 2.2.1, table 2.2). Further, due to the obvious ethical complication of purposefully subjecting participants to a state of overtraining, data of over trained elite athletes is limited. The influence

of shorter periods (1 to 4 weeks) of overreaching on autonomic nervous system activity is unclear - parasympathetic activity has been shown to increase, decrease or remain unchanged (Buchheit, 2014). In a case study of an over trained junior cross-country skier (2 months total rest to recover), parasympathetic activity was increased and heart rate accordingly reduced during over training when compared to pre or recovered values suggesting parasympathetic dominance during prolonged overtraining (Hedelin *et al.*, 2000). In a more recent example, a world championship rower underperformed at the world championships following prolonged periods (~6-7 weeks) of significant reduction below baseline values of LnRMSSD (Plews, Laursen and Buchheit, 2016). Taken together, it is suggested that LnRMSSD will be reduced in the initial stages of negative adaption before rising significantly and taking much longer to reduce to baseline as overtraining becomes more established (Buchheit, 2014). In order to observe significant deviations from the baseline it is crucial to have a significant amount of concomitant baseline heart rate variability and accurate training load data (Plews, Laursen and Buchheit, 2016).

2.4. The immune system and its role in coping with training and competition load.

2.4.1. Overview of immune system

It is not within the scope of this review to detail the immune system structure and function in its entirety (see Peake *et al.* (2017), Simpson *et al.* (2015) for full review), rather the components which are most relevant to exercise, performance and recovery will be focused upon. The immune system is generally divided into two broad arms; innate (natural, nonspecific) and adaptive (specific, repetitive) immunity. This considered, both branches of the immune system often function synergistically to mount an overall immune response (Simpson *et al.*, 2015). Importantly, formation of the adaptive immune response takes in the order of 4-7 days and thus considerably longer than the innate immune response (order of hours; Murphy and Weaver, 2017). White blood cells (WBC) have varied functions in the immune system, consisting of granulocytes (60-70% circulating blood), monocytes (10-15%) and lymphocytes (20-25%). Neutrophils are the predominant immune cell in

peripheral human blood, composing ~60% of total WBC count with a primary function to destroy antigens and pathogens (Gleeson, Bishop and Walsh, 2013). Neutrophils also have the ability to function as antigen presenting cells to CD4 T-cells, thus contributing to adaptive immunity (Koup *et al.*, 2017; Lin and Loré, 2017). Constituting 20-25% of circulating WBC count lymphocytes possess highly variable antigen receptors on their surface which becomes a unique variant of a prototype antigen receptor upon maturation. This results in a large repertoire of highly diverse cells which are capable of recognising in excess of 10^8 different antigens meaning the body is capable of mounting a response to almost any pathogen (Nikolich-Žugich, Slifka and Messaoudi, 2004). Subsets of lymphocytes include B-cells, T-cells and natural killer (NK) cells. T-cells and NK cells will be the focus of this review as they are the most responsive to exercise (Gleeson, Bishop and Walsh, 2013). Derived from common lymphoid progenitor cells generated in the bone marrow, T-cells later differentiate in the thymus and are identified by presence of a membrane bound T-cell receptor (TCR) complex (Yanagi *et al.*, 1984). The TCR gene rearrangement on T-cells is highly diverse and thus allows recognition of vast numbers of specific antigens. The TCR is also used to bind to the major histocompatibility complex (MHC) class I and II molecules (Murphy and Weaver, 2017). The two main classes of T-cell are cytotoxic T-cells and helper T-cells, defined by the presence of cell surface proteins CD8 and CD4 retrospectively (Singer, Adoro and Park, 2008). CD8 T-cells have the capacity to directly kill infected cells, can only recognise antigen presented in MHC class I molecules and are particularly important in the defence against viral infection. CD4 T-cells can only recognise antigen presented in MHC class II molecules and can release chemical mediators which activate many immune functions which will target intracellular and extracellular bacteria, viruses, fungi, and parasites (Zhu and Paul, 2009).

Costimulatory receptors CD27 and CD28 are often used as markers of T-cell differentiation (Hamann *et al.*, 1997). CD28 binds with CD86 to promote T-cell proliferation, cytokine production (particularly IL-2) and cell survival. CD27 binds with CD70, loss of expression is irreversible and associated with terminal effector T-cell differentiation (Azuma, Phillips and Lanier, 1993; Hamann *et al.*,

1997). CD62L is an adhesion molecule that enables cells to extravagate from the peripheral blood compartment to secondary lymphoid tissues via endothelial venules (Kansas, 1996; Krüger and Mooren, 2007). Central memory T-cells also express CD62L and reside in the secondary lymphoid tissues waiting to re-encounter their specific antigen (Lipp *et al.*, 1999). Effector and senescent T-cells lack expression of CD62L, instead migrating to peripheral and non-lymphoid tissues where they utilise potent cytotoxic effector functions upon exposure to antigen (Sallusto, Geginat and Lanzavecchia, 2004). CD45RA augments cell signalling through the TCR and has widely been used to determine stage of differentiation whereby expression is observed only in naïve and senescent populations (Okumura *et al.*, 1996; Romero *et al.*, 2007; Ingram *et al.*, 2015). This combination of cell surface receptors allows the following identification of T-cell populations; naïve (N; $CD45RA^{+}/CD62L^{+}$), central memory (CM; $CD45RA^{-}/CD62L^{+}$), effector memory (EM; $CD45RA^{-}/CD62L^{-}$), senescent (S; $CD45RA^{+}/CD62L^{-}$), early ($CD27^{+}/CD28^{+}$), intermediate ($CD27^{-}/CD28^{-}$), late ($CD27^{+}/CD28^{-}$) as described in figure 2.1 below (Romero *et al.*, 2007; Simpson, 2011). Naïve T-cells have not been stimulated by antigen and have the ability to extravagate from the peripheral blood compartment. Central memory cells have been stimulated by their cognate antigen but still recirculate between the peripheral blood and secondary lymphoid tissues (Campbell *et al.*, 2009). Effector memory and senescent T-cells are highly cytotoxic and migrate between the circulating blood and peripheral tissues. Senescent cells have a greater history of antigen exposure but reduced proliferative capacity compared to effector memory T-cells (Mahnke *et al.*, 2013). Similarly, early T-cells are those which have not experienced their cognate antigen, intermediate T-cells have antigen experience but retain the ability to proliferate while late T-cells are

antigen experienced but lack the ability to proliferate (Azuma, Phillips and Lanier, 1993; Hamann *et al.*, 1997).

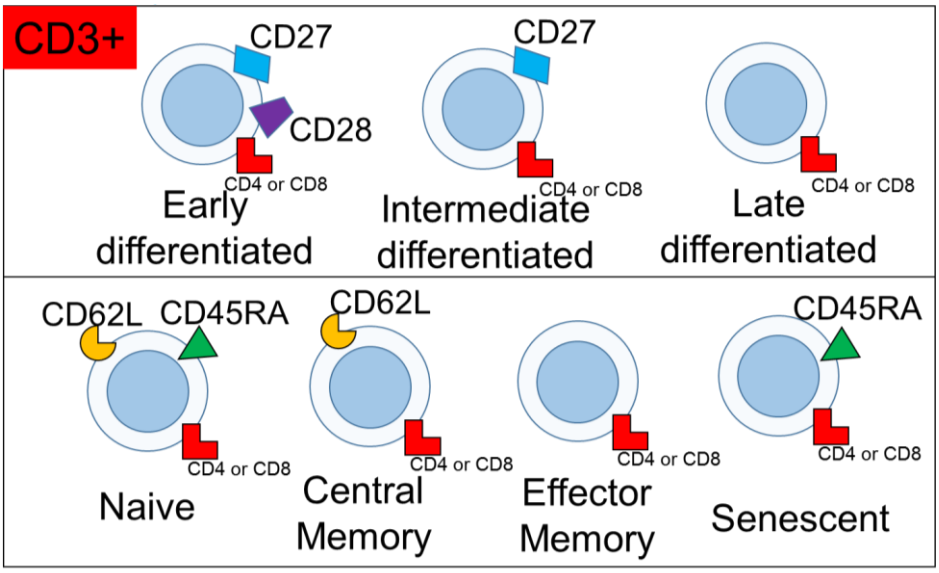

Figure 2.1: Cell surface markers used to define functionally distinct subsets of T-cells (adapted from Simpson, 2011)

Natural killer (NK) cells constitute 10-15% of lymphocytes at rest in circulating blood, are derived from the same common lymphocyte progenitor cells as T- and B-cells but do not feature a specific antigen receptor (Murphy and Weaver, 2017). NK cells are phenotypically characterised by a lack of CD3 associated with the TCR and the presence of CD56 on the cell surface ($CD3^-/CD56^+$; Cooper, Fehniger and Caligiuri, 2001). Further distinct populations of NK cells are characterised by cell surface density of CD56 expression. 90% of NK cells express low cell surface density of CD56 ($CD56^{dim}$) and are more cytotoxic against recognised targets at rest compared to the remaining 10% of NK cells with high surface density of CD56 ($CD56^{bright}$) cells (Cooper, Fehniger and Caligiuri, 2001; Cooper *et al.*, 2013). Cell surface expression of CD57 signifies the latter stages of maturation in peripheral blood NK cells (Nielsen *et al.*, 2013). NK cells expressing CD57 ($CD3^-/CD56^+/CD57^+$) increase in number in the presence of CMV infection, are highly cytotoxic, and may offer protective mechanisms against non-communicable diseases (Nielsen *et al.*, 2013).

2.4.2. Immune system and exercise

The human body is an incredibly complex system that has developed a multitude of innate survival mechanisms throughout its evolution. Such mechanisms are designed to offer protection during periods of increased stress by inducing movement to escape or face the challenge, dubbed the 'fight or flight' response (Cannon, 1922). Accordingly, it is not surprising that immune function is altered during periods of exercise where risk of injury is inherently increased. Early studies reported an increase in self-reported incidence of upper respiratory tract infection (URTI) in athletes compared to controls in the week following marathon (Nieman, Johanssen, *et al.*, 1990) and ultramarathon running (Peters and Bateman, 1983). Around a similar time, no increase in URTI was found in 5km, 10km or half marathon race participants suggesting exercise duration was the key factor in increased incidence of URTI (Nieman, Johanssen and Lee, 1989).

2.4.3. J-shaped curve

Incidence of self-reported URTI symptoms post-race was also shown to rise in those with higher pre-race training volume and the fastest race times (Nieman, Johanssen and Lee, 1989; Nieman, Johanssen, *et al.*, 1990). At around the same time, moderate exercise such as walking was shown to reduce incidence of URTI compared to sedentary behaviour (Nieman, Nehlsen-Cannarella, *et al.*, 1990). Together, this data led to the formulation of the 'J-shaped curve' shown in figure 2.2 (Nieman, 1994). This hypothesis infers that if sedentary individuals were considered to be at medium risk of URTI, those undergoing long durations of heavy training were at high risk of URTI whilst moderately active individuals were at low risk (Nieman, 1994).

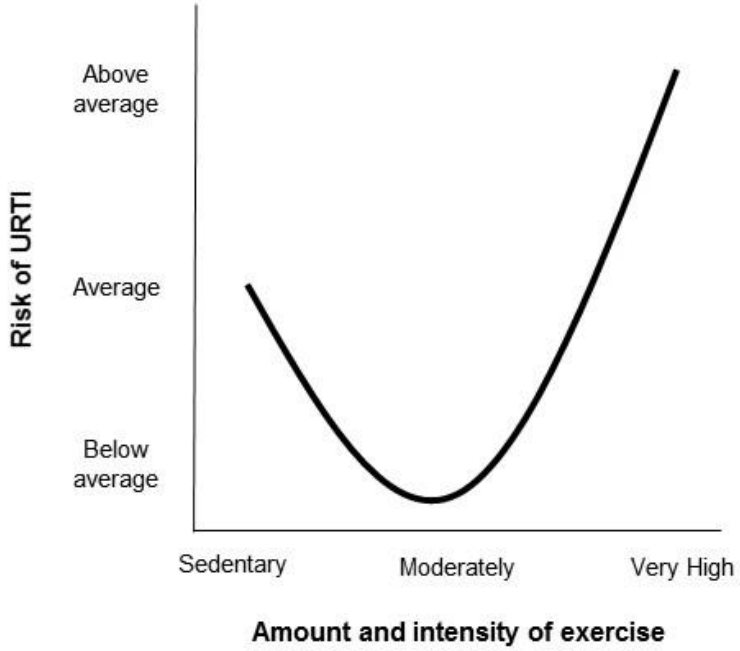

Figure 2.2: The 'J-shaped curve' showing proposed relationship between upper respiratory tract infection (URTI) and amount and intensity of exercise (adapted from Nieman, 1994).

2.4.4. S-shaped curve

Following the development of the J-shaped curve hypothesis an important study by Spence et al. (2007) found very few laboratory verified infections in athletes reporting URTI symptoms during 5 months of training and competition. Indeed, only 30% of self-reported URTI symptoms were corroborated by a positive laboratory diagnosis of viral, bacterial, chlamydial or mycoplasmal infection. This led to the proposal of non-infectious factors such as allergy, asthma, non-specific mucosal inflammation or airway inflammation due to inhalation of cold air or increased ventilation being responsible for URTI-like symptoms (Gleeson, 2007; Campbell and Turner, 2018). Additionally, attendance of any mass participation event of any nature is likely to increase the odds of acquiring a novel pathogen (Choudhry *et al.*, 2006). As the 'J-shape curve' hypothesis was formulated on the basis of data taken from competitive events it is also possible that pre competitive anxiety or psychological stress may compromise immunity and contribute to risk of URTI (Glaser and Kiecolt-Glaser, 2005; Edwards *et al.*, 2018). Taken

together, these findings suggest that acute bouts of endurance exercise may not have the immunosuppressive effects initially proposed (Campbell and Turner, 2018). Accordingly, Malm (2006) updated the “J-shaped curve” to include an elite population with a URTI infection odds ratio similar to that of a sedentary population in the “S-shaped curve” as shown in figure 2.3 below.

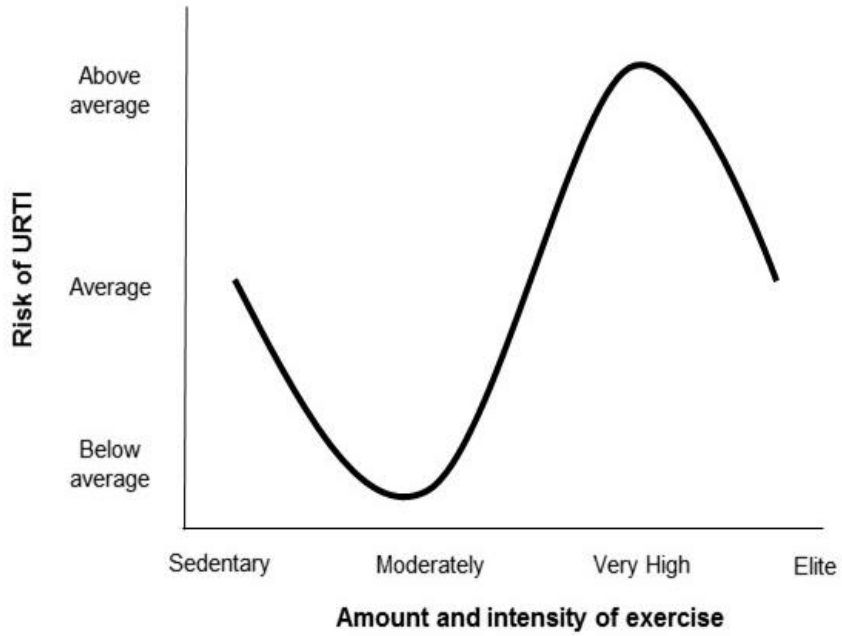

Figure 2.3: The ‘S-shaped curve’ showing proposed relationship between risk of upper respiratory tract infection (URTI) and amount and intensity of exercise (adapted from (Malm, 2006).

The ‘S-shaped curve’ model is supported by national athletes showing higher prevalence of URTI than more elite international counterparts, proposed to be due to improved lifestyle habits (e.g. awareness of avoiding those who are ill, reduced stress/increased recovery through not working full time; Hellard *et al.* (2015)). The same study showed that incidence of illness reduced year on year over 4 years of monitoring elite swimmers thus suggesting that the athletes were learning means to reduce incidence of illness throughout the duration of the study. Odds risk of infection was also nearly doubled during winter compared to summer training, similar to that observed in sedentary populations (Hellard *et al.*, 2015). These findings are corroborated by an inverse correlation between training volume and sick days assessed via

detailed illness diaries in a small (n=11) group of elite endurance athletes (Mårtensson, Nordebo and Malm, 2014). Finally, ultramarathon runners undertaking extremely high training volumes (up to 3,876 ± 1,526km per annum) reported 1.5 – 2.8 illness days per annum, less than the 4.4 illness days per year reported by the US department of Health and Human services report in 2009 (Hoffman and Fogard, 2012; Hoffman and Krishnan, 2014). Together these findings suggest that athletes undergoing the largest training volumes compared to non-elite counterparts are better able to cope with the demands of their training.

2.4.5. Open window and repeated bouts theory

As previously discussed in section 2.2.1, it is recommended that training is not increased beyond 5-10% per week in order to maintain satisfactory immune function in athletes, particularly in winter (Walsh, 2018). These findings also emphasise the importance of utilising accurate measures of training load in elite athlete populations in order to manage risk of illness. This notion is supported by the 'open window theory' proposed by (Pedersen and Ullum, 1994; Pedersen, Rohde and Ostrowski, 1998). It is well acknowledged that overall immune function (e.g. antibody production) is transiently increased from baseline immediately following exercise. Immune function then decreases temporarily in the recovery period (~post-1h) before returning to baseline 3-72h later, depending on the duration and intensity of the exercise bout (Pedersen and Ullum, 1994; Simpson, 2011). This transient decrease in immune function during the recovery from exercise is proposed to increase the chances of infection gaining a foothold, hence the term 'open window theory' (Pedersen and Ullum, 1994).

If a subsequent exercise bout is undertaken without allowing sufficient recovery, the process starts from a lower baseline and ultimately results in a lower immune function during the subsequent recovery period (Pedersen, Rohde and Ostrowski, 1998). If this pattern continues for weeks, months or years without allowing sufficient time for immune function to recover, it is proposed this could lead to chronic suppression of immune function offering

further increased potential for infection. This is summarised in the repeated bouts theory as shown in figure 2.4 and further highlights the importance of assessing immune recovery relevant to measures of training load in order to ensure adequate recovery time is permitted between bouts (Pedersen, Rohde and Ostrowski, 1998; Simpson, 2011).

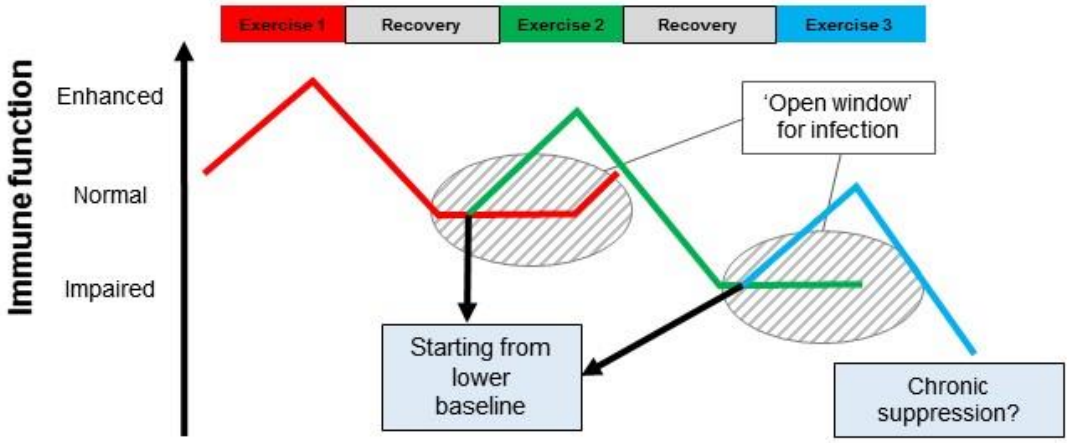

Figure 2.4: Open window theory (Pedersen and Ullum, 1994) and repeated bouts theory, adapted from Simpson *et al.* (2015)

It should however be considered that the immune response to exercise is dependent on exercise duration and intensity relative to the individuals training status (Peake *et al.*, 2017). Therefore, it is possible to train the immune response to exercise alongside increases in training status, provided the increases in training load are not excessive and sufficient rest is prescribed (Hellard *et al.*, 2015). Further, a recent review has refuted the open window theory, suggesting that the redistribution of leukocyte subpopulations from circulation to secondary lymphoid tissues is protective to the host (Krüger and Mooren, 2007; Campbell and Turner, 2018). Further, while the innate response is typically mounted in an order of hours, the adaptive response takes 4-7 days and can thus be difficult to directly attribute to a bout of exercise. This is particularly pertinent when the few cases of clinically confirmed URTI presented in the literature suggest an incidence of infection similar to that of the general population (Mäkelä *et al.*, 1998; Spence *et al.*,

2007; Campbell and Turner, 2018). Together, recent literature suggests endurance exercise training may not be as detrimental to immunity as previously thought, however more research is required to corroborate this assertion.

2.4.6. Leukocyte subset response to exercise

One of the most replicated findings in exercise immunology is a transient increase in leukocytes immediately post exercise. The magnitude of leucocytosis is typically ~3-5 fold and is determined by exercise intensity and duration (Simpson *et al.*, 2005; Gleeson, 2007). These increases are driven by increased numbers of neutrophils and lymphocyte subsets, particularly subsets exhibiting potent cytotoxic effector functions, high tissue migrating ability, and low proliferative ability (Shephard, 2003; Simpson *et al.*, 2008; Campbell *et al.*, 2009). In the recovery period following exercise, leucocyte count is influenced by reduction in lymphocyte values whilst neutrophil values may continue to rise due to release of neutrophil reservoirs from the bone marrow.

2.4.7. Neutrophils

Neutrophil count increases immediately following exercise and the magnitude of increase is dependent on exercise duration more so than intensity. A sustained elevated neutrophil count may be observed due to cortisol induced release of neutrophil reservoirs in the bone marrow (Bishop *et al.*, 2001). Moderate intensity exercise increases neutrophil respiratory burst activity and chemotaxis but not ability to adhere to the endothelium, which is the first step in migration to infected tissue (Ortega *et al.*, 1993). Neutrophil respiratory burst capacity is also enhanced immediately following moderate intensity (50% VO_{2peak}) exercise while it is attenuated following high intensity (80%VO_{2peak}) exercise (Dziedziak, 1990). During the recovery period, oxidative burst is impaired following prolonged or exhaustive exercise but enhanced following moderate exercise (Pyne, 1994; Suzuki *et al.*, 2003). Neutrophil phagocytosis and spontaneous degranulation is enhanced following an acute bout of exercise, though degranulation in response to a bacterial challenge is impaired (Bishop *et al.*, 2002).

2.4.8. Lymphocytes

Circulating lymphocyte cell number can increase ~4-5 fold above resting values immediately after exercise (Nieman *et al.*, 1993, 1994; Simpson *et al.*, 2008). Values may then drop below baseline values 1h-post exercise if exercise intensity and/or duration has been sufficient (Nieman *et al.*, 1994). Upon commencement of exercise, sympathetic nervous system activity is increased, activating the hypothalamo-pituitary-adrenal (HPA) axis, in turn releasing catecholamines which increase heart rate (Shephard, 2003). Increased shear stress due to elevated cardiac output dislodges lymphocytes from the marginal pools and into circulating peripheral blood (Shephard, 2003). In addition to non-specific mechanical stress, increases in populations of highly cytotoxic lymphocytes is proposed to be due to beta 2 (β_2)-adrenergic receptor activation which downregulates adhesion molecules expressed in abundance on these cells (Sanders, 2011). β_2-adrenergic receptors are activated in a linear fashion dependant on the concentration of adrenaline present in the peripheral blood (Sanders, 2011). Furthermore, exercise lymphocytosis is partially blunted by administration of β_2-adrenergic receptor antagonists (Murray *et al.*, 1992; Mills *et al.*, 1999, 2000). Thus, any situation in which the HPA axis is activated likely results in an increased number of circulating lymphocytes expressing β_2-adrenergic receptors. Exercise intensity has a more profound effect than duration on the biphasic response, whereby increasing intensity results in larger increases in lymphocytes post exercise and larger decreases during the recovery period 1-3h post exercise (Nieman *et al.*, 1994).

2.4.9. T-cells

Antigen experienced $CD8^+$ T-cell populations exhibiting high cytotoxicity, high tissue migrating potential and low proliferative ability (e.g. $CD8^+$ EM) are preferentially mobilised in response to exercise (Simpson *et al.*, 2008; Simpson, 2011). High intensity (20 mins at 85% peak power) exercise elicits a larger ingress of $CD8^+$ T-cells compared to low intensity (20 mins at 35% peak power) exercise (Campbell *et al.*, 2009). Of those $CD8^+$ cells mobilised, magnitude of cell mobilisation immediately post exercise was increased in

order of chronological maturation stage determined by phenotype (Campbell *et al.*, 2009). In comparison, $CD4^+$ T-cell populations show little response to exercise (Campbell *et al.*, 2008). Provided exercise duration or intensity has been sufficient, peripheral blood levels of CD8 T-cells may reduce below baseline values during recovery from exercise (Simpson, Florida-James, *et al.*, 2006; Simpson *et al.*, 2008) producing a biphasic response (figure 2.5).

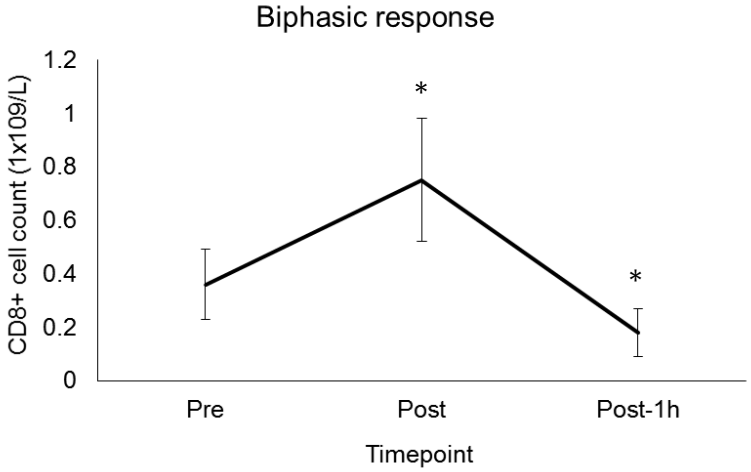

Figure 2.5: An example of the biphasic CD8+ T-cell response to exercise. * denotes significant difference from pre exercise values ($p < 0.05$). Adapted from the data of (Simpson, Florida-James, Cosgrove, *et al.*, 2007).

These changes are driven primarily by relocation of senescent and effector memory cells and lesser so by central memory T-cells from the peripheral blood compartment (Simpson *et al.*, 2008). Initial reports suggested apoptosis as a major contribution to lymphocyte alterations following exercise, though this has been widely disproven in subsequent reports (Simpson, Florida-James, Whyte, *et al.*, 2007). Instead, in a mouse model, bone marrow, Peyer's patches and lung are suggested to be the main destinations of effector–type T-cells leaving the peripheral blood compartment (Krüger *et al.*, 2008). As these tissues are potential entry points for antigen, the redistribution of effector-type cell subsets is proposed to be beneficial to the host (Simpson *et al.*, 2015). In the presence of cytomegalovirus infection CD8+ T-cells are mobilised in significantly greater numbers following exercise, primarily driven by increased numbers of effector memory and senescent CD8+ T-cell subpopulations (Turner *et al.*, 2010). Meanwhile, little or no changes are seen in CD4+ T-cells or total lymphocyte population in CMV seropositive individuals

(Turner *et al.*, 2010; Lavoy *et al.*, 2014). Interestingly, psychological stress induces a preferential redistribution of effector –type T-cell subsets in a similar manner to exercise (Atanackovic *et al.*, 2006; Anane *et al.*, 2009). This suggests that the 'fight or flight' response may also enhance immune surveillance before any potential increased risk antigen challenge has even begun. Additional acute stressors placed upon the body such as disrupted sleep, and carbohydrate availability can also augment T-cell redistribution following exercise (Braun and Von Duvillard, 2004; Ingram *et al.*, 2015). Interestingly, a significant relationship between psychological stress and susceptibility to the common cold has also been shown repeatedly (Cohen, 2005). In one particularly important study individuals completed questionnaires to assess the degree of psychological stress and were exposed to the common cold virus via nasal spray and subsequently quarantined for seven days. The highest stress quartile were shown to be at almost double the risk of infection compared to the lowest risk quartile even when controlling for age, weight, education and season (Cohen, Tyrrel and Smith, 1991). This is particularly interesting in the context of athletes during competition where psychological stress is likely to be increased, thus potentially compromising immunity (Edwards *et al.*, 2018).

T-cell function can also be influenced by exercise intensity. T-cell proliferative capacity was shown to be impaired by 50% and 25% following 2 hours of exercise at 80% and 60% VO_{2peak}, respectively (Nieman *et al.*, 1994). However, this finding may be confounded by the concurrent biphasic response in T-cell number and proportion previously discussed. In another elegant study by Bishop *et al.* (2009), participants ran at 60% VO_{2peak} for 2 hours, once with CHO supplementation and once with a placebo solution. Results showed that *ex vivo* T-cell migration towards supernatants of human rhinovirus infected airway epithelial cells was reduced following the exercise protocol. However, CHO supplementation during exercise was associated with a smaller reduction in T-cell migration towards infected cells than placebo, thus CHO supplementation may offer increased immune protection to the host. This is supported by evidence showing glucose to be an important energy substrate for activated leukocytes (MacIver *et al.*, 2008) which may increase stress as

maintenance of blood glucose is increasingly challenging as exercise duration increases (Coyle, 1999).

2.4.10. NK cells

NK Cells are the most exercise responsive lymphocyte subset, showing a 50-1000% increase immediately post exercise (Shek *et al.*, 1995; Campbell *et al.*, 2009). $CD56^{dim}$ cells (cytotoxic) are mobilised preferentially to $CD56^{bright}$ (regulatory) cell subsets (Campbell *et al.*, 2009). This is once again proposed to be a protective mechanism during increased risk of injury during stressful situations. NK cell numbers during recovery can also reduce below baseline values for 1 – 24h following very intense or prolonged duration exercise (Shephard and Shek, 1999). NK cell cytotoxic function is assessed by culturing PBMCs with a tumour target cell line where the number of dead target cells expressed relative to the number of NK cells present in the PBMC fraction provides a measure of NK cell cytotoxic function (Simpson *et al.*, 2015). Several reports have shown that overall NK cell activity is increased immediately following exercise before showing reductions in the recovery period following exercise (Shephard and Shek, 1999). However, these changes are largely due to the biphasic response in circulating numbers of NK-cells in the peripheral blood as supported by evidence of cell-by-cell cytotoxic activity remaining largely unchanged by acute bouts of exercise in CMV seronegative individuals (Nieman *et al.*, 1993). However, in CMV seropositive individuals, NK cell redistribution and cytotoxic activity is attenuated following exercise indicating potential reduction in NK-cell mediated immunosurveillance (LaVoy *et al.*, 2013).

Aside from exercise induced changes to cellular components of the immune system, concentrations of several cytokines and glucocorticoids are also significantly altered during exercise and the recovery period from exercise. Concentrations of interleukin (IL)-6 rise in a more marked and more consistent fashion than any other cytokine response measured to date (Nehlsen-Cannarella *et al.*, 1997; Reihmane *et al.*, 2013). Exercise, environmental and psychological stress also stimulates activation of the HPA axis (Duclos and

Tabarin, 2016) resulting in the release of glucocorticoids, including cortisol (Persson *et al.*, 2008). When exercise intensity and duration is sufficient, cortisol accumulates in the plasma and peak levels are typically observed upon exercise cessation or shortly thereafter (Hill *et al.*, 2008). In response to an acute stressor cortisol has primarily immunosuppressive and anti-inflammatory which is suggested to prevent an overreaction of the immune response to muscle damage or oxidative stress associated with acute bouts of exercise (Duclos and Tabarin, 2016). Both IL-6 and cortisol increase energy substrate availability to working muscles; cortisol by increasing blood glucose concentration, stimulating of hepatic gluconeogenesis and mobilising lipids via lipolysis in fat cells, and IL-6 by inducing upregulation of fat oxidation (Van Hall *et al.*, 2003) and hepatic glucose production (Febbraio *et al.*, 2004). Thus, increased quantities of IL-6 and cortisol may offer performance benefits during prolonged exercise by promoting delivery of energy substrate to working muscle and thus delaying the onset of fatigue. IL-6 is produced in response to tissue injury and infection acting as a potent mediator of inflammation and immune response functions including developmental differentiation of lymphocytes (Hodge, Hurt and Farrar, 2005; Tanaka, Narazaki and Kishimoto, 2014) During the initial stage of inflammation, IL-6 is produced in a local lesion before inducing differentiation of naïve CD4 and CD8 T-cells (Korn *et al.*, 2009; Okada *et al.*, 2019) thus linking the innate and acquired immune response. Together, this section of this review has shown the immune response to be dependent on the duration, intensity, and the subsequent energy demands of the exercise. Therefore, to increase the understanding of the immune system to enduro mountain biking, it is important to fully detail the demands of the event.

2.5. Vibration

Thus far, a majority of research has focused on the workload demands of mountain biking in the field by measurement of power output, heart rate (Stapelfeldt *et al.*, 2004; Hassenfratz, Ravier and Grappe, 2012) and more recently, oxygen uptake (Hays *et al.*, 2018). Most studies have therefore omitted the influence of vibration, despite evidence of excessive vibration

exposure in road and commuter cycling (Chiementin *et al.*, 2013; Taylor, Edgar and Raine, 2018) which contributes to overall workload demands of cycling (Rønnestad *et al.*, 2018). The term vibration refers to an oscillatory motion, therefore motion is not constant (Griffin, 1990). The displacement of the oscillation determines the magnitude of vibration and the rate of repetition of cycles of oscillation per second is referred to as the frequency of vibration, measured in Hertz (Hz). Research in human exposure to vibration linked the incidence of various diseases affecting the blood vessels, nerves, bones, joints, muscles or connective tissues habitual use of power tools that emit vibration in a professional setting (Andréu *et al.*, 2011; Shen and House, 2017). As a result of these findings, a plethora of guidelines intended to standardise quantification of vibration exposure and subsequently limit vibration exposure in the workplace have been formed (BSI, 2001; ISO, 2017). However, it is not possible to define the specific vibration exposure required to cause such diseases and as such it is not possible to define a safe exposure range in which vibration related diseases will not occur (ISO, 2017). Although no safe vibration exposure range can be guaranteed, it appears advantageous to the health of the individual to reduce excessive vibration exposure at any opportunity (Griffin, 1990). Hand arm vibration (HAV) exposure concerns vibration exposure at the hands and transferred to the arm and shoulder, whilst whole body vibration (WBV) exposure concerns situations where the entire body is exposed to vibration (e.g. passenger in vehicle; Griffin, 1990). The assessment of HAV and WBV have different complex methodological requirements with respect to recording rate, measurement location and statistical analyses (BSI, 2001; ISO, 2017). Briefly, assessment of HAV requires an accelerometer recording at a minimum recording frequency of 2800Hz in three axes located as close to the point of interaction between the hand and vibration as possible. Assessment of WBV requires an accelerometer recording at a minimum of 160Hz in three axes located as close to the point of interaction between contact point and vibration source. Full extent of methodological requirements can be found in ISO regulations (BSI, 2001; ISO, 2017) and will be addressed later in general methods chapter.

2.5.1. Vibration exposure in cycling

Excessive levels of both HAV and WBV have been reported in road cycling on cobbled terrain. One study assessed WBV exposure during the Paris-Roubaix course and found that the exposure limit value was exceeded in less than a minute (52 ± 14s) in to a race lasting 90 minutes (Duc, Puel and Bertucci, 2016). Comparatively, HAV exposure limit value was exceeded in only 7.5min at 35km.h^{-1} over similar terrain (Chiementin *et al.*, 2013). Both studies also reported that increased vibration exposure was correlated with increased velocity over rough surfaces, thus suggesting vibration exposure is greater for faster riders and during faster sections of courses. These data were collected over challenging terrain on a road bike with no suspension and low profile tyres, factors which are together likely to increase vibrations transferred to the rider. Terrain covered in enduro mountain biking is often more challenging than previous studies in road cycling, suggesting vibration exposure to the rider may be altered (Taylor, Edgar and Raine, 2017). However the bicycles used for enduro racing feature front and rear suspension systems designed to mitigate accelerations transferred to the rider, suggesting further investigation of vibration exposure in enduro racing is warranted This is particularly pertinent when the overall duration of an enduro race is considered where prolonged periods of descending on challenging terrain may be sufficient to expose the rider to harmful levels of vibration. Such findings are concerning due to findings of musculoskeletal, neurological and vascular pathological disorders proposed to be related to vibration exposure exceeding ISO exposure limit values (ELV) in road and commuter cycling (Haloua, Collin and Coudeyre, 1987; Rtaimate *et al.*, 2002; Munera *et al.*, 2014; Taylor, Edgar and Raine, 2018).

In a study investigating the vibrations associated with road vs mountain bike cycling, Macdermid, Fink and Stannard (2015) reported that heart rate, VO2, and vibrations assessed at the handlebar, arm, leg and seat post were greater in mountain biking compared to road cycling at the same speed. However, this finding is limited by insufficient data recording frequency (128Hz), lack of technical downhill terrain, and use of the same bicycle with the same suspension and tyre pressure for every participant regardless of weight which

can alter impact attenuation (Macdermid *et al.*, 2015). Indeed, equipment can have a large influence on vibration exposure experienced by the rider whereby different frames, wheels and suspension forks can all influence transference of accelerations from the terrain to the rider (Lépine, Champoux and Drouet, 2015; Macdermid, Fink, *et al.*, 2017). Despite inherent limitations previously discussed, the current body of research indicates that vibration exposure during cycling tasks can exceed potentially harmful levels therefore further research is required to investigate the full extent of the issue in different cycling populations such as those racing enduro (Taylor, Edgar and Raine, 2017).

2.5.2. Vibration and performance

In addition to being potentially harmful to health, vibration exposure can also influence physical and cognitive performance (Samuelson, Jorfeldt and Ahlborg, 1989b; Sperlich *et al.*, 2009; Yung *et al.*, 2017). Cycling time to exhaustion is reduced by 21% or 13 ± 2.9 minutes when vibration is added to the same constant workload compared to constant workload alone (46.9 ± 5.3 min vs 59.5 ± 7.7 min respectively; (Samuelson, Jorfeldt and Ahlborg, 1989b). Unfortunately, the workload selected was based on a workload participants expected they could continue with for around 45 minutes rather than a fixed power output. This is important as lack of power output measurement means it is not possible to differentiate the fatigue induced by the addition of vibration from the fatigue induced by the generation of power. More recently, vibration exposure during fixed power output cycling was shown to increase mean VO_2 but not mean HR in well trained cyclists, therefore suggesting an elevated workload (Rønnestad *et al.*, 2018). This suggests that measurement of heart rate and power output without inclusion of vibration exposure may underestimate physiological workload during periods of vibration. This finding may be particularly important given that measurements of heart rate and power output are the most popular means to assess training load in cycling athletes (Padilla *et al.*, 2001; Sanders *et al.*, 2017). Work from the same group showed that vibration exposure decreases endurance of maximal voluntary contraction in the thigh muscle by 6.7 ± 1.8 seconds from 22.5 to 15.8 seconds on average (Samuelson, Jorfeldt and Ahlborg, 1989b). This finding is pertinent

as isometric contractions are associated with controlling mountain bikes on rough downhill terrain, typical duration of which exceeds endurance of maximal contraction reported in the previous study (Hurst *et al.*, 2012). This is further supported by field data showing that maximal grip strength in Scottish elite downhill mountain bikers was reduced in the right hand by 29.1 ± 8% and in the left hand by 22.8 ± 4.9 in the left hand after one run on a World Cup DH track and additionally the decrement in grip strength was linked with the cumulative load (number of impacts) exposure from the previous day's riding (Florida-James, Ball and Westbury, 2010). Reduction in grip strength results in a compromised ability to effectively use the brakes or grip the handlebars, which may result in a crash or reduction in performance (Miller *et al.*, 2018). Accordingly, whilst the general reduction in grip strength is likely partly attributable to vibrations and impacts, differences between loss of strength in hands is postulated to have arisen due to the right hand operating the front brake which is proposed to be used more often in DH riding (Lopes and McCormack, 2017). The front brake is used almost exclusively during hard braking in motorcycle racing as almost all vehicle load is transferred to the front wheel, though further investigation in mountain biking is required (Corno *et al.*, 2008). In summary, every previous study which has attempted to assess vibration exposure in mountain biking was limited by equipment (insufficient recording rate), incorrect accelerometer position (not between or close to point of contact), incorrect analysis or a combination of some or all factors. As a result, it is pertinent to assess vibration exposure in mountain bike racing in accordance with internationally recognised guidelines and standards, and further to explore links between vibration exposure and performance outcomes.

2.6. Immune and heart rate variability responses to combined exercise and vibration

The majority of previous exercise immunology and heart rate variability (HRV) research discussed in this review has focused on the response to bouts of exercise that have been quantified by heart rate or power output and are free from excessive levels of vibration (Simpson *et al.*, 2005, 2008; Witard *et al.*,

2012; Plews, Laursen and Buchheit, 2016). Transient changes in frequency domain parameters of HRV have been shown in response to acute exposure to low level vibration associated with driving tasks (Jiao *et al.*, 2004). Reductions in frequency domain parameters of HRV have been shown in response to between 6 and 8 weeks of concurrent vibration and strength training compared to strength training alone (Wong and Figueroa, 2018). No data of responses of time domain parameters of HRV to vibration are available, particularly in an endurance training context. However, considering the alterations to frequency domain parameters of HRV in response to vibration, it is conceivable that time-domain parameters utilised in the monitoring of athletes such as LnRMSSD (see section 2.3) may be altered by vibration exposure. There is also a lack of research available on the immune response to the addition of vibration to cycling tasks. However, a limited number of studies have explored the immune response to passive vibration exposure. Exposure to three minutes of hand arm vibration (r.m.s = 2.5ms^{-2}) was shown to reduce CD4+ T-cell concentration and increase CD8+ T-cell concentration; overall reducing the CD4:CD8 ratio and suggested to be primarily immunosuppressive (Noguchi and Ando, 2002). The addition of whole body vibration to squats was shown to reduce the proliferative capacity of CD4+ T-cells in elderly patients with osteoarthritis with no significant change observed in CD8+ T-cells (Tossige-Gomes *et al.*, 2012). In this population, the effect of vibration was proposed to be beneficial in mediating T-cell immunity, potentially minimising the progression of osteoarthritis (Tossige-Gomes *et al.*, 2012), however no information of magnitude of vibration was provided. Purposefully exposing humans to excessive doses of vibration is not ethically viable; hence the only data available of immune responses to excessive levels of vibration are data from studies conducted in the rat model. Chronic vibration exposure (horizontal vibration of 5.0*g*, frequency 20Hz) for 3 hours per day over 3 months reduced lymphocyte count and increased corticosterone concentration in albino rats (Gunasekaran, 2001). This suggests that vibration is a potent stressor of the immune system, and that further work is required to understand the potential immune response in humans. This may have pertinent implications in the recovery of the immune system following off-road cycling where vibration exposure may be excessive

and training load underestimated when using currently available models of training load (Sanders *et al.*, 2017; Rønnestad *et al.*, 2018). It is therefore a key aim of this thesis to investigate the immune response to mountain bike racing with respect to the influence of vibration exposure in addition to traditional means of heart rate based training load.

2.7. Summary of literature

To summarise, this literature review suggests that accurate measures of training stimulus are necessary to accurately determine response of the immune system and ANS to prescribed training stimuli. Previous literature has successfully quantified heart rate, oxygen uptake, and power output associated with other off-road cycling disciplines, to date there is still a lack of data on enduro. Vibration exposure has been widely suggested to contribute to the physiological demands of off-road cycling disciplines, however attempts to determine vibration exposure during mountain biking have been widely compromised by poor methodology. Accurate training load models are established in road cycling, assisting with successful training programming alongside well established information on the recovery of the immune system following periods of cycling. However, these findings have all been reported in the absence of vibration which is shown to increase physiological workload and be a potent stressor of the immune system. It is also not known if the physiological and immunological response to vibration is trainable. Therefore, further investigation of the physiological and immunological response to mountain biking and the associated vibration will be a focus of this thesis.

Chapter 3: General material and methods

3.1 Introduction

This chapter is divided in to 3 sections describing different components of methodology common to multiple chapters within this thesis. Data of chapters 4-6 was collected as part of one longitudinal study during 2016-2017 while data of chapter 7 was collected separately in 2018. An overview of the experimental design is provided in section 3.2. Details of participants included in chapters 4-6 are described in section 3.3 and laboratory exercise protocols common to chapters 4-6 are described in section 3.4. Chapter 7 includes a standalone methods chapter. Statistical analysis and any other methodological details specific to individual chapters are included therein.

3.2 Experimental design

Participants (detailed in section 3.3) attended Edinburgh Napier University laboratory on three occasions to complete anthropometric assessment (section 3.4.1), an incremental exercise protocol (section 3.4.2), and blood sampling (chapter 6, section 6.2.1.). Each visit aligned with the start of winter training 2016/17 (baseline), the mid-point in the 2017 race season (mid-season) and after the final race of the 2017 season (off-season). The timing of each visit was negotiated with each athlete and their respective coach depending on wellness, training status and planned race events meaning that the actual date of each visit varied between participants. Details of time between each laboratory visit for each participant are provided in table 3.2. As part of their 2017 race season, all participants completed a one-day international mountain bike enduro race where heart rate, GPS data (Chapter 4, section 4.2.1-4.2.3) and blood samples (chapter 6, section 6.2.1) were also collected.

Table 3.1: Details of days between athlete testing and days in each training phase

Athlete	Days between tests			Days in phase				
	Period 1	Period 2	Total	GP	PC	CT	CR	RP
1	191	132	323	106	14	174	14	17
2	244	86	330	142	14	130	11	35
3	235	57	292	53	14	174	14	39
4	173	141	314	91	14	174	14	23
5	186	122	308	87	14	174	14	21
6	-	-	344	90	14	174	14	54
7	265	69	334	70	14	188	15	49
8	161	98	259	75	14	130	10	32
9	192	60	252	72	14	130	11	27

Note: participant 6 did not complete a mid-season test due to racing, training and illness commitments.

3.3 Participants

Eight male (*n*=8, age= 25 ± 3 years, height=181 ± 6cm, mass=80 ± 11kg) and one female (*n*=1, age = 23, height = 160cm, mass = 56.6kg) elite mountain bike athletes agreed to participate in this study. All participants had previously finished in the top 85 positions at an Enduro World Series (EWS) race, five of whom had previously finished in the top 10 at a EWS race. Ethical approval for the study was granted from the ethics committee of Edinburgh Napier University. Subsequently, both oral and written consent was obtained from all participants. Participants were free from any perceived illness for two weeks prior to each laboratory visit, were not using medication, and were required to refrain from consuming alcohol or caffeine in the 24h preceding the laboratory visit and race event. Details of individual participant gender, age and EWS results from the season over which this research was conducted (2017) are detailed in Table 3.1 below.

Table 3.2: Details of participant gender, age and 2017 EWS results

Athlete	Gender	Age	Best EWS result (2017)	Years of training history
1	Male	23	3rd	10
2	Male	30	46th	11
3	Female	23	2nd	12
4	Male	25	1st	8
5	Male	27	26th	17
6	Male	23	12th	11
7	Male	23	102nd	4
8	Male	22	61st	10
9	Male	23	47th	6
Mean ±SD	-	24 ± 2	30 ± 35	10 ± 4

3.4 Laboratory exercise protocols

This section describes the protocol for each laboratory visit. Participants arrived in the laboratory at 9am having consumed the same breakfast prior to each visit. The exercise protocol began between 10.30am and 11am to minimise the influence of circadian variation (Atkinson and Reilly, 1996). A detailed timeline of events for each laboratory visit is presented in figure 3.1 below.

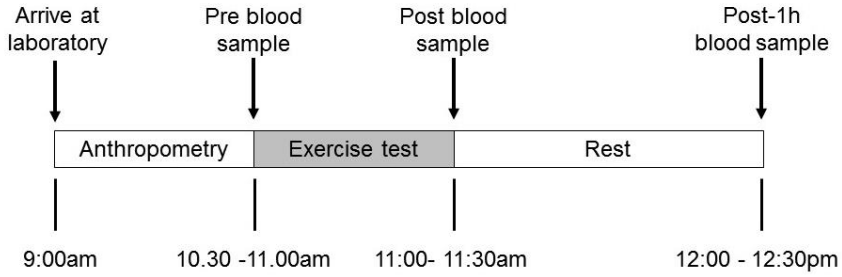

Figure 3.1: Laboratory visit timeline

3.4.1 Anthropometry

The ISAK (International Society for the Advancement of Kinanthropometry, 2010) restricted profile was used to collect anthropometric data on the second visit to the laboratory. Corrected measurements of calf, thigh, and upper arm girth were used to calculate estimated muscle mass (Martin *et al.*, 1990). In addition, thigh girth 1cm distal of the gluteal fold and grip strength of both hands was collected due to previous relationships with downhill racing performance (Florida-James, Ball and Westbury, 2010; Hadden and Florida-James, 2011). Body mass was measured using scales (Seca 761, Germany) and stature was measured using a stadiometer (Holtain Limited Harpenden Portable, UK).

3.4.2 Lactate threshold and VO_{2peak} protocol

Power output and heart rate at fixed blood lactate concentrations of 2 and 4mmol.L^{-1} (FBLC2 and FBLC4; HR_{FBLC2} and HR_{FBLC4}, respectively) was assessed using an incremental exercise test using a bicycle suited to the height of the participant (Surprise, size large, Pinarello, Italy; Alpha 1.2, size 56cm, Trek, Taiwan; Mira, size small, Litespeed, USA) mounted on a cycle ergometer (Computrainer Pro 3D, RacerMate, Seattle, WA, USA). Blood samples taken from the ear lobe were analysed for blood lactate concentration using the Lactate Pro 2 Meter (Arkray LT-1730, Japan) which has been previously reported as a reliable (CV ≤ 1.0%) measure of blood lactate concentration (Bonaventura *et al*., 2015). The initial workload was set at 110W increasing 30W every 3 minutes. Samples were taken within the last 30s of each workload until lactate concentration was ≤4mmol.L^{-1}. At this point the workload increased by 20W every minute until volitional exhaustion or when cadence >60rpm could not be maintained. Online gas analysis was used to determine oxygen uptake (Metalyzer 3B, Cortex, Germany; Hans Rudolph V2, Germany). VO_{2peak} was taken as the highest 8-breath average from raw breath-by-breath data. Power and HR at respiratory exchange ratio equal to 1 (RER ≥ 1; $HR_{RER \geq 1}$, respectively) were taken where 8-breath average RER value exceeded 1.00. Rating of perceived exertion (RPE) (Borg, 1970) was assessed in the last 10s of every stage and HR (Polar, Finland) was recorded at 5s intervals throughout the two tests.

Chapter 4: Physiological requirements of international enduro racing

4.1 Introduction

Due to the recent creation of enduro mountain bike racing as a discipline there is a lack of empirical literature available. To date, researchers have focused on the demands of the race stages as they contribute directly to general classification (20-40mins) thus omitting the demands of the transition stages. Race stages have consistently been reported to be very high intensity, evidenced by high mean HR values (>90%HR_{max} and elevated blood lactate values (~15mmol.L^{-1}) upon completion (Hassenfratz, Ravier and Grappe, 2012; Kirkwood *et al.*, 2017). Though time limited transition stages do not contribute directly to general classification, they compose the majority of the overall race duration (3-9h total) lasting up to 2.5h per transition stage and covering up to 2000m vertical per day (Enduro World Series, 2018). Importantly, male and female riders complete the same courses with the same time limits applied (Enduro World Series, 2018) a situation driven by the athletes themselves. Elevated blood lactate concentrations have been observed following transition stages, suggesting they contribute significantly to the demands of the racing event (Hassenfratz, Ravier and Grappe, 2012). This may influence recovery following race stages and impact on performance during subsequent race stages, hence further research is warranted on the demands of transition stages and the impact of these demands on race stage performance.

Previously defined physiological characteristics of male elite enduro riders showed that enduro riders had VO_{2peak} and power at VO_{2peak} values comparable to that of national level XCO athletes (Impellizzeri, Marcora, *et al.*, 2005). Concurring with the findings of Hassenfratz, Ravier and Grappe (2012), elite athletes were also shown to produce greater absolute and relative power output at fixed blood lactate concentrations of 2 and 4mmol.L^{-1} (FBLC2 and FBLC4, respectively) when compared to non-elite enduro athletes (Kirkwood *et al.*, 2017). This suggests that the elite athletes have greater potential to complete transition stages at submaximal workload and conserve energy for the race stages thus contributing to successful performance. This considered, the findings of Kirkwood *et al.* (2017) were based on data collected 1-2 years after the conception of EWS in 2013 when most riders had switched to enduro

from XCO or DH. Now, many athletes and coaches have focused solely on enduro events for several years. It is therefore possible that athletes and coaches have used their experience and previous research to adapt training regimes specifically to optimise enduro racing performance which in turn may alter the physiological characteristics of elite athletes.

Alongside traditional measures of heart rate and power output, measurement of terrain induced accelerations which must be mitigated before reaching the torso have been proposed as a measure of physiological workload in mountain biking (Florida-James, Ball and Westbury, 2010; Macdermid, Fink and Stannard, 2014). Indeed, dissipation of terrain induced accelerations has also been shown to contribute to physiological workload experienced by enduro riders, whereby faster riders must absorb larger magnitude accelerations (Kirkwood *et al.*, 2017). In addition to assessment of magnitude of accelerations previously presented, it would be useful to estimate vibration exposure based on international organisation for standardisation (ISO) guidelines (ISO, 2017). This is crucial as recent findings demonstrate that vibration exposure has primarily negative effects on cycling performance (Duc, Puel and Bertucci, 2016; Rønnestad *et al.*, 2018) and must be measured and analysed following specific criteria in order to measure any physiological responses (ISO, 2017; Taylor, Edgar and Raine, 2017). Therefore, more detailed analysis of vibration exposure in enduro mountain biking may allow greater understanding of the performance demands of enduro racing in context of other cycling disciplines (Chiementin *et al.*, 2013; Duc, Puel and Bertucci, 2016). Combined, these findings provide rationale to assess the demands of the transition stages, potential impact of transition stages on GC result, and the overall physiological demands of the race event including estimates of whole body vibration. Therefore, the aims of this study were to:

1: Investigate the current physiological characteristics of international elite enduro athletes;

2: Investigate the physiological demands of current international elite enduro racing including transition stages and whole body vibration exposure; and

3: Investigate relationships between physiological characteristics and enduro race performance.

4.2 Methodology

Please refer to chapter 3 for details of participants, lactate threshold and VO_{2peak} protocol. To detail the physiological characteristics of competitive elite enduro mountain bike athletes, the results of the mid-season laboratory test protocols previously detailed (chapter 3, section 3.4) are reported in this chapter. To assess the demands of international competition and the relationship between race performance and physiological characteristics all participants also completed an international race event, details of which are provided below.

4.2.1 Race event protocols

A one-day international enduro race in the United Kingdom consisting of 5 race stages was chosen for the race analysis in chapter 4. Mean distance, elevation, and percentage gradient ($100\ x\ \frac{\Delta elevation\ (m)}{distance\ (m)}$) of the race stages, transition stages, and entire course are available in figure 4.1 and table 4.1. This data was not provided by organisers and is instead calculated from the GPS device of each athlete hence coefficient of variation (CV) is also provided as a measure of reliability. Stages 1, 2 and 4 featured only technical terrain; stages 3 and 5 featured both technical and non-technical terrain. Technical and non-technical terrain are described in detail in chapter 2 section 2.1.2; in brief technical terrain is composed of challenging features such as jumps and drops while non-technical terrain is easier to navigate, often comprised of forest roads.

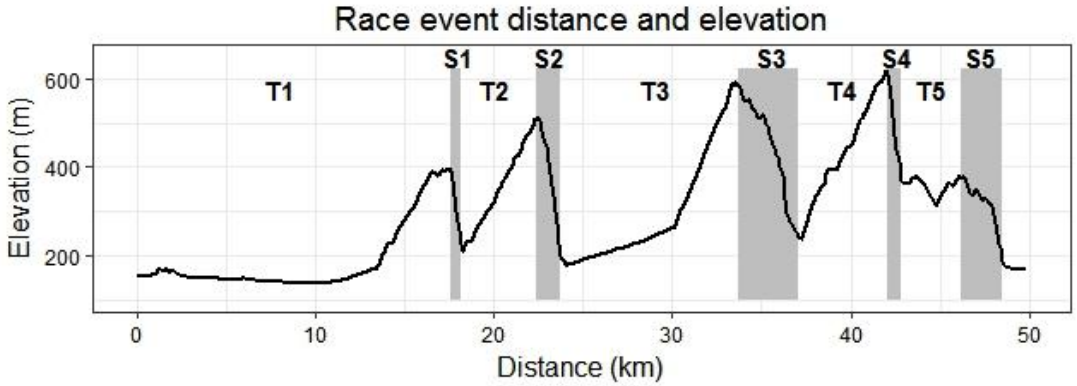

Figure 4.1: Distance and elevation details for the entire course with stage (S) and transition (T) sections overlaid.

Table 4.1: Details of distance, elevation and gradient for individual stages (all data presented as mean and coefficient of variation (CV).

Section	Distance (km)		Elevation (m)		Gradient	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
Entire course	**50.0**	**1.1**	**1451**	**3.9**	**N/A**	**N/A**
Transition 1	17.3	2.2	281.3	3.1	1.6	1.7
Stage 1	0.7	0.7	300.6	1.2	-44.5	1.0
Transition 2	4.3	3.8	299.0	4.3	6.9	4.4
Stage 2	1.5	2.0	351.2	1.3	-22.7	2.4
Transition3	10.0	2.6	409.1	4.2	4.1	5.7
Stage 3	4.0	3.3	436.4	0.6	-10.8	3.3
Transition 4	5.5	9.1	379.7	4.1	6.9	10.7
Stage 4	0.9	1.8	483.7	1.3	-51.2	2.4
Transition 5	3.5	7.4	85.0	7.6	2.4	11.6
Stage 5	2.5	0.8	298.7	0.7	-11.9	1.3

4.2.2 GPS accelerometer and heart rate data

A GPS device featuring 100Hz triaxial accelerometer (Catapult Optimeye S5, Catapult Innovations, Melbourne, Australia) was fixed to the seat post of each participant's bicycle in the orientation specified by the manufacturer. Location and elevation was measured at 10Hz using both global positioning system (GPS) and global navigation satellite system (GLONASS). All participants wore a wireless HR monitor strap (Wearlink, Polar, Finland) encoded to their individual GPS units recording HR at 1Hz. The 100Hz triaxial accelerometer was used to calculate bicycle load and accumulated bicycle load, the validity and reliability of these parameters have been reported elsewhere (Boyd, Ball and Aughey, 2011b; Van Iterson *et al.*, 2016). Due to the limited battery life of the GPS units and the long first transition, participants were instructed to turn the units on 10 minutes prior to the start of the first stage. This facilitated measurement of the terrain induced acceleration during all race stages which has previously been linked with performance. The distance and elevation of the first transition stage was calculated using data from the participants' own GPS cycling computers (Garmin Edge 500, Garmin, USA). Upon return to the event headquarters the catapult GPS accelerometer unit was connected to a laptop and the data downloaded for later analyses. To determine relative

intensity of each race and transition stage percentage time spent $<HR_{FBLC2}$, HR_{FBLC2} - HR_{FBLC4} and $>HR_{FBLC4}$ was calculated in addition to time spent $>HR_{RER \geq 1}$ which was used to determine time spent utilising carbohydrate as primary source of energy.

4.2.3 GPS analysis

Previous methodology to analyse Catapult files relied upon the mapping feature of Catapult Sprint which no longer functions in the software. Therefore, GPS data was initially downloaded to Catapult Sprint 5.1 software (Catapult Innovations, Melbourne, Australia) and exported to .csv file for further analyses in R version 3.4.2 (R Core Team, 2018). Technical (e.g. single track with rocks and roots) and non-technical (e.g. gravel road) sections of each race stage were identified by the research team in consultation with the athletes using visual cues (e.g. tree line). The latitude and longitude files for each participant were then overlaid on terrain maps using an online map (Google Earth, 2017) to determine the mean latitude and longitude of the start and end of each race stage, technical and non-technical section. R package RANN (Arya *et al.*, 1998, 2018) was then used to match the mean latitude and longitude identified on google earth to the closest latitude and longitude in each participant's GPS accelerometer file. This was completed to identify the start and end point of every race stage and every technical and non-technical section. Maximum, mean, and accumulated bicycle load and mean and maximum velocity was then calculated for each race stage, technical and non-technical section.

GPS data (10Hz sample rate) was used to calculate overall run time (s) in agreement with event organiser's time and percentage time spent in velocity zones (5km.h^{-1} increments ;. 0-5, 5-10…45-50, 50+ (km.h^{-1})). Bicycle load used here has been previously defined (Kirkwood *et al.*, 2017) and is calculated using the formula available in the Catapult Sprint software manual (Catapult Innovations, 2013, page 80). Accelerometer data (100Hz sample rate) was used to calculate percentage time spent in bicycle load zones (1*g* increments; 0-1, 1-2…14-15, >15(*g*)), and accumulated bicycle load for each race stage and terrain type (technical and non-technical). Based on ISO

analysis guidelines (ISO, 2017) magnitude of whole body vibration was calculated, defined as root-mean-square of accelerations (r.m.s, ms^{-2}) acting on the bicycle seat post calculated from the resultant acceleration of all 3 axes.

4.2.4 Statistical analyses

All statistical analysis was performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). All data of physiological characteristics were presented as mean ± range for the elite male population (n=8). Data of the female athlete presented as an outlier (more than 2 times standard deviation (SD) from mean value) and is therefore presented as a case study. For ramp test data, power is presented as absolute (watts, W), relative (watts/kilogram body mass, W/kg) and relative total (watts/kilograms total cycling mass). Two-way repeated measures analysis of variance (ANOVA) was used to assess differences in stage type (stage or transition) and stage number for bicycle load and heart rate variables (stage type x stage number). Between terrain differences for velocity (terrain x velocity) and bicycle load (terrain x bicycle load) were assessed using two-way repeated measures ANOVA. Greenhouse Geisser correction was used to adjust the degrees of freedom if the assumption of homogeneity was violated. In the case of a significant interaction, Bonferroni corrected paired t-tests were utilised to determine terrain differences. All data were presented as mean ± standard deviation (SD) unless otherwise stated. Relationship between overall race time, vibration (r.m.s) and physiological characteristics were investigated using Pearson's correlation coefficient. As the physiological characteristics of the female athlete presented as an outlier, her data were removed from correlation analysis. Spearman's rank correlation was used to determine relationships between time spent in heart rate zones and overall finishing position within the population investigated. The magnitude of correlation coefficients was considered as trivial (r<0.1), small (0.1<R<0.3), moderate(0.3<R<0.5), large (0.5<R<0.7), very large (0.7<R<0.9), almost perfect (R>0.9) or perfect (R=1; Hopkins, 2002). Significance was accepted at the 95% confidence interval (p=0.05) throughout.

4.3 Results

4.3.1 Physiological characteristics

All participants completed the laboratory tests with complete datasets. Physiological characteristics of male (n=8) and female (n=1) elite enduro athletes from the 2017 season are presented in table 4.2. Mean coefficient of variation for all anthropometry measures was 1.5%.

Table 4.2: Physiological characteristics elite male (n=8) and female (n=1) enduro athletes. All data presented as mean and range.

Parameter (unit)	Mean (male, n=8)	Male Range	Female (n=1)
Relative VO_{2peak} ($ml.kg.min^{-1}$)	61.1	50.7 – 68.0	55.1
Relative total VO_{2peak} ($ml.total\ kg.min_{-1}$)	49.2	42.2 – 53.2	39.8
Absolute VO_{2peak} ($L.min^{-1}$)	4.8	4.5 – 5.4	3.1
Power VO_{2peak} (W)	410.9	390.0 – 440.0	290.0
Relative power VO_{2peak} (W/kg)	5.2	4.2 – 5.9	5.1
Relative total power VO_{2peak} (W/total kg)	4.2	3.5 – 4.7	3.7
Power FBLC $4mmol.L^{-1}$ (W)	318.4	292.0 – 350.0	175
Power FBLC $2mmol.L^{-1}$ (W)	283.6	256.0 – 330.0	205
Relative power FBLC $4mmol.L^{-1}$ (W/kg)	4.0	3.3 – 4.4	3.6
Relative power FBLC $2mmol.L^{-1}$ (W/kg)	3.6	2.9 – 3.9	3.1
Relative total power FBLC $4mmol.L^{-1}$(W/total kg)	3.2	2.7 – 3.5	2.6
Relative total power FBLC $2mmol.L^{-1}$(W/total kg)	2.9	2.4 – 3.2	2.2
HR FBLC $4mmol.L^{-1}$ ($\%HR_{max}$)	89.7	87.1 – 92.6	90.1
HR FBLC $2mmol.L^{-1}$ ($\%HR_{max}$)	83.5	80.0 – 87.4	82.2
Absolute RER ≥ 1 (W)	298.1	255.0 – 320.0	185.0
Relative RER ≥ 1 (W/kg)	3.8	2.4 – 4.3	3.3
Relative total RER ≥ 1 (W/total kg)	3.1	2.0 – 3.4	2.4
Anthropometry			
Body mass (kg)	79.9	69.2 – 105.9	56.6
Total mass (rider + bike; kg)	99.2	86.9 – 127.1	78.4
Skeletal muscle mass (kg)	48.1	40.2 – 66.7	31.3
Skeletal muscle mass (%)	60.0	56.6 – 63.0	55.3
Thigh girth 1cm distal gluteal fold (cm)	60.2	57.1 – 70.2	54.9
Sum of 6 skinfolds (mm)	53.3	36.8 – 91.5	70
Sum of 8 skinfolds (mm)	70.0	47.3 – 129.3	90.8
Grip strength left (kg)	50.3	35.5 – 60.0	30.0
Grip strength right (kg)	53.0	40.0 – 61.0	30.0

4.3.2 Physiological demands of race event

One participant failed to finish the race event due to a mechanical failure and was removed from subsequent analyses. Stage 1 was removed from analyses as the GPS accelerometer units failed to pick up signal for three participants. One participant was removed from stage 5 due to a crash but included for all other stages. Heart rate and bicycle load variables for each stage and transition section are displayed in table 4.3.

Table 4.3: Mean (n=8) heart rate and bicycle load variables between race stages (S) and transition stages (T) for all participants. All data mean ± SD. Two-way repeated measures ANOVA was used to assess differences in stage type (stage or transition) and stage number for bicycle load and heart rate variables (stage type*stage number). # denotes significant difference between stage and transition values in the presence of a significant interaction (stage type*stage number).

Stage	Peak HR (%HR_{max})	Mean HR (%HR_{max}) #	Time <HR_{FBLC2} (%) #	Time HR_{FBLC2}-HR_{FBLC4} (%)	Time >HR_{FBLC4} (%)	Time below $HR_{RER \geq 1}$ (%)	Peak bicycle load (*g*)	Mean bicycle load (*g*)	Accumulated bicycle load (A.U.)	Whole body vibration (r.m.s, ms^{-2})
T1	84.8 ± 5.8	66.9 ± 6.0	99.6 ± 0.7	0.4 ± 0.7	0.0±0.0	99.3 ± 1.6	-	-	-	-
S1	98.9 ± 4.2	90.4 ± 2.9#	15.8 ± 6.0	21.1 ± 24.1	63.1 ± 29.9	30.4 ± 17.1#	14.6 ± 1.8	6.4 ± 0.9#	175.0 ± 26.5	35.1 ± 3.7#
T2	90.6 ± 5.8	71.0 ± 6.1	92.1 ± 8.4	7.1 ± 8.4	0.9 ± 0.6	98.4 ± 1.3	7.8 ± 3.8	1.1 ± 2.0	305.1 ± 35.2	10.4 ± 0.1
S2	96.8 ± 2.5	92.8 ± 2.1#	4.7 ± 1.7	12.7 ± 21.4	82.6 ± 22.9	9.6 ± 7.4#	13.3 ± 1.7	5.6 ± 0.6#	371.5 ± 24.7	32.6 ± 2.7#
T3	92.0 ± 5.9	75.1 ± 5.5	84.1 ± 16.0	14.8 ± 15.5	1.0 ± 0.7	94.4 ± 9.3	9.3 ± 0.9	0.4 ± 0.0	444.4 ± 34.5	10.4 ± 0.1
S3	98.2 ± 2.5	94.0 ± 2.0#	3.2 ± 1.3	8.4 ± 15.4	88.4 ± 16.2	6.3 ± 3.2#	14.4 ± 1.6	4.8 ± 0.8#	735.6 ± 64.2	28.4 ± 2.4#
T4	91.2 ± 5.2	76.8 ± 4.5	76.6 ± 20.9	19.4 ± 19.8	4.1 ± 7.5	88.4 ± 13.5	7.6 ± 3.0	0.5 ± 0.1	477.6 ± 37.3	10.5 ± 0.2
S4	94.1 ± 1.8	89.5 ± 2.6#	11.8 ± 4.4	24.9 ± 27.7	63.3 ± 31.1	28.7 ± 28.1#	14.1 ± 1.8	6.3 ± 0.6#	256.0 ± 19.3	34.2 ± 2.7#
T5	89.9 ± 7.8	73.9 ± 6.5	85.7 ± 15.2	7.8 ± 10.3	6.5 ± 9.6	91.4 ± 13.2	8.7 ± 0.9	0.8 ± 0.1	262.1 ± 18.5	11.3 ± 0.2
S5	97.1 ± 2.2	93.0 ± 2.1#	5.0 ± 2.2	8.3 ± 10.0	86.7 ± 10.6	11.4 ± 12.1#	13.8 ± 1.6	4.3 ± 0.5#	377.4 ± 47.1	26.2 ± 2.4#

There was a significant interaction between stage type and mean HR (F(4, 20) = 31.49, p<0.001). Analysis of simple main effects revealed significantly higher mean HR during all race stages compared to transition stages (p <0.05). Mean and individual data for transition and race stages are presented in figure 4.2 below.

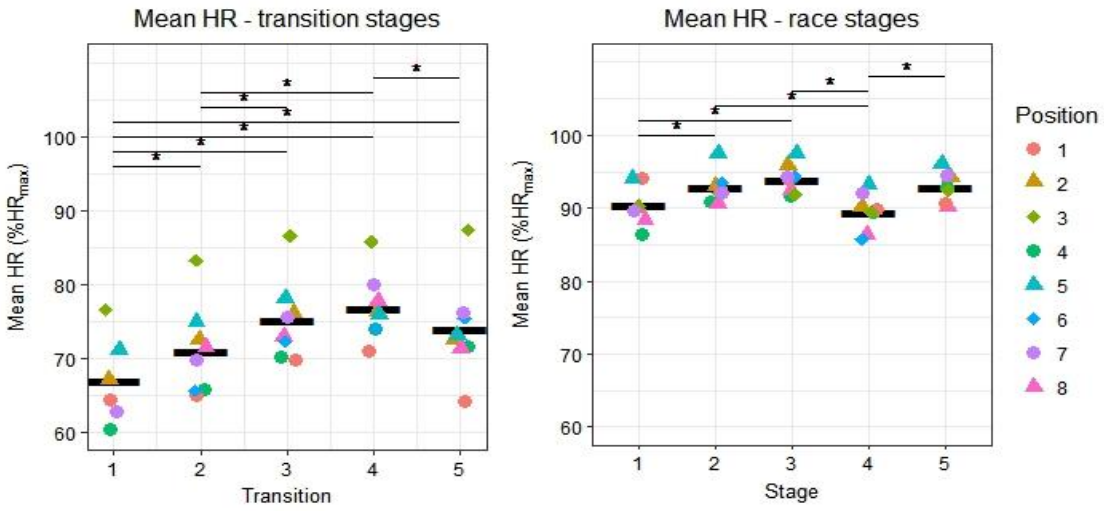

Figure 4.2**:** Mean (n=8) and individual HR (%HR_{max}) for transition and race stages. Black line represents group mean per section. Two-way repeated measures ANOVA was used to assess differences in stage type (stage or transition) and stage number for mean heart rate (stage type*stage number). *denotes significant difference between stage number (p <0.05). A significant effect of stage type was observed as detailed in table 4.3 above.

There was a significant interaction between stage type and percentage time spent <HR_{FBLC2} (F(4, 20) = 3.52, p = 0.025). Analysis of simple main effects revealed significantly more time spent <HR_{FBLC2} during all transition stages compared to race stages (p <0.05). A significant interaction was observed between stage type and percentage time spent <$HR_{RER \geq 1}$ (F(4, 20) = 4.55, p = 0.009). Analysis of simple main effects revealed significantly more time spent <$HR_{RER \geq 1}$ during all transition stages compared to race stages (p <0.05). No significant interaction was discovered between stage type and time spent HR_{FBLC2} – HR_{FBLC4} or time spent >HR_{FBLC4}. Mean and individual data for time spent in HR zones during transition stages are presented in figure 4.3 below.

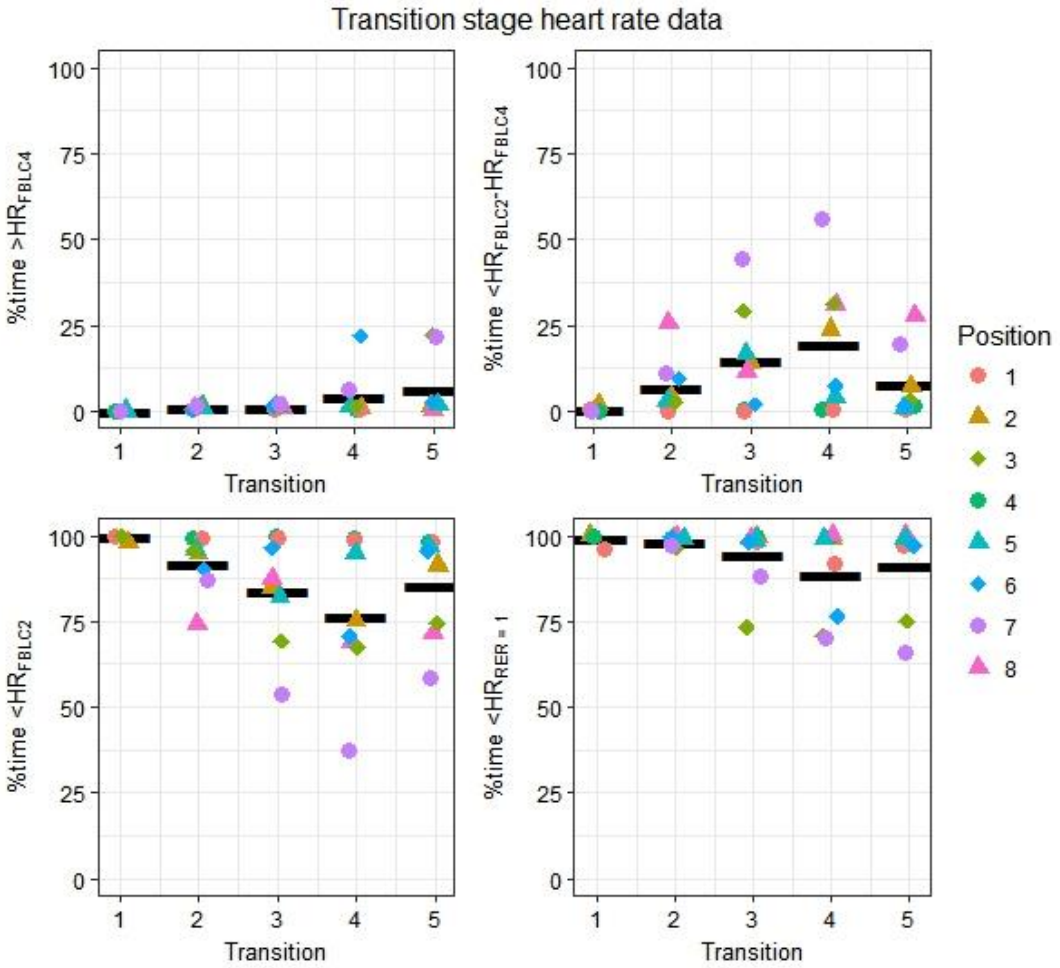

Figure 4.3**:** Individual data of time spent below HR corresponding to fixed blood lactate concentrations (FBLC) of 2mmol.L^{-1}, 2-4mmol.L^{-1}, >4mmol.L^{-1} and power at respiratory exchange ratio (RER) = 1 assessed from laboratory test. Black bar represents group mean (n=8) for each section.

There was a significant interaction between stage type and mean bicycle load (F(3, 18) = 55.347, p<0.001) and stage type and vibration (F(3,18) = 267.23, p<0.001). Analysis of simple main effects showed both mean load and vibration to be significantly higher for all race stages compared to transition stages. No significant interaction was observed between stage type and peak bicycle load or stage type and accumulated bicycle load.

4.3.3 Performance correlates

Several physiological characteristics displayed significant correlations with race time, details of which are provided in figure 4.4. The strongest correlations (R>0.9; almost perfect effect) were observed between race time and absolute power at RER ≥ 1, FBLC2 and VO_{2peak}.

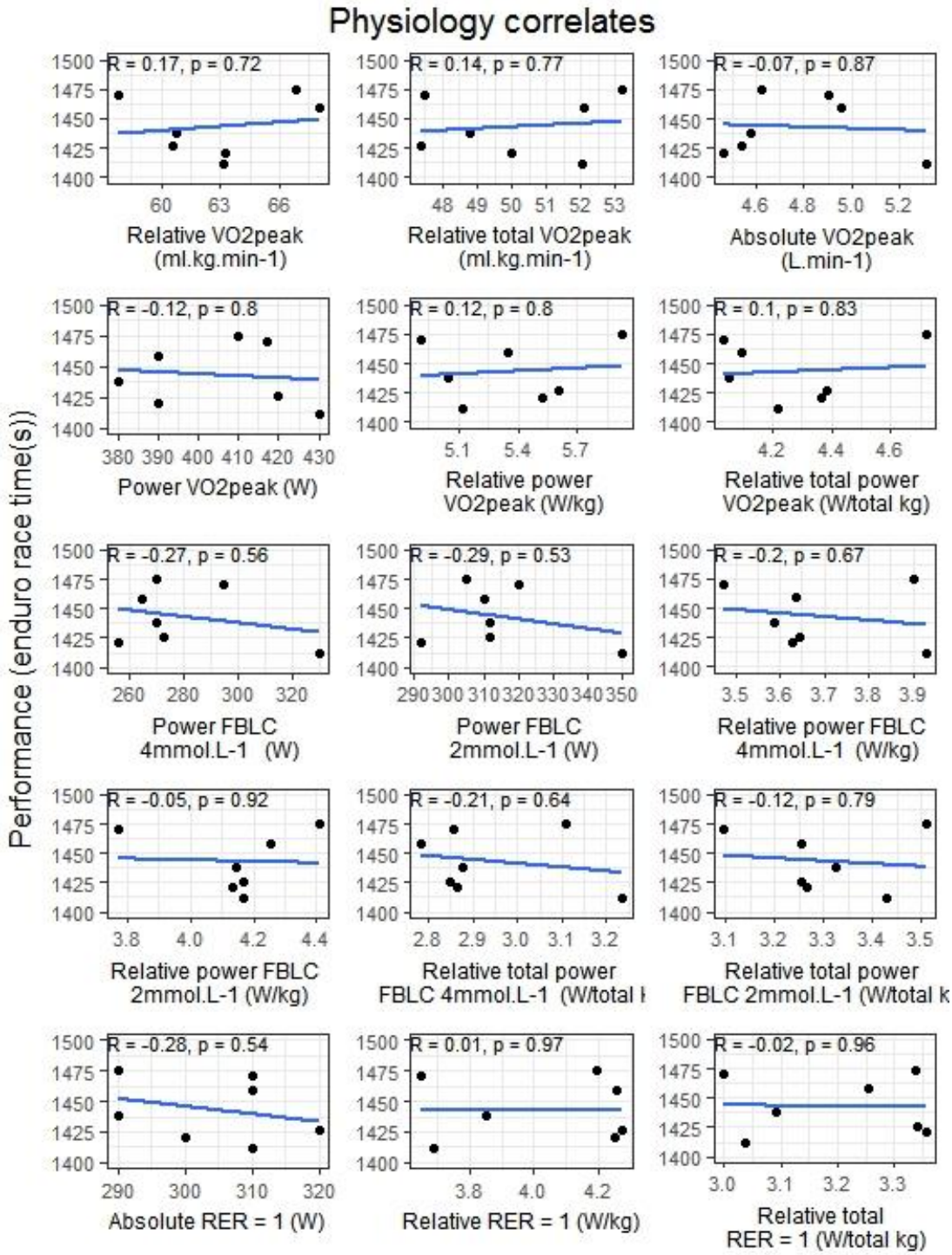

Figure 4.4: Pearson bivariate correlations between performance (enduro race time (s)) and physiological parameters assessed in the laboratory.

Significant correlations were also found between several anthropometry variables and overall race performance, as detailed in figure 4.5. Very large effects (R>0.7) were seen between race performance and body mass, total mass and right hand grip strength.

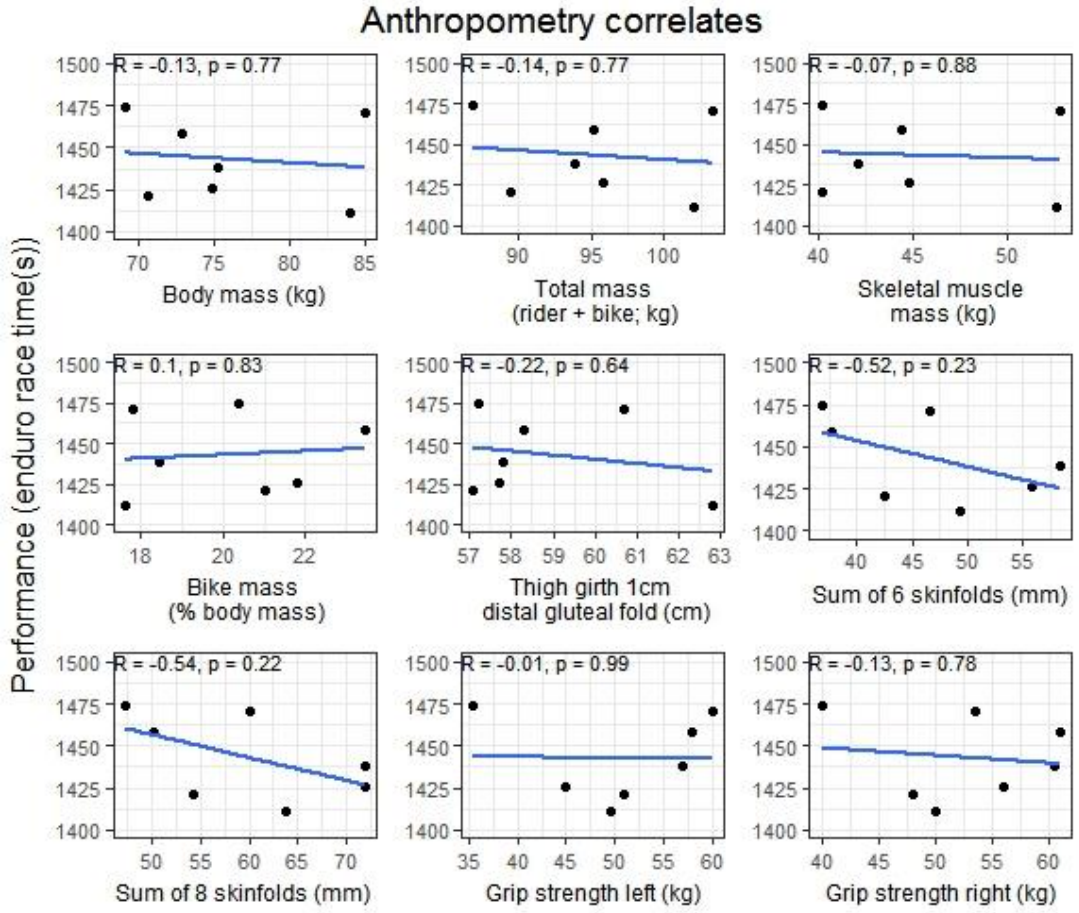

Figure 4.5: Pearson bivariate correlations between performance (enduro race time (s)) and anthropometry parameters assessed in the laboratory.

Spearman's rank correlations revealed a nearly significant relationship between overall within-group finishing position and time spent $<HR_{FBLC2}$ and HR_{FBLC2} - HR_{FBLC4} during transition sections, as shown in figure 4.6 below.

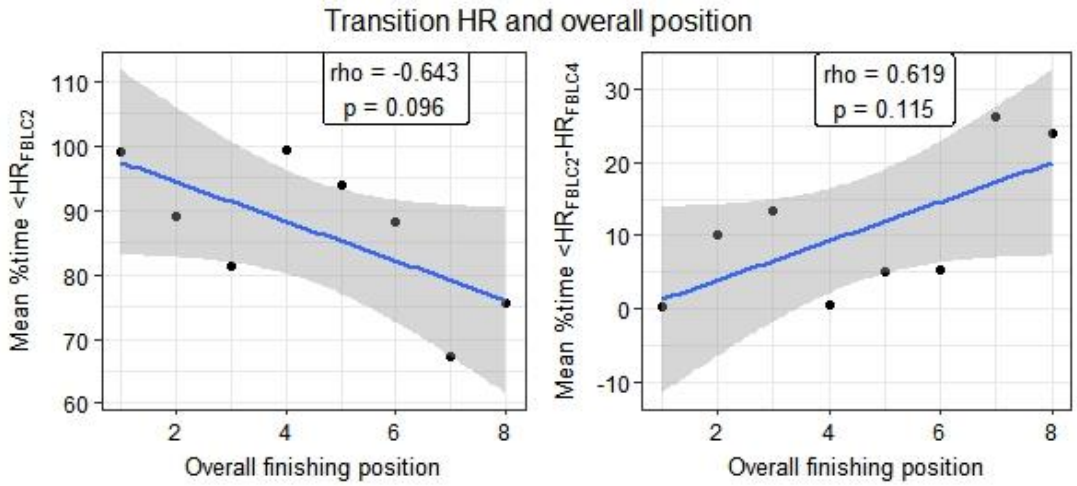

Figure 4.6**:** Spearman's rank correlation between overall finishing positon and mean percentage time spent $<HR_{FBLC2}$ (left) and $HR_{FBLC2} - HR_{FBLC4}$ (right).

4.3.4 Loading and vibration

Terrain based differences in bicycle load and velocity are presented in figure 4.7 and 4.8 respectively. There was a significant interaction between terrain type and percentage time in player load bands ($F(1.55, 10.87) = 44.97$, $p = 0.000$). Analysis of simple main effects revealed significant differences between terrain type and time spent in every bicycle load band except >15g ($p < 0.05$). Significantly more time was spent in bicycle load zones <4*g* during non-technical terrain compared to technical terrain, with most time spent in bicycle load zone 2-3*g*. Conversely, significantly more time was spent in bicycle load zones over 4*g* during technical terrain compared to non-technical terrain, with most time spent in bicycle load zone 5-6*g*.

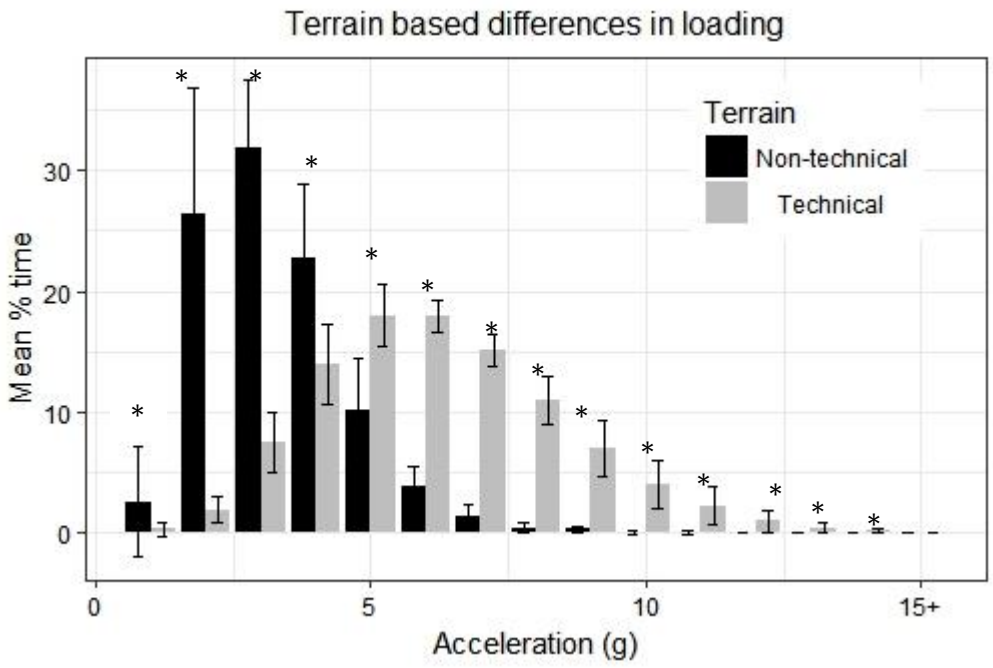

Figure 4.7: Percentage time spent in bicycle load bands during technical and non-technical terrains of enduro race stages (mean ± SD). Between terrain differences for velocity (terrain x velocity) were assessed using two-way repeated measures ANOVA. *denotes significant difference (p<0.05, Bonferroni corrected) between terrain type.

There was also a significant interaction between terrain type and percentage time in velocity bands (F(1.55, 10.83) = 6.88, p = 0.016). Analysis of simple main effects revealed significant differences between terrain type and percentage time in velocity bands 15-20, 25-30, 30-35, 40-45 and 45-50 km.h^{-1} (p<0.05). Piloting across technical terrain, the largest proportion of time was spent at velocity of 10-15 km.h^{-1} while non-technical terrain saw most time spent between 15-20 km.h^{-1}.

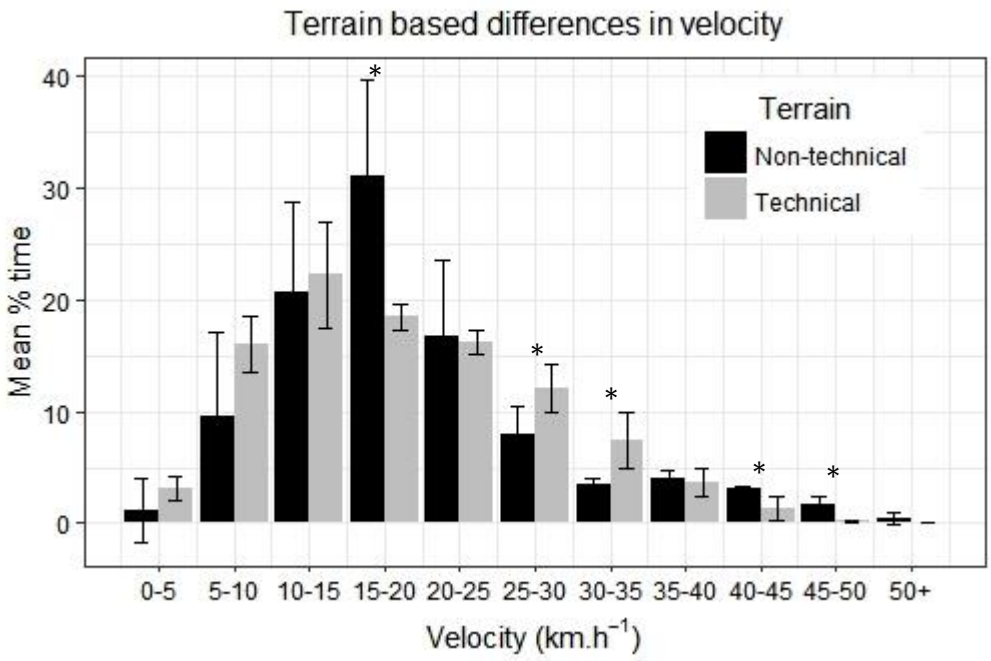

Figure 4.8**:** Percentage time spent in velocity bands during technical and non-technical sections of enduro race stages (mean ± SD). Between terrain differences for velocity (terrain x bicycle load) were assessed using two-way repeated measures ANOVA. *denotes significant difference (p<0.05, Bonferroni corrected) between terrain type.

No significant correlation (R= -0.51) was found between whole body vibration (r.m.s, ms^{-2}) and overall finishing time as shown in figure 4.9.

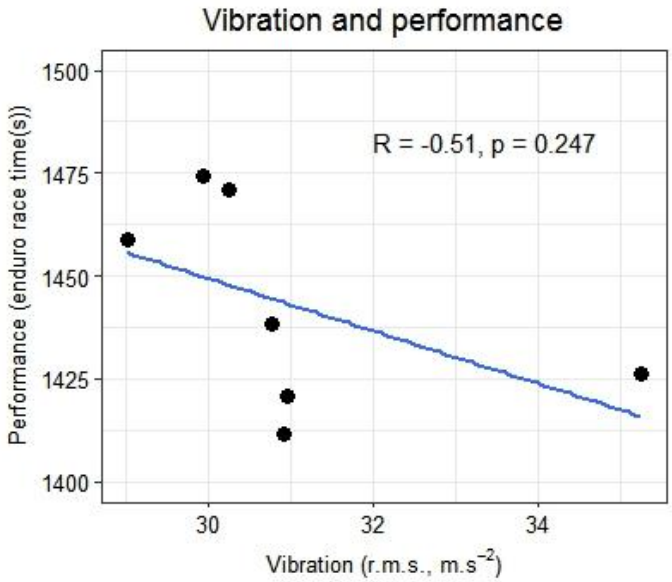

Figure 4.9**:** Pearson's bivariate correlation between mean whole body vibration (RMS) and overall finishing time.

4.4 Discussion

This study aimed to assess the physiological characteristics of elite enduro riders, quantify the demands of an international enduro race (transition and race stages) and finally, investigate relationships between physiological characteristics and race performance. The main findings of this study showed that elite enduro riders exhibit a substantial aerobic capacity combined with the ability to produce considerable power at submaximal intensities alongside large grip strength values. Transition stages placed considerable physiological demands on athletes which subsequently influenced race stage performance. Terrain induced accelerations were greater during technical sections than non-technical sections showing a significant physiological workload associated with navigating technical terrain. Lastly, no significant correlations were observed between race performance, physiological characteristics or whole body vibration.

Relative $VO_{2peak,}$ power at $VO_{2peak,}$ and relative power at FBLC2 and FBLC4 values of elite enduro riders remain comparable to national level elite XCO athletes (Impellizzeri, Rampinini, *et al.*, 2005; Hays *et al.*, 2018; Novak *et al.*, 2018), highlighting the requirement for a large aerobic capacity in elite enduro riders. The importance of this aerobic capacity was previously attributed to facilitating recovery via increased PCr resynthesis between bouts of anaerobic power output in other cycling disciplines (Novak and Dascombe, 2014). However, findings presented here suggest a large aerobic capacity is also essential in recovery between stages in order to maximise time below HR_{FBLC2} during transition sections. Significantly lower mean HR and mean time spent below HR_{FBLC2} demonstrate transition stages are of lower intensity than race stages. However, analysis of individual data revealed large variations in workload intensity during the transition stages particularly as the race progressed. For example, the race winner was able to complete all of the transition stages almost entirely $<HR_{FBLC2}$ whilst the lowest position male rider (seventh overall) spent >40% time $>HR_{FBLC2}$ during the final 3 transition stages. This assertion is corroborated by an almost significant (p=0.09) correlation between overall within-group finishing position and mean time spent $<HR_{FBLC2}$ during transition stages, whereby faster riders spent less time

>HR_{FBLC2} during transition sections. These findings suggest that transition stages can potentially place greater accumulated demands on enduro athletes with insufficient submaximal workload capacity, potentially reducing subsequent race stage performance. This further reinforces the importance of power at FBLC2 and FBLC4 in enduro athletes being comparable to that of XCO athletes for whom ascending is a key component of successful performance (Abbiss *et al.*, 2013). Previous research (Kirkwood *et al.*, 2017) showed that elite riders presented a larger power output at workload equivalent to RER ≥ 1, an observation now supported by the current dataset showing absolute and relative total power at RER ≥ 1 to correlate significantly with overall race performance. The current findings show a majority of the race stage time (>70%) is completed above $HR_{RER \geq 1}$ whilst almost all (>88%) of the transition stages are completed <$HR_{RER \geq 1}$. Carbohydrate is the only fuel source utilised at intensities beyond RER ≥ 1, therefore increased power output at RER ≥ 1 facilitates greater time spent at intensity below RER ≥ 1 during transition stages. This preserves the limited resources of carbohydrate stores within the body for utilisation during the high intensity race stages, thus benefiting performance (Venables, Achten and Jeukendrup, 2005).

Race stage intensity reported in this study is very high (~90-95% HR_{max}), similar to previous reported values in international enduro racing (Hassenfratz, Ravier and Grappe, 2012; Kirkwood *et al.*, 2017). Athletes in the current study spent a majority of race stage time (>63%) above $HR_{FBLC4,}$ concurring with previous findings of elevated blood lactate values (~15mmol.L^{-1}) immediately following race stages (Hassenfratz, Ravier and Grappe, 2012). This data would suggest that physiological characteristics such as blood lactate response to increasing workload may correlate with race performance, however no significant relationship was observed between any physiological characteristics and race time. These findings are in agreement with previous research showing very limited physiological predictors of performance in a cohort of elite male XCO athletes (ref impel). The difference in performance between the highest ranked male rider (race time = 1411s) and lowest ranked male rider (race time = 1474s) is only 4.5%, therefore it is not surprising that no significant correlation between physiological characteristics and race time

is evidenced in this study. Instead, the current dataset suggests that the physiological characteristics reported in the current study are a prerequisite for elite enduro performance. This has been suggested previously (Kirkwood *et al.*, 2017) and but does not mean that individual improvements in physiological characteristics will not improve enduro performance. This is particularly pertinent for female athletes that will likely display a lower absolute VO2peak than male counterparts (Tanaka et al., 1993). Accordingly, the female participant of the current study produces less absolute power at VO2peak, FBLC2, FBLC4 and RER ≥ 1, less relative power at VO2peak, and less relative total power at FBLC2 and FBLC4 than the lowest respective male value. It is therefore likely that the demands of the race are greater for the female athlete than the male athletes but as there was only one female athlete included in the current study cohort, much more work is required in this population to make firm these conclusions. Several additional factors such as rider skill, technique, psychology and equipment may offer further explanation of performance in elite enduro mountain bike racing, however analysis of these factors was beyond the scope of the current study.

As the race stages are predominantly downhill, and thus the influence of gravity reduced, the ability to produce greater absolute power may contribute to race performance as it has been shown to in downhill racing (Hadden and Florida-James, 2011). Conversely, the current study did not show any correlation between absolute power, body mass, or estimated muscle mass and race performance, further suggesting enduro requires a different physiological repertoire than DH. Additionally, the method used to estimate skeletal muscle mass from skin fold data used in this study is less accurate than dual-energy X-ray absorption methods, thus offering a direction for future research aiming to explore this relationship further (Stewart and James Hannan, 2000).

Differences in time spent in bicycle load bands presented here are similar to previous findings where riders spend more time in higher bicycle load bands during technical terrain compared to non-technical terrain (Hurst *et al.*, 2012; Kirkwood *et al.*, 2017). As these accelerations must be reduced to similar

levels before reaching the torso in order to maintain control of the bicycle and protect the central nervous system, it is proposed that greater non-propulsive workload is associated with navigating technical terrain compared to non-technical terrain (Hurst *et al.*, 2012, 2013). Interestingly, the stages featuring the largest vibration values (1, 2 and 4) feature only technical terrain yet have the lowest mean HR values, whilst stages featuring non-technical terrain (stages 3 and 5) present the lowest vibration values and the highest HR values. This supports previous findings showing the addition of vibration to cycling tasks increases oxygen uptake but not HR in laboratory settings, however it was not feasible to burden the athletes with measurement of oxygen uptake in the current study (Samuelson, Jorfeldt and Ahlborg, 1989b; Sperlich *et al.*, 2009; Rønnestad *et al.*, 2018). It is therefore suggested that HR does not provide a sufficient measure of overall workload in the presence of vibration, warranting further investigation between vibration exposure, oxygen uptake and HR in a field setting. Furthermore, as all current models to assess training load utilise HR or power as the sole determinant of workload, training load is likely to be underestimated in the presence of vibration exposure such as that reported in the current study. The levels of whole body vibration reported here (r.m.s.; ms^2) are similar to equivalent values reported during road riding which exposed the athlete to harmful levels of vibration when assessed in accordance with ISO guidelines for whole body vibration (ISO, 2017). Unfortunately it is not possible to calculate the equivalent ISO values with the equipment used in the current study due to insufficient recording rate (100Hz; >160Hz required) as suitable equipment was not available for use in the field at the time of data collection (ISO, 2017). It is therefore recommended that further research focuses on detailing vibration exposure in mountain biking, particularly with reference to hand arm vibration exposure.

In summary, this study shows that elite enduro athletes present a large submaximal workload capacity combined with large maximal aerobic capacity in both absolute and relative terms. These physiological characteristics facilitate the preservation of carbohydrate stores and reduced reliance on glycolysis during transition stages, in turn improving race performance. No

significant relationship was reported between any physiological parameter and race performance, though the demands of the race were higher for the female athlete due to a clear physiological disadvantage. Potentially harmful levels of whole body vibration exposure were reported during the race event, however more work is required to fully detail vibration exposure in mountain bike enduro racing. In conclusion, successful enduro performance requires suitable conditioning to withstand substantial vibration exposure whilst retaining a large maximal aerobic capacity supported by a large submaximal workload capacity expressed relative to total cycling mass.

Chapter 5: Training characteristics and cardiac autonomic nervous system response in the international enduro athletes

5.1 Introduction

The training characteristics of elite athletes in endurance sports such as cross country skiing, triathlon and marathon running have been reported extensively in the past (Billat *et al.*, 2001; Neal, Hunter and Galloway, 2011; Mujika, 2014; Solli, Tønnessen and Sandbakk, 2017). Such characteristics typically show large volumes of training specific to the physiological demands of the event with additional focus placed on skill development, for example in sports such as Nordic combined and biathlon (Tnønessen *et al.*, 2014; Tønnessen *et al.*, 2016). Detailing training characteristics of elite athletes provides useful information for aspiring coaches and athletes aiming to improve sporting performance. As the physiological requirements of enduro mountain biking are unique and have only recently been assessed, it is not currently known how elite athletes train to develop the physiological characteristics necessary to meet the demands of the events.

In Chapter 4 section 4.3.1, physiological prerequisites for elite enduro performance were described. Individual improvements in these physiological characteristics may therefore offer an increase in performance, however, little detail is available in the literature regarding appropriate training stimulus for elite off-road cyclists and none in enduro mountain biking. A dose-response relationship between training impulse (TRIMP) and improvements in performance characteristics of elite road cycling athletes offers a promising means of prescribing appropriate training stimulus to elicit important physiological changes in elite riders (Sanders *et al.*, 2017). TRIMP models have also shown a superior ability to predict changes in performance characteristics compared to duration of sessions or session RPE (session duration x rating of perceived exertion; Foster *et al.*, 1996; Sanders *et al.*, 2016). The upper limit of training stimulus where excessive training stimulus combined with insufficient recovery leads to stagnation or reduction in performance measures has however not been identified (Meeusen *et al.*, 2013). Furthermore, mountain biking is associated with greater vibration exposure to the rider, which may elicit greater training stimulus that is not reflected in heart rate measures (Rønnestad *et al.*, 2018). More work is required to investigate the suitability of currently available TRIMP models in

assessment of training load and dose-response relationships with performance in enduro mountain bike applications. Enduro racing also features a large component of technically challenging downhill terrain, which must be navigated quickly for successful performance (Hassenfratz, Ravier and Grappe, 2012). This requires development of specific skills associated with descending such as cornering (Lopes and McCormack, 2017), energy conservation (Miller *et al.*, 2016) and braking (Miller *et al.*, 2018), in addition to important physical adaptations such as increased grip strength (Burr *et al.*, 2012). There is a lack of information available regarding optimal training volume, intensity, or specificity in elite enduro mountain bike athletes.

As discussed in chapter 2 (section 2.2), the main aim of any training programme is to prescribe sufficient stimulus to promote physiological adaptation while allowing sufficient rest to prevent excessive fatigue resulting in prolonged reductions in performance. Measures of resting heart rate (RHR; bpm), natural logarithm of the square root of the mean of the sum of the squares of differences between adjacent normal R–R intervals (LnRMSSD; ms) and LnRMSSD to R-R interval ratio (LnRMSSD:RR) have been previously used in the literature to monitor the adaptation of endurance athletes in response to increased weekly training load (Plews, Laursen, Stanley, *et al.*, 2013; Plews, Laursen and Buchheit, 2016). Changes in resting heart rate measures have been observed during both positive and negative adaptations to training load (Manzi, Castagna, *et al.*, 2009; Buchheit, 2014; Flatt *et al.*, 2017). Data from previous studies has however often been collected in a controlled setting such as a team camp or laboratory, training load standardised for all participants and heart rate recordings lasting between 5 minutes and 8 hours (nocturnal) (Manzi, Castagna, *et al.*, 2009; Buchheit *et al.*, 2010). In contrast, many athletes and coaches in the field rely on ultra-short term recordings (55 seconds) with large variation in training load, frequent travel and little to no control for environmental factors known to inform daily training programming decisions. It is, therefore, beneficial to understand if resting heart rate measures reflect changes in training load and subsequent adaptation in free running field settings. Resting heart rate measures have also been shown to correlate with maximal aerobic running speed in

recreational runners, though no comparable data is available in an elite cycling population (Buchheit *et al.*, 2010). This relationship is proposed to offer a simple means to track changes in peak aerobic power without the cost and time constraints of continual laboratory testing, though more research is required in cycling populations.

In summary, there is a lack of information regarding the training characteristics employed by elite enduro athletes to meet the physiological demands of the race events. The application of TRIMP models to monitor training load or predict physiological changes within enduro mountain bike athletes is not known, nor is the use of resting heart rate measures to monitor elite athletes' response to training load in a free running field setting. Therefore, the aims of this chapter are

1) To determine the training characteristics of elite mountain bike athletes throughout training and competition.
2) To assess how these training characteristics relate to the demands of enduro and to identify the training load measure that shows the strongest relationship with changes in key physiological characteristics defined in Chapter 4.
3) To assess resting cardiac autonomic system parameters in relation to performance, changes in training load, and life stress in a free running field setting.

5.2 Methodology

5.2.1 Participants

Eight male (*n*=8, age= 25 ± 3 years, height=181 ± 6cm, mass=80 ± 11kg) and one female (*n*=1, age = 23, height = 160cm, mass = 56.6kg) elite mountain bike athletes agreed to participate in this study. All participants had previously finished in the top 85 positions at a EWS race, five of whom had previously finished in the top 10 at a EWS race as shown in table 5.1 below. Ethical approval for the study was granted from the ethics committee of Edinburgh Napier University. Subsequently, both oral and written consent was obtained from all participants. Participants were free from any illness for two weeks prior to each laboratory visit, were not using medication, and were required to refrain from consuming alcohol or caffeine in the 24h preceding the laboratory visit and race event.

Table 5.1: details of participant characteristics.

Athlete	Gender	Age	Best EWS result (2017)	Years of training history
1	Male	23	3rd	10
2	Male	30	46th	11
3	Female	23	2nd	12
4	Male	25	1st	8
5	Male	27	26th	17
6	Male	23	12th	11
7	Male	23	102nd	4
8	Male	22	61st	10
9	Male	23	47th	6
Mean ±SD	-	24 ± 2	30 ± 35	10 ± 4

5.2.2 Laboratory testing protocols

Participants attended Edinburgh Napier University laboratory on three occasions to complete an anthropometric assessment (chapter 3 section 3.3.1) and an incremental exercise protocol (chapter 3 section 3.3.2) on each occasion. Each visit aligned with the start of winter training (baseline), the mid-point in the race season (mid-season) and after the final race of the season (off-season). The timing of each visit was negotiated with each athlete and their respective coach depending on wellness, training status and planned race events meaning that the actual date of each visit varied between participants. However, a majority of baseline and post-season tests were completed with a 3 week window and mid-season tests were completed between June and September depending on race season plans. The time between baseline and mid-season is attributed to 'Period 1' while time between mid-season and off-season is attributed to 'Period 2'. Details of time between each laboratory visit for each participant are provided in table 5.2 (section 5.2.3) below.

5.2.3 Training data collection and analysis

Daily training data (heart rate, location, velocity and elevation) was collected remotely for the entire duration of the study using the participant's personal cycling computers (Garmin Edge 500, Garmin, USA or Polar V650, Polar, Finland). The relevant GPS files (.tcx format) were input to Golden Cheetah Version 3.4 for further analysis. In addition to duration, distance and elevation, custom scripts were developed and written by the author within the Golden Cheetah software to allow calculation of all TRIMP measures (iTRIMP, LuTRMP, bTRIMP and EdTRIMP; as described in section 2.2.2.) for each individual participant. Based on data from other endurance training studies (Tønnessen *et al.*, 2015; Solli, Tønnessen and Sandbakk, 2017), training time was divided into phases based on competitive season. General preparation (GP) lasted from the first laboratory visit until two weeks prior to the first race. Pre-competition (PC) included the two weeks prior to the first major race of the season, as confirmed by discussion with the athlete. The competition season (C) spanned from the first major race event to the last, and the period between

the final race of the season and the final laboratory test was labelled the regeneration phase (RP). Data was also extracted for each race for each participant during competition phase and presented as mean values per day for the same variables. Details of days included in each phase for each athlete are presented in table 5.2 below. Training data from each training phase is presented as mean per week unless stated otherwise.

Table 5.2: Details of duration (days) between tests and in each phase of training for each athlete with mean (SD).

Athlete	Days between tests			Days in phase			
	Period 1	Period 2	Total	GP	PC	CT	RP
1	191	132	323	106	14	188	17
2	244	86	330	142	14	141	35
3	235	57	292	53	14	188	39
4	173	141	314	91	14	188	23
5	186	122	308	87	14	188	21
6	-	-	344	90	14	188	54
7	265	69	334	70	14	203	49
8	161	98	259	75	14	140	32
9	192	60	252	72	14	141	27
Mean	205.9	95.6	306.2	87.3	14.0	173.9	33.0
(SD)	(37.2)	(33.0)	(32.6)	(25.6)	(0.0)	(25.4)	(12.6)

Note: Athlete 6 did not perform a mid-season test due to illness and racing commitments.

To calculate the ability of training load models to predict changes in physiological parameters at each laboratory test, mean training load values per week were calculated between laboratory tests for each training load metric and compared to relative change (%Δ) in physiological characteristics (Sanders *et al.*, 2017). As a result, mean training load and relative change in performance is calculated for period 1 and period 2 for each athlete and both measures included in overall correlation analysis. To assess the difference in training load dependant on training phase, mean weekly load was calculated for each training phase. Time spent mountain biking (MTB), road cycling (road) or doing other training activities (other) is also presented as percentage time

per week, as group mean for each training phase and on an individual basis for the overall duration of the study. Strength training data for every session was requested from the athletes in any format available.

5.2.4 Resting heart rate measures

Participants were instructed to complete a resting heart rate recording lasting 55 seconds to provide RHR (bpm) and LnRMSSD (ms) every morning upon waking (Plews *et al.*, 2014; Bellenger *et al.*, 2016). All participants completed the recording in the supine position with the exception of athletes 3 and 4 who completed the readings in the seated position due to a previously established routine developed by their coach. These participants were included for individual analysis between training load and resting heart rate measures but removed from group analysis of relationship between resting heart rate measures and peak aerobic power. Participants completed the heart rate recording using a Bluetooth 4.0 heart rate monitor strap (Polar H7, Polar, Finland) and a smartphone application (Ithlete™, HRV Fit, England). This method has previously been shown to be accurate and reliable means of assessing resting heart rate and LnRMSSD when compared to the gold standard measurement of 5-lead electrocardiogram (Flatt and Esco, 2013; Esco, Flatt and Nakamura, 2016; Giles, Draper and Neil, 2016). The readings were uploaded to a secure online cloud system and subsequently exported to .csv file for further analysis in R (version 3.4.2, The R Foundation for Statistical Computing, 2017) where LnRMSSD:RR (ms) was also calculated. All resting heart rate data was offset by one day, meaning the heart rate reading followed the training load or other events of the day prior. All heart rate measures were then analysed using week mean values due to large variance reported in standalone daily measures (Plews, Laursen, Kilding, *et al.*, 2013). If a week contained less than three readings the week was removed from further analysis (Plews *et al.*, 2014; Bellenger *et al.*, 2016).

5.2.5 Relationship between heart rate measures and training load

To assess the relationship between resting heart rate measures and training load, percentage of mean load for total duration of study and percentage change to previous week was calculated for each training load measure (iTRIMP, bTRIMP, LuTRIMP, EdTRIMP, duration, distance) for each training week for each participant. Weekly values for each of the training load measures were then compared to the corresponding mean weekly value and percentage change in mean weekly value for all resting heart rate measures (LnRMSSD, RHR, LnRMSSD:RR). Analysis of relationships between changes in training load and heart rate measures was all performed on an individual athlete basis. To investigate the relationship between resting heart rate measures and peak aerobic power, mean resting heart rate measures from the week following baseline and mid-season tests were calculated in between tests for analysis of relationships with peak aerobic power (relative power VO_{2peak} (W/kg)). Relative change (%Δ) in mean weekly resting heart rate measures was also calculated to investigate relationship with relative changes (%Δ) in peak aerobic power.

5.2.6 Performance measures

To provide a measure of field performance, participants were ranked in the order of best international result within the current study population (i.e. 9 athletes). This method is designed to account for the true ability of the athlete and remove the influence of mechanical issues, crashes or non-completion of overseas events associated with use of overall world ranking. The female rider is included in this analysis ranked by overall time at her best performance in comparison to the male participants at the same race event (female athletes complete the same course as their male counterparts in elite mountain bike enduro races and thus permits this comparison).

5.2.7 Statistical analysis

All data is presented as group mean ± SD unless otherwise stated. Total training volume is expressed in hours per year calculated using the following equation: (total hours training completed/ total days participated in study) x 365. A repeated measures one way analysis of variance (ANOVA) was used to determine group mean differences between physiological characteristics at each laboratory test (physiological characteristic * test) and training characteristics during each training phase (training characteristic * phase). In the occurrence of a significant interaction (test or phase), Bonferroni corrected post-hoc tests were used to identify phase or test differences. Spearman's rank correlations are used to investigate relationships between overall study rank and training characteristics. Pearson's bivariate correlation was used to investigate relationship between mean weekly training load (distance, duration, iTRIMP, LuTRIMP, bTRIMP, EdTRIMP) variables and percentage changes (%Δ) in physiological characteristics determined to be most crucial for enduro performance (VO_{2peak}, power at FBLC2, FBLC4, VO_{2peak} and RER ≥ 1) as defined in chapter 4. Pearson's bivariate correlation was also employed to explore relationship between training load variables and changes in weekly average of resting heart rate measures. The magnitude of correlation coefficients was considered as trivial (r<0.1), small (0.1<r<0.3), moderate (0.3<r<0.5), large (0.5<r0.7), very large (0.7<r<0.9), almost perfect (r>0.9) or perfect (r=1; Hopkins, 2002). Significance was set at the 95% confidence interval (p=0.05) throughout. All Pearson's correlations are provided with 95% confidence intervals (95%CI) unless otherwise stated.

5.3 Results

Athlete 8 was removed from all analysis as insufficient resting heart rate data and training data was reported (<10% adherence). Additionally, athlete 6 was removed from resting HR analyses as no recordings were completed by the participant. Athlete 6 is also removed from analysis of changes in performance as no mid-season test was performed due to training and racing schedule, however this athlete is still included in analysis of training characteristics. No strength training data was returned by any athlete.

5.3.1 Group mean difference between laboratory tests

No significant differences were evident in group mean physiology or anthropometric variables between laboratory visits, which is shown in table 5.3 below.

Table 5.3: Group mean ± SD physiological characteristics from laboratory tests.

Physiological characteristic	Baseline (n = 9)	Mid-season (n = 8)	Off season (n = 9)
Power FBLC2 (W)	259.1 ± 39.7	268.0 ± 48.4	266.0 ± 53.2
Power FBLC4 (W)	296.8 ± 41.1	302.9 ± 48.0	302.1 ± 55.7
Relative power FBLC2 (W/kg)	3.4 ± 0.3	3.5 ± 0.5	3.5 ± 0.4
Relative power FBLC4 (W/kg)	4.0 ± 0.4	4.0 ± 0.4	3.9 ± 0.5
Relative $VO2_{peak}$ ($ml.kg.min^{-1}$)	60.1 ± 5.2	60.8 ± 6.3	57.3 ± 4.7
Relative P_{max} (W)	5.2 ± 0.4	5.2 ± 0.5	5.2 ± 0.5
Absolute P_{max}(W/kg)	387.5 ± 55.2	391.4 ± 49.1	393.1 ± 50.4
Absolute $P_{RER \geq 1}$ (W)	283.1 ± 60.2	277.1 ± 44.7	250.6 ± 63.8
Relative $P_{RER \geq 1}$ (W/kg)	3.7 ± 0.5	3.7 ± 0.7	3.3 ± 0.7
Body mass (kg)	75.7 ± 14.0	76.5 ±15.4	76.9 ± 13.3
Skeletal muscle mass (kg)	44.9 ± 11.4	45.5 ± 11.3	44.9 ± 10.0
Sum of 6 skinfolds (mm)	52.2 ± 17.0	56.3 ± 20.4	60.5 ± 21.3
Sum of 8 skinfolds (mm)	66.9 ± 21.9	74.2 ± 29.4	81.6 ± 29.9

5.3.2 Training characteristics – total season

A total of 3841 person hours training data was collected over 1649 sessions on 1318 person days. On average, athletes completed 561.2 (range: 360.2 to

896.8) hours per year total volume of training, equivalent to 10.7 hours training per week (range: 6.9 to 17.1 hours) throughout the study period. Mean weekly measures of training duration, distance, modality (MTB, road, other) and all TRIMP models (iTRIMP, LuTIMP, bTRIMP, EdTRIMP) for elite enduro mountain bike athletes are presented in Table 5.4 below.

Table 5.4: Overview of training duration, mean weekly training volume and modality for individuals with group mean (SD).

Athlete	Best EWS result	Rank within study	Total hours (h/yr)	Weekly mean						Mode (% weekly duration)		
				Duration (h)	Distance (km)	iTRIMP (A.U.)	LuTRIMP (A.U.)	bTRIMP (A.U.)	EdTRIMP (A.U.)	MTB	Road	Other
1	3rd	2	896.8	17.1	198.7	731.3	663.0	740.4	1327.6	76.2	23.8	0.0
2	46th	5	360.2	6.9	68.0	361.7	359.6	458.5	815.5	85.0	11.2	3.8
3	2nd(F)	8	434.3	8.0	68.4	458.0	446.2	552.1	990.2	76.0	15.0	9.1
4	1st	1	624.1	11.9	106.3	593.3	597.0	664.5	1146.8	77.7	10.6	11.7
5	26th	4	464.7	8.9	67.7	329.0	489.3	456.5	915.6	87.6	12.4	0.0
6	12th	3	688.3	13.2	110.5	237.7	557.8	470.0	812.4	85.8	13.1	1.1
7	102nd	7	453.1	8.7	80.1	480.0	442.1	592.8	991.4	83.6	14.5	1.8
9	47th	6	567.8	10.7	95.3	394.7	471.1	456.4	815.5	75.3	22.7	2.0
Mean	30	-	561.2	10.7	99.4	448.2	503.3	548.9	976.9	80.9	15.4	3.7
(SD)	(35)	-	(173.3)	(3.3)	(43.7)	(156.4)	(97.2)	(109.2)	(183.3)	(5.1)	(5.1)	(4.4)

Abbreviations: iTRIMP = individualised training impulse, LuTRIMP = Lucia's training impulse, bTRIMP = Banister's training impulse, EdTRIMP = Edward's training impulse, MTB = mountain bike, A.U. = arbitrary units, EWS = Enduro World Series.

Athletes recorded a mean ascent of 2811m (range: 1975 – 4968m) and mean descent of 3107m (range: 2031 – 5792m) descent per week. Descent is greater than ascent due to removal of mechanical uplift time (e.g. chairlift). Data for individual athletes expressed as mean metres per week is shown in figure 5.1.

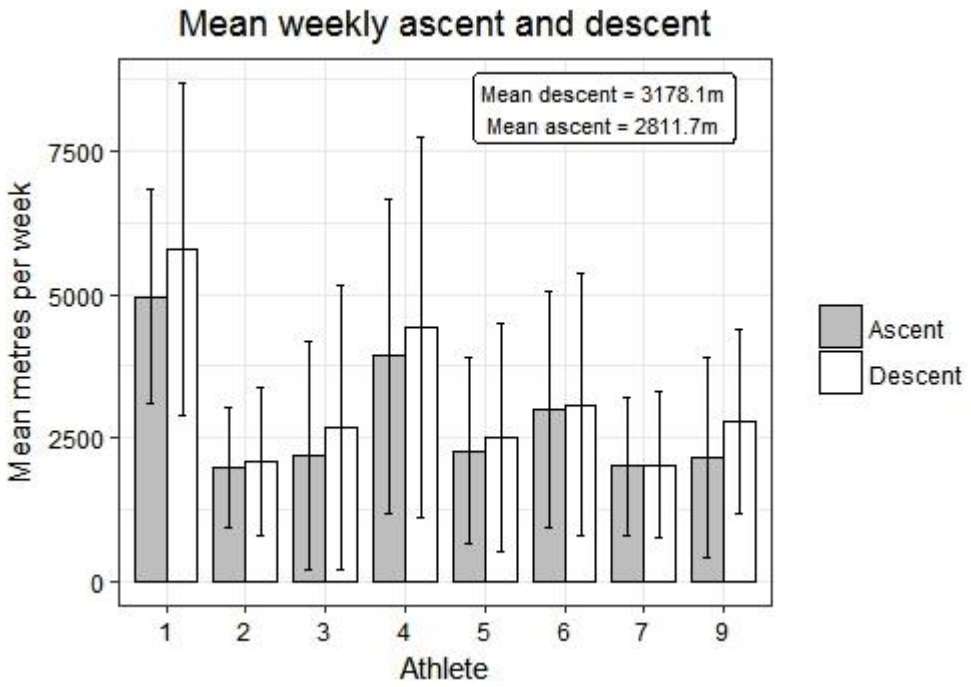

Figure 5.1: Mean (± SD) ascent and descent (metres per week) for individual athletes.

A large relationship was found between overall study rank and mean descent per week on mountain bike (rho = -0.81, p = 0.022) but not mean descent per week completed when road cycling (rho = -0.67, p = 0.083). Similarly, a large relationship was shown between overall study rank and mean weekly ascent completed on mountain bike (rho = -0.81, p = 0.022) but not on road (rho = -0.64, p = 0.022), as shown in figure 5.2 below.

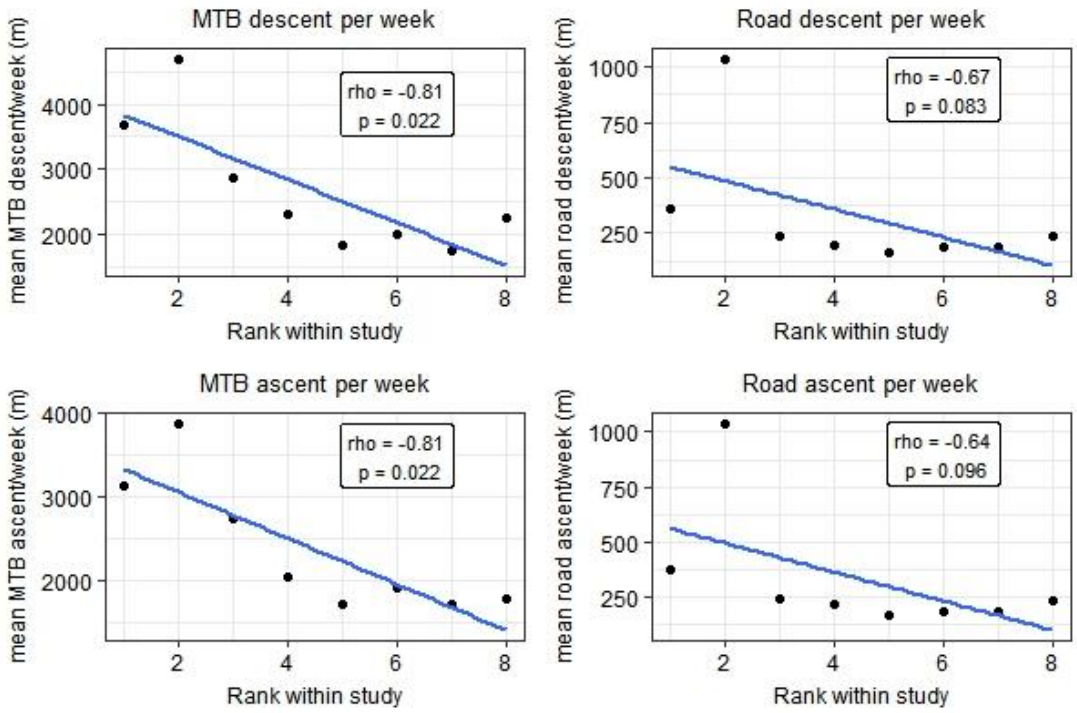

Figure 5.2: Spearman's rank correlations between overall study rank and mean weekly ascent and descent completed by mountain bike (MTB) or road.

5.3.3 Training characteristics – phase of season

Significant interactions were observed between several training variables and training phase as presented in Table 5.5. In the event of significant interaction between training variable and training phase, Bonferroni corrected post-hoc tests are used to show details of differences between phases, also shown in Table 5.5. Mean duration per day for each race was 4.9 ± 1.0h, 84.3 ± 7.0% of which was $<HR_{FBLC2}$, 9.2 ± 8.1% was at $HR_{FBLC2-4}$ and 6.5%was $>HR_{FBLC4}$. All races were completed by MTB and mean ascent and descent per race day was 1465 ± 341.2m and 1540.6 ± 267.3m, respectively. Mean iTRIMP (231.1 ± 94.4), LuTRIMP (223.3 ± 84.5), bTRIMP (270.8 ± 97.0) and EdTRIMP (484.7 ± 165.9) values for each race day are just under half of the group mean values for training load per week (see table 5.5)

Table 5.5: Weekly training volume, training intensity, elevation changes and mode throughout general preparation (GP), pre-competition (PC), competition (C), regeneration phase (RP).

		Main effect of phase	GP	PC	C	RP
Weekly training volume	Duration (h)	$F_{(3,21)}$ = 13.6, p = 0.000	11.1 ± 3.7	14.3 ± 3.2[d]	11.2 ± 3.6[d]	5.6 ± 3.9[b,c]
	iTRIMP (A.U.)	$F_{(3,21)}$ = 7.9, p = 0.001	542.7 ± 306.7	555.4 ± 209.4[d]	443.0 ± 121.3[d]	152.8 ± 168.0[b,c]
	LuTRIMP (A.U.)	$F_{(3,21)}$ = 7.7, p = 0.001	565.4 ± 218.3	668.5 ± 193.5[d]	508.7 ± 90.7	223.8 ± 231.8[b]
	bTRIMP (A.U.)	$F_{(3,21)}$ = 11.7, p = 0.000	676.6 ± 221.2	821.3 ± 256.2[d]	618.1 ± 114.0[d]	234.6 ± 236.5[b,c]
	EdTRIMP (A.U.)	$F_{(3,21)}$ = 10.4, p = 0.000	1111.3 ± 399.9	1331.1 ± 424.4[d]	981.3 ± 127.1[d]	363.8 ± 369.2[b,c]
Intensity (h/wk)	<HR_{FBLC2}	$F_{(3,21)}$ = 10.8, p = 0.000	6.9 ± 2.3	9.3 ± 2.8[d]	6.4 ± 1.5[d]	2.7 ± 2.3[b,c]
	HR_{FBLC2} – HR_{FBLC4}	$F_{(3,21)}$ = 7.7, p = 0.001	0.8 ± 0.4	0.8 ± 0.6[d]	0.6 ± 0.5[d]	0.2 ± 0.3[b,c]
	>HR_{FBLC4}	$F_{(1.3,9.0)}$ = 2.9, p = 0.119	0.4 ± 0.4	0.4 ± 0.3	0.3 ± 0.2	0.1 ± 0.2
Intensity (%time/ week)	<HR_{FBLC2}	$F_{(1.0,7.2,)}$ = 1.6, p = 0.243	85.9 ± 7.0	88.4 ±5.9	86.1 ± 7.5	67.3 ± 42.1
	HR_{FBLC2} – HR_{FBLC4}	$F_{(3,21)}$ = 5.8, p = 0.005	9.5 ± 5.3	7.5 ± 4.8	9.1 ± 6.5	4.5 ± 5.2
	>HR_{FBLC4}	$F_{(1.4,9.5)}$ = 0.6, p = 0.623	4.6 ± 3.3	4.2 ± 3.1	4.8 ± 2.5	3.1 ± 3.1
Elevation (m/week)	Ascent	$F_{(3,21)}$ = 8.2, p = 0.001	3023.0 ± 1343.1	3785.6 ± 1493.0[d]	3769.3 ± 1705.9[d]	1284.2 ± 498.6[b,c]
	Descent	$F_{(3,21)}$ = 8.8, p = 0.001	3108.7 ± 1509.2	3949.2 ± 1443.8[d]	3592.5 ±1539.7[d]	1181.4 ± 539.7[b,c]
Mode (% time)	MTB	$F_{(1.3,9.0)}$ = 1.4, p = 0.266	72.8 ± 10.2	79.1 ± 16.6	86.4 ± 4.4	71.2 ± 23.4
	Road	$F_{(3,21)}$ = 1.5, p = 0.261	20.1 ± 11.0	12.7 ± 9.5	12.4 ± 3.9	23.0 ± 22.1
	Other	$F_{(1.5,10.1)}$ = 0.9, p = 0.413	7.1 ± 8.7	8.3 ± 17.3	1.2 ± 1.6	5.8 ± 9.8

Abbreviations: iTRIMP = individualised training impulse, LuTRIMP = Lucia's training impulse, bTRIMP = Banister's training impulse, EdTRIMP = Edward's training impulse, MTB = mountain bike, A.U. = arbitrary units. A repeated measures one way ANOVA was used to determine group mean differences between training characteristics during each training phase (training characteristic * phase). [a] = significantly different to GP,[b] = significantly different to PC, [c] = significantly different to C, [d] = significantly different to RP, all p <0.05 (Bonferroni corrected).

Group mean and individual data of time spent in intensity zones for each phase is shown in figure 5.3 below. Athlete 1 (ranked 2nd) completed the largest volume of low intensity training during GP (10.5h) while athlete 4 (ranked 1st) completed the largest volume of training >HR_{FBLC4} during GP and PC phases (1.2 h in both).

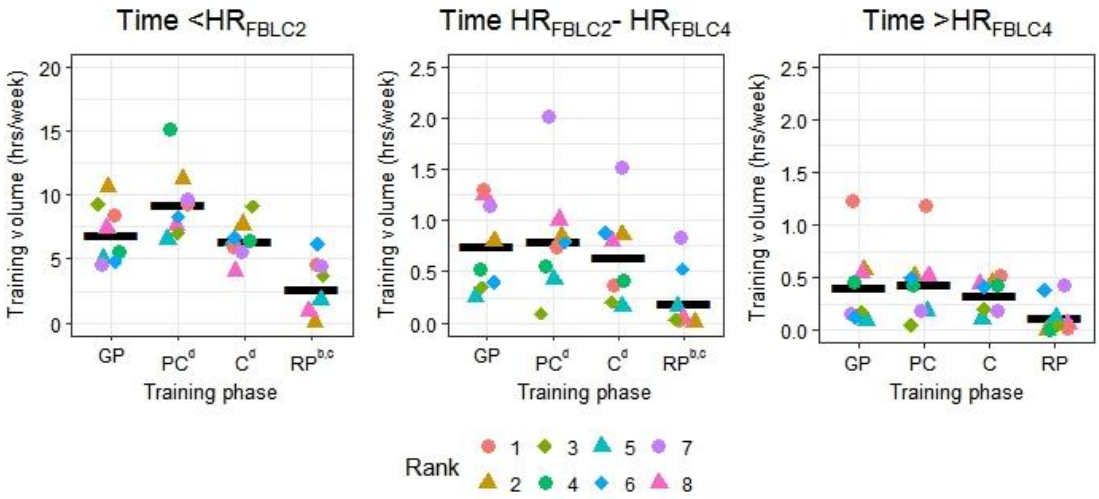

Figure 5.3: Individual time in heart rate zones corresponding to <FBLC2, FBLC-FBLC4, >FBLC4. Black horizontal lines represent group mean (n=8) value for each phase. A repeated measures one way ANOVA was used to determine group mean differences between training characteristics during each training phase (training characteristic * phase). GP = General preparation; PC = pre-competition; C = competition; RP = regeneration phase. [b] = significantly different to PC; [c] = significantly different to C; [d] = significantly different to RP, all p <0.05 (Bonferroni corrected).

5.3.4 Correlations between training load and individual performance

Despite no significant changes in group mean physiological characteristics, analysis of individual data revealed differences in individual physiological characteristics between tests. Accordingly, dose response relationships between training load and changes in individual physiological characteristics are shown in table 5.6 where several significant relationships between TRIMP models and changes in individual physiological characteristics are evident. Of particular interest, the largest correlations were found between LuTRIMP and $\%\Delta P_{FBLC2}$ (R = 0.68 [95%CI: 0.23 to 0.89]), $\%\Delta P_{FBLC4}$ (R = 0.67 [95%CI: 0.22 to 0.89]), and $\%\Delta P_{RER \geq 1}$ (R = 0.56 [95%CI: 0.05 to 0.84]), as shown in table 5.6. $\%\Delta VO_{2peak}$ showed the largest correlation with bTRIMP (R = 0.57, [95%CI: 0.05 to 0.84]; see figure 5.4).

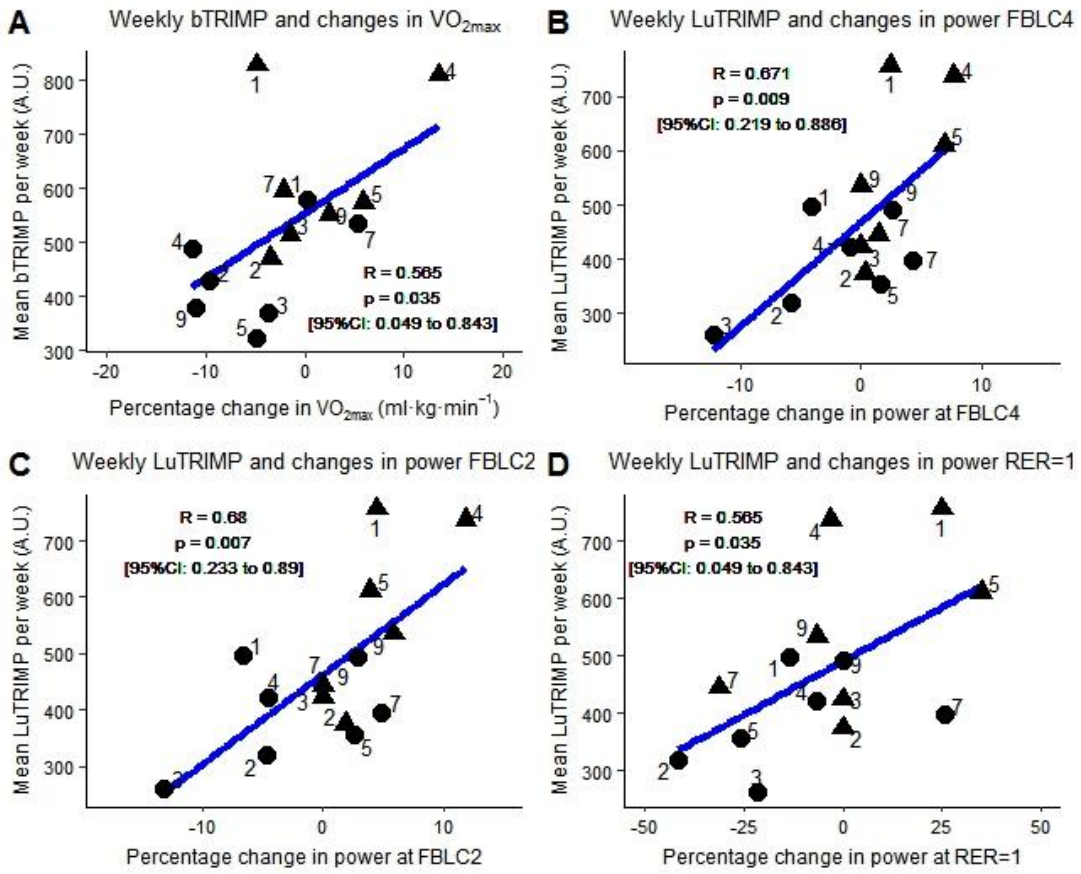

Figure 5.4: Relationship between weekly bTRIMP and changes in VO_{2peak} (A), and weekly LuTRIMP and percentage changes in power output at FBLC4 (B), FBLC2 (C) and RER ≥ 1 (D) presented with Pearson's bivariate correlation coefficient (R) with 95% confidence interval (CI) and probability value (*p*). ▲= period 1 (baseline to mid-season, n=8), ● = period 2 (mid-season – post-season, n=8).

Large correlations were also observed between $\%\Delta VO_{2peak}$ and iTRIMP (R = 0.55, [95%CI: 0.03 to 0.84]), and $\%\Delta VO_{2peak}$ and EdTRIMP (R = 0.56, [95%CI: 0.04 to 0.84]). No mean weekly measures of training load were able to predict changes in either FBLC2 or $VO_{2max,}$ and no significant relationship was shown between training impulse model and changes in peak aerobic power (see table 5.6).

Table 5.6: Relationship between mean weekly training measures and percentage changes in physiological characteristics. Pearson's bivariate correlation coefficients are presented with 95% confidence intervals.

	iTRIMP (A.U.)	LuTRIMP (A.U.)	bTRIMP (A.U.)	EdTRIMP (A.U.)	Duration (h)	Distance (km)
%Δ Power FBLC2 (W)	0.44 [-0.12 to 0.79]	0.68* [0.23 to 0.89]	0.52 [-0.01 to 0.86]	0.52 [-0.01 to 0.83]	0.28 [-0.29 to 0.28]	0.24 [-0.33 to 0.69]
%Δ Power FBLC4 (W)	0.35 [-0.22 to 0.74]	0.67* [0.22 to 0.89]	0.50 [-0.04 to 0.81]	0.51 [-0.02 to 0.82]	0.34 [-0.23 to 0.74]	0.24 [-0.33 to 0.68]
%Δ $VO2_{peak}$ ($ml.kg.min^{-1}$)	0.55* [0.03 to 0.84]	0.49 [-0.06 to 0.81]	0.57* [0.05 to 0.84]	0.56* [0.04 to 0.84]	0.09 [-0.46 to 0.59]	0.10 [-0.45 to 0.60]
%Δ P_{max} (W)	0.21 [-0.36 to 0.67]	-0.12 [-0.44 to 0.61]	0.14 [-0.42 to 0.62]	0.18 [-0.39 to 0.65]	-0.03 [-0.55 to 0.51]	0.06 [-0.49 to 0.57]
%Δ P_{RER} (W)	0.34 [-0.23 to 0.74]	0.56* [0.05 to 0.84]	0.44 [-0.12 to 0.79]	0.52 [-0.02 to 0.82]	0.38 [-0.19 to 0.76]	0.40 [-0.17 to 0.77]

Abbreviations: iTRIMP = individualised training impulse, LuTRIMP = Lucia's training impulse, bTRIMP = Banister's training impulse, EdTRIMP = Edward's training impulse, A.U. = arbitrary units.

5.3.5 Resting heart rate measures

A total of 971 resting heart rate samples were recorded across a total of 2756 person days, resulting in a mean adherence of 35.3% equivalent to one recording every three days. Once data was assessed for valid training weeks (Mon-Sun) which contained three or more heart rate recordings, 151 person weeks of data were included in further analysis. No significant relationship was found between any measure of training load (iTRIMP, LuTRIMP, bTRIMP, EdTRIMP, Distance, Duration) and any resting HR measure (LnRMSSD, RHR, LnRMSSD,RR ratio). For example, see figure 5.5 for percentage changes in weekly training load and percentage changes in resting LnRMSSD.

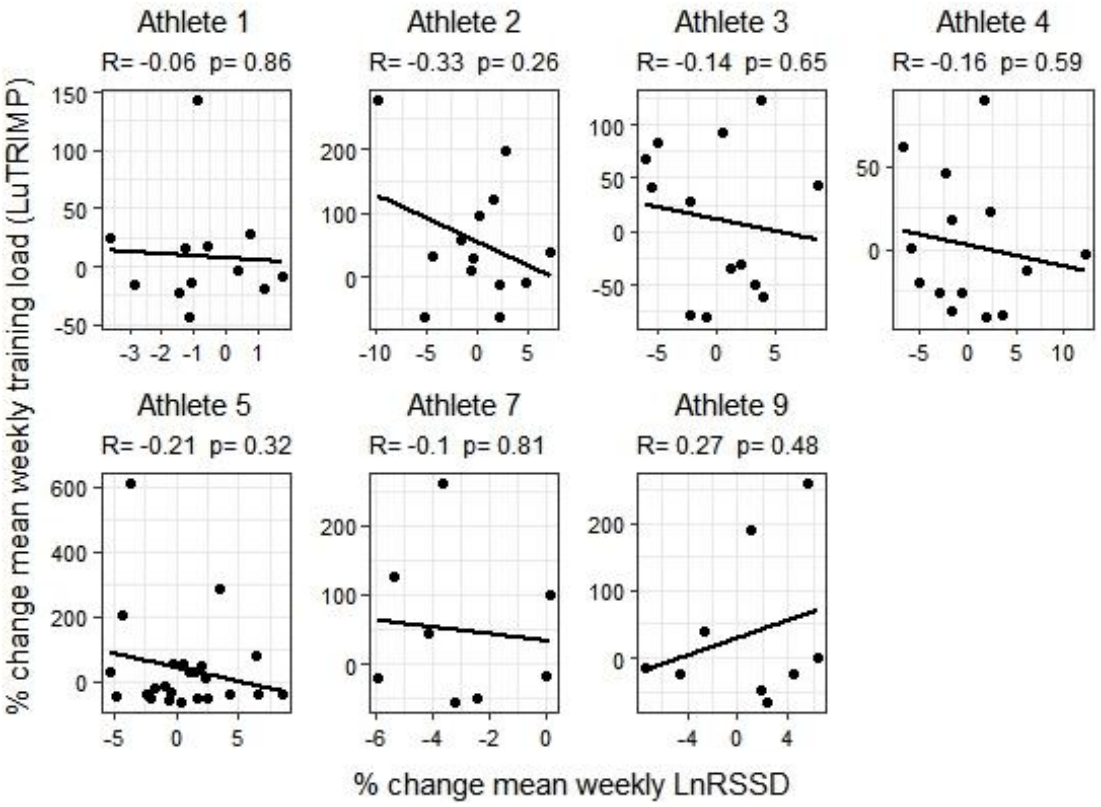

Figure 5.5: Percentage changes in training load (mean bTRIMP/week) and percentage changes in weekly resting LnRMSSD (ms) presented with corresponding Pearson's bivariate correlation coefficient value and probability value (*p*).

No relationship (R = 0.00 [95%CI: -0.57 to 0.57], *p* = 0.99) was found between resting LnRMSSD values and maximal aerobic power, as shown in figure 5.6. Further, no correlation was found between change in aerobic power output and change in resting heart rate or heart rate variability.

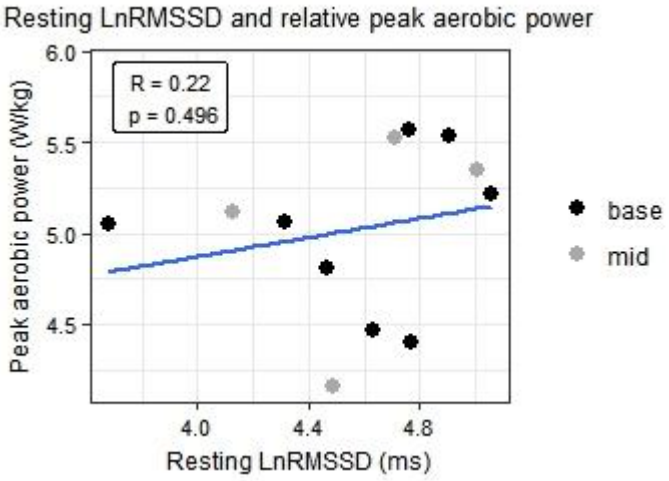

Figure 5.6: Relationship between resting mean weekly heart rate variability and peak aerobic power output at baseline and mid-season testing.

5.4 Discussion

The aims of this chapter were to 1) determine the training characteristics of elite enduro mountain bike athletes, 2) establish if these training characteristics influence changes in key physiological characteristics and 3) investigate the cardiac autonomic nervous system response changes in response to training load and life stress. The main findings of this study demonstrate that enduro mountain bike athletes completed a mean of 561 hours per year training of which a majority (~85-90% total volume) was completed <$HR_{FBLC2,}$ with mountain biking the predominant training modality (~80% total volume). Mean weekly training impulse models showed stronger relationships with changes in physiological variables compared to mean distance or duration per week, though no training load values could account for changes in peak power output. To the best of our knowledge, the current data set of resting heart rate measures is larger than any other data set provided by elite athletes in a longitudinal study available in the literature. Results showed resting heart rate measures (RHR, LnRMSSD, LnRMSSD:RR) did not correspond with changes in any measure of training

load (duration, distance, iTRIMP, LuTRIMP, bTRIMP, EdTRIMP). There was also no evidence of a relationship between aerobic capacity or performance and resting heart rate measures, thus presenting novel findings that will be discussed in more detail later in this chapter.

The results of the current study show that elite enduro athletes complete large total training volumes (h/week) similar to those completed by other endurance athletes such as Ironman triathletes (Neal, Hunter and Galloway, 2011) and orienteering world champions (Tønnessen *et al.*, 2015). Distribution of training intensity (%time) shows that 85-90% of training time is spent below HR_{FBLC2} which is also comparable to observations in other endurance sports such as cross country skiing (Solli, Tønnessen and Sandbakk, 2017). Mountain biking was the predominant training modality (75-88% training time) for all participants with road cycling accounting for a majority of the remaining training time (10-24%). This signifies increased specificity in terms of modality when compared to other endurance sports such as swimming or skiing, though reasons for this remain unclear at present (Hellard *et al.*, 2015; Solli, Tønnessen and Sandbakk, 2017). Mean values of training volume, intensity, and specificity did not differ between general preparation and competition phases, suggesting the training regime of the elite enduro athletes in the current study accurately reflects the demands of the competitive season. This is proposed to promote physiological adaptations specific to the demands of competition and familiarisation with the physiological stress of the competitive season, together offering benefit to the athlete (Pierce *et al.*, 1990; Tønnessen *et al.*, 2016).

A non-significant increase in low intensity training volume during pre-competition phase compared to general preparation and competition phases is surprising, as optimal tapering strategies in endurance sport typically feature a reduction in low intensity training volume whilst retaining high intensity training volume (Bosquet *et al.*, 2007; Hellard *et al.*, 2013). This was not found in the current study where low intensity training volume showed a trend to increase and high intensity ($>HR_{FBLC4}$) volume remained unchanged. This may be explained by the changeable terrain associated with enduro, as the

first race of the year for 5 of the 8 athletes was Enduro World Series (EWS) round one, located in New Zealand. Personal communication with the athletes revealed that the terrain and hence skill demands associated with this event were notably different to other races, thus considerable time during the pre-competition phase was spent on attempting to adapt to the unique demands of this event. This finding is not likely to be limited to the first race as at least one day of practice is completed at every race to allow athletes to see the racetracks. Therefore, it is feasible that enduro athletes prepare for events differently to other endurance sports; however further investigation is required to explore this assertion. Training loads (total duration, duration HR_{FBLC2} and $HR_{FBLC2\text{-}FBLC4}$, all TRIMP models, ascent and descent) were significantly reduced during regeneration phase compared to all other phases which is proposed to allow psychological and physiological recovery following the competitive season (Solli, Tønnessen and Sandbakk, 2017). Similar results are reported in other endurance sports and are proposed to be of benefit to athletes by reducing the risk of developing a state of non-functional overreaching (NfOR; Meeusen *et al.*, 2013).

There were no significant differences in training modality (mountain biking, road cycling, other) between all phases, however there was a trend for increased mountain bike time during competition phase. This is likely due to all races being completed on a mountain bike and may show a trend towards athletes training specifically for the demands of the event during the competitive season. Similarly, mean ascent and descent per week was only lower during regeneration phase, remaining similar for all other phases. Together, these findings support the assertion that athletes train during general preparation with appropriate specificity to meet the demands of competition before allowing appropriate recovery during regeneration phase. Crucially, mountain biking ascending and descending time was correlated with performance within this study but not the equivalent values for road cycling, thus signifying the importance of specificity within the training regime of elite enduro mountain bike athletes. As enduro race events typically feature substantial elevation changes (~half of the group mean weekly descent in one day) and the race stages are predominantly on descending terrain, the faster

riders are able to gain an advantage by training more specifically to meet the demands of the race (Hassenfratz, Ravier and Grappe, 2012; Chidley *et al.*, 2015). Though specificity appears to be a key component of training for enduro, riders still completed large volumes of road cycling. Road cycling may offer reduced vibration exposure, in turn reducing total workload during recovery activities and reduced injury risk when compared to mountain biking in addition to reducing training monotony and thus helping to balance training (Ashwell *et al.*, 2012; Macdermid, Fink and Stannard, 2015). In contrast, increased MTB descending time is also very likely to expose the riders to greater levels of vibration and increased muscle activity (Hurst *et al.*, 2012, 2013), which may offer additional training stimulus specific to the demands of enduro racing such as maintenance of grip strength. Furthermore, riding descending terrain is likely to develop or maintain rider skill (Chidley *et al.*, 2015) and increases bicycle load values specific to negotiating technical terrain at higher velocities, which we have previously shown to be key components of performance in enduro mountain biking (Kirkwood *et al.*, 2017).

Power output at FBLC2, FBLC4, VO_{2peak} and RER ≥ 1 have also previously been shown (chapter 4) as key physiological characteristics required for successful performance in enduro racing. The current study aimed to compare the strength of relationships between the currently available measures of training load and individual changes in the physiological characteristics above. This allows investigation of the efficacy of each training load measure for use in monitoring training load in enduro mountain bike athletes. Furthermore, this approach allows comparison of dose-response relationships previously observed in road cycling (Sanders *et al.*, 2017). Relationships between training impulse models and changes in power output at FBLC2 and FBLC4 are slightly weaker in the current elite enduro cohort than an elite road cyclist population (R = 0.81 vs 0.68 (FBLC2), R = 0.77 vs 0.67 (FBLC4), all $p<0.05$) (Sanders *et al.*, 2017). Conversely, mean bTRIMP showed a stronger relationship with changes in $VO2_{peak}$ in the enduro cohort (R = 0.57, $p<0.05$) compared to the elite road population (R = 0.39, $p >0.05$). This is also the first study to show a strong dose-relationship between LuTRIMP and changes in power at RER ≥ 1, shown to be a key physiological characteristic required for

successful enduro performance as shown in chapter 4 of this thesis. Weekly duration and distance were not correlated with change in any physiological variable suggesting these markers are not sensitive enough to monitor training load in enduro mountain bike athletes.

While training volume and intensity are comparable to other sports, training load assessed by training impulse (TRIMP) measures per week were approximately half of the values reported previously in national level road cyclists of comparable physiological characteristics (Sanders *et al.*, 2017). However, very similar physiological characteristics (Sanders vs current data: VO_{2peak} 61 vs 61ml.kg.min^{-1}; Power VO_{2peak} 391 vs 411W) and changes in performance characteristics (-10 to +15% in both studies) are shown in the study of Sanders et al. (2017) and the current study. This suggests athletes of Sanders *et al* (2017) are of similar ability and thus would require a similar training stimulus to evoke similar changes in performance. Yet, similar changes in performance physiology are reported in both studies despite considerably lower training load values presented for the enduro athletes of the current study. For example, mean iTRIMP (448 ± 156 vs. 1005 ± 229), bTRIMP (549 ± 109 vs. 1090 ± 220), LuTRIMP (503 ± 97 vs. 891 ± 200) and EdTRIMP (977 ± 183 vs. 2142 ± 432) were all lower during the current study compared with Sanders et al. (2017) respectively. Taking all this information together, it appears that current training impulse models underestimate training load in elite enduro mountain bike athletes compared to elite road cycling athletes and subsequently load values are not comparable between these disciplines. The largest difference between road cycling and mountain biking is the increased vibration exposure associated with mountain biking (Macdermid, Fink and Stannard, 2015). Previous reports show that vibration is increased during mountain biking, and it is known that vibration can decrease performance and increase oxygen consumption but not heart rate values (Samuelson, Jorfeldt and Ahlborg, 1989b; Rønnestad, Slettaløkken Falch and Ellefsen, 2016). As all of the training impulse measures analysed here are based on heart rate, it is proposed that the effect of vibration accounts for a substantial proportion of the nearly two-fold discrepancy in training impulse values between elite road cyclists and enduro athletes. Accordingly,

further research is required to accurately quantify the vibration exposure during mountain biking, and understand the physiological response to vibration exposure in an exercise training context. As a whole, these findings suggest that LuTRIMP, although by no means perfect, is the most suitable model currently available for monitoring training load with respect to physiological changes in elite enduro athletes. However, no model could predict changes in peak power, which also has a very strong relationship with enduro race time, hence an ability to predict improvements using a model that takes cognisance of the above factors would be useful to both enduro athletes and coaches. This indicates more work is required to develop a load model designed to monitor training load in elite enduro riders and that such a model should necessarily include the influence of vibration exposure. The lack of strength training data reported in the current study is also a limitation to assessing overall training load and should be considered in any future research.

Although a dose-response relationship is evident between training load and physiological characteristics associated with performance in the current study similar to previous findings, analysis of individual data from the current study reveals a more complex relationship. During period 1 of the current study, athlete 1 and athlete 4 reported 30% greater training load values per week, ~30% more than any other athletes. Athlete 1 saw a ~5% reduction in VO_{2peak} with only ~3% increase in power at FBLC2 and FBLC4, recording a 10^{th}, 19^{th} and 13^{th} finishing position at EWS races during period 1. Conversely, athlete 4 underwent similar training load during period 1 yet saw the largest improvements in power at FBLC2, FBLC4, and $VO2_{peak}$ (up to 15% improvement) and was world number 1 at the end of period 1 (5^{th}, 2^{nd} and 1^{st} at EWS races during period 1). An increase in performance suggests that athlete 4 showed positive adaptations to training stimulus (functional overreaching or acute fatigue) while reductions in performance for the same stimulus suggest athlete 1 has reached a state of NfOR as described previously in chapter 2, section 2.2.1 (Meeusen *et al.*, 2013). Conclusive reasons for this remain unclear, however differences in training response may be explained by differences in total training volume and distribution of training

intensity. For example, athlete 1 (ranked 2nd in study) also completed the largest total training volume (897h/yr, 17h/wk), comparable to training volumes of elite cross country skiers and biathletes (Tnønessen *et al.*, 2014; Solli, Tønnessen and Sandbakk, 2017), elite Nordic combined athletes (Tønnessen *et al.*, 2016), and world class rowers (Guellich, Seiler and Emrich, 2009). Comparatively, both athlete 4 (ranked 1st) and athlete 6 (ranked 3rd) completed around 30% less total volume (624 and 688 h/yr respectively) than athlete 1 (ranked 2nd). During GP phase, athlete 1 also completed considerably more low intensity (h/wk) training volume (10.5h vs 8.5 h) but much less high intensity (0.6h vs 1.2h h/wk >HR_{FBLC4}) training volume than athlete 4, respectively. This considered, athlete 1 made the largest improvement in power RER ≥ 1, likely to be partly due to the very high volume of training time spent <HR_{FBLC2} (Laursen and Jenkins, 2002). Although power RER ≥ 1 shows the strongest correlation with enduro performance, it appears that athlete 1 has become physiologically adapted to perform optimally at lower intensity while negating the high intensity capacity required for successful performance in enduro racing (Seiler and Tønnessen, 2009). This suggests that Athlete 1 completed excessive volumes of low intensity training <HR_{FBLC2} (and hence total training volume) which the findings of the current chapter suggest does not offer the most benefit to enduro racing performance. While similar LuTRIMP values were reported between athletes, distribution of training intensity was the crucial difference between these athletes and as such must be monitored alongside LuTRIMP to reduce risk of NfOR, particularly when high values (~800 A.U.) are reached. In addition to NfOR, distribution of training intensity is also shown to be important for performance as exemplified by the data of the highest ranked rider in the current study. Athlete 4 (rank 1) completed the highest volume of training >HR_{FBLC4} (1.2h/wk or ~15% of weekly load) in GP and PC phases (~2 fold more than any other athlete) while low and medium intensity training volume was similar to other athletes. As around 90% of race stages are spent >HR_{FBLC4}, increased training time at this intensity would appear to offer physiological adaptations specific to the demands of the race event.

Despite evidence that athlete 1 was presenting symptoms of NfOR (Meeusen *et al.*, 2013) while athlete 4 was presenting signs of positive adaptation, no differences were found in resting heart rate measures in response to changes in training load for any athlete. These findings contradict previous literature showing significant changes in resting heart rate measures during positive or negative adaptation to increases in training load (Manzi, Castagna, *et al.*, 2009; Plews, Laursen, Kilding, *et al.*, 2013; Nakamura *et al.*, 2018). This is particularly interesting as athletes routinely exceeded the recommended 10% increase in training load per week, yet this was not reflected in any change in resting heart rate measures (Hellard *et al.*, 2015; Nakamura *et al.*, 2018; Walsh, 2018). These previous studies have generally been of shorter duration (~4weeks), training load has been controlled between participants, and/or data collection completed during training camps where athletes are exposed to the same environmental stressors (Buchheit *et al.*, 2010; Plews, Laursen, Kilding, *et al.*, 2013). Accordingly, the contradictory findings presented here may arise due to methodological considerations such as ultra-short (55sec) vs short (5-10min) term recording and no control of environmental conditions which can effect heart rate measures, such as light exposure, heat, altitude, and time of day (Plews, Laursen, Kilding, *et al.*, 2013; Bellenger *et al.*, 2017). The methodology employed in the current study is very similar to that of coaches and athletes in the field due to the low time cost for the athlete, though adherence could be considered poor compared to other studies (Buchheit *et al.*, 2010; Plews, Laursen, Kilding, *et al.*, 2013). This was particularly evident during the competitive season, where personal communication from the athletes showed the heart rate monitor strap to be easily forgotten when travelling. As previous studies have shown clear influence of training load on resting heart rate measures in more controlled environments, it is possible that confounding variables such as life stress and environmental factors masked any influence of training load. In summary, it is suggested that heart rate variability does not offer any inference regarding adaptation to training load in elite enduro athletes in the field without taking into account life stress and environmental factors.

The current study is the first to compare resting heart rate measures and performance in elite enduro cyclists. No relationship between resting heart rate measures and aerobic power was observed, nor change in resting heart rate measures and change in physiological characteristics of athletes. These findings differ from previous findings showing a relationship between peak aerobic power (maximal aerobic running speed) in recreational endurance runners (Buchheit *et al.*, 2010). Within the population of recreational runners, the non-responder group (no change in peak aerobic power or LnRMSSD following training) also presented higher pre-training performance values (10km run time, maximal aerobic speed; Buchheit *et al.*, 2010). It therefore appears that a relationship between resting LnRMSSD and performance is only evident in recreational athletes starting from lower fitness levels, with no relationship observed between resting LnRMSSD and performance in well trained and elite populations. There was also no evidence in the current study to suggest that changes in fitness were accompanied by changes in LnRMSSD values in the elite population. This further supports the notion that changes in physiological characteristics are not reflected by changes in LnRMSSD in elite populations in the same way they appear to be linked in recreational athletes (Buchheit *et al.*, 2010). This may be the result of parasympathetic saturation due to the long history of endurance training associated with all elite athletes included in this study (Plews, Laursen, Stanley, *et al.*, 2013; Plews, Laursen and Buchheit, 2016). Previous reports suggest the use of LnRMSSD:RR to negate the influence of parasympathetic saturation, however once again, no relationship was found suggesting a link between LnRMSSD:RR and training stimulus or adaptation (Plews, Laursen and Buchheit, 2016). Furthermore, contrasting previous findings, no correlation between change in resting heart rate measures and change in maximal aerobic power was observed in the current study (Buchheit *et al.*, 2010; Plews, Laursen, Kilding, *et al.*, 2013). In summary, ultra-short term resting heart rate measures collected in a free running field setting do not appear to offer a reliable means of assessing response to training load when environmental and life stress factors are not controlled.

In conclusion, elite enduro athletes meet the demands of the race events by completing large volumes of training (~650/yr), of which ~15% was spent >HR_{FBLC4} by optimal performers. Mountain biking was the main modality of training, with a particular trend observed in successful athletes to cover ascent and descent similar to that of races in order to develop the appropriate skills and physiological adaptations required to negotiate technical terrain at high speed. LuTRIMP appears to be the most effective TRIMP model currently available to estimate training load in enduro, and it is evidenced that excessive low intensity training volume during training weeks exceeding 800 A.U. LuTRIMP may increase risk of NfOR, however more research is required. Resting heart rate measures are not shown to offer any inference of positive or negative adaptation to training load in the field, likely due to poor control of confounding variables. As signs of overreaching were not reflected in resting heart rate measures, other measures such as immune response may offer more valuable insight to recovery in response to training load and racing in elite athletes. The ability of TRIMP models to predict dose-response relationships between training load and performance in enduro is still reduced compared to road cycling, hence further work is required to develop training load models in mountain biking. Lastly, TRIMP values appear to underestimate training load in mountain biking compared to road cycling which may be due to greater vibration induced workload during mountain biking, however more work is required.

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Chapter 6: Seasonal changes in white blood cell subset redistribution in response to maximal laboratory based exercise testing and international enduro mountain bike racing

6.1 Introduction

Elite athletes complete large volumes of training and compete regularly for several months of the year as detailed in chapter 5 for elite enduro athletes, the focus of this thesis. Completion of such large volumes of training load and subsequent performance in competition relies on minimising the number of days missed due to illness, hence highlighting the importance of healthy immune function in athletic populations. Previously, greater total training volume (h/year) has been associated with reduced number of self-reported sick days, however mechanisms for this relationship are unknown (Mårtensson, Nordebo and Malm, 2014). Similarly, incidence of illness reduced incrementally over a 4-year period of observation in elite swimmers. Evidence such as this supports the S-curve theory which puts elite athletes at lower risk of infection than highly active populations (Malm, 2006). Whilst this theory is well accepted in current literature, the mechanisms responsible for reduced incidence of illness in elite athletic populations are not fully understood at present. Lymphocyte subset redistribution is altered in response to repeated bouts of intense exercise if insufficient recovery time is permitted, potentially leading to prolonged periods of suppressed immunity following the basis of the open window theory (Pedersen and Ullum, 1994). For example, over four days of repeated bouts of mountainous running, reductions in lymphocyte populations 1 hour after exercise were only observed on the first two days of exercise (Simpson, Guy, *et al.*, 2006). All subsets were shown to return to baseline levels 24h following all four days of exercise, suggesting an impaired response to exercise after the first two bouts of exercise despite no changes in resting values (Simpson, Guy, *et al.*, 2006). These findings may be due to reductions in exercise induced beta-2 (β_2) -adrenergic receptor expression on the surface of cytotoxic (NK cell, $CD8^+$ T-cell) lymphocyte subsets (Schaller *et al.*, 1999) during periods of heavy training, though this has only been shown in moderately trained individuals. Further, glucocorticoid receptor sensitivity has previously been shown to reduce with training (Duclos, Gouarne and Bonnemaison, 2002), which may also account for reduced extravasation of lymphocytes during recovery from exercise in trained individuals. Together, these findings contribute to the well accepted theory that athletes risk prolonged immunosuppression if high

training volumes are combined with insufficient periods of recovery (Hellard *et al.*, 2015; Simpson *et al.*, 2015). Conversely, a recent review has refuted the open window theory, suggesting that the reduction in absolute numbers of leukocyte populations in the peripheral blood following exercise is protective to the host (Campbell and Turner, 2018). This argument is supported by data showing that the immune response to exercise is potentially beneficially altered with training status and fitness level (Soppi *et al.*, 1982; Kendall *et al.*, 1990). For example, the lymphocyte response of trained (VO_{2max} >60ml.kg.min^{-1}) and untrained (VO_{2max}<50ml.kg.min^{-1}) participants showed trained participants to exhibit a smaller increase in absolute number of CD8+ T-cells immediately after exercise but no changes in percentages of cell subsets mobilised in response to exercise (Kendall *et al.*, 1990; Moyna, 1996). Similarly, lymphocyte response to exercise was blunted following 6 weeks of aerobic exercise training when compared to responses to exercise at baseline or in control participants (Soppi *et al.*, 1982). This data shows an attenuated disturbance to the immune system in response to the same stressor as training status increases, potentially allowing athletes to complete larger volumes of training while maintaining immune function (Hellard *et al.*, 2015; Walsh, 2018).

Limited research is available to corroborate relationships between high training loads, insufficient recovery and chronic immunosuppression in relation to lymphocyte cell subsets present in the peripheral blood; a key component of adaptive and innate immunity. One study showed increases in senescent cell phenotypes at rest during an intensified training period within 6 months preparation for an Ironman triathlon in club level triathletes (Cosgrove *et al.*, 2012). Other studies have shown no changes in lymphocyte or neutrophil count at rest during heavy training periods in an elite cycling population provided (Ferrari, Gobatto and Manchado-Gobatto, 2013) or in response to a maximal exercise test in elite skiers (Ronsen *et al.*, 2001). No further detail of lymphocyte phenotype was provided in either study, however. These findings initially suggest elite athletes may be more robust in response to increasing training loads, supporting the S-curve hypothesis previously discussed in Chapter 2 section 2.4.4 (Malm, 2006; Hellard *et al.*, 2015). Resting values of lymphocyte cell subsets are unable to predict potential changes in factors

influencing lymphocyte cell subset redistribution such as reduction in exercise induced PBMC β_2-adrenergic receptor expression, which can only be evaluated in response to an acute stressor such as exercise (Schaller *et al.*, 1999; Krüger *et al.*, 2008; Graff *et al.*, 2018). Further, details of lymphocyte phenotype offer greater insight to the immune response and the ageing of the immune system compared to total lymphocyte count (Campbell *et al.*, 2008; Graff *et al.*, 2018), and hence further research is warranted in this area.

In addition to training stressors, knowledge of the recovery of the immune system in response to competition allows coaches and athletes to make informed decisions about returning to training following competition while minimising the risk of non-functional overreaching (NfOR) or chronic immunosuppression (Meeusen *et al.*, 2013; Simpson *et al.*, 2015). To date, a majority of studies have detailed the immune response to acute exercise in a laboratory based environment (Simpson, Florida-James, *et al.*, 2006; Nieman *et al.*, 2014) while fewer studies have focused on competitive events in the field (Castell *et al.*, 1997; Kratz *et al.*, 2002; Nieman *et al.*, 2003; Suzuki *et al.*, 2003). The psychological stress (Cohen, 2005; Atanackovic *et al.*, 2006; Edwards *et al.*, 2018) and environmental factors (McFarlin and Mitchell, 2003; Patterson *et al.*, 2008) associated with outdoor competition may alter the immune response meaning conclusions cannot be drawn from laboratory data only. Accordingly, several studies have detailed the influence of marathon (Kratz *et al.*, 2002; Suzuki *et al.*, 2003) half marathon (Lippi *et al.*, 2010; Zimmer *et al.*, 2016) and hill running (Simpson *et al.*, 2005) competition on the immune system. Concentrations of IL-6 and cortisol are consistently increased following marathon running (Haq *et al.*, 1993; Suzuki *et al.*, 2003), likely as a mechanism to deliver energy substrate to working muscle. In contrast to the lymphocytosis commonly observed following laboratory based exercise, peripheral blood lymphocyte count is reported to increase or decrease following a half-marathon (Lippi *et al.*, 2010; Zimmer *et al.*, 2016) and remain unchanged following marathon running (Haq *et al.*, 1993; Castell *et al.*, 1997; Suzuki *et al.*, 2003). This discrepancy may arise due to difficulties in collecting a blood sample immediately upon cessation of exercise in a competitive environment, meaning lymphocyte subset counts may already be

declining in peripheral blood (Rooney *et al.*, 2017). In the subsequent recovery (1-4h) from marathon racing, lymphocyte count is reduced below baseline levels before returning to baseline levels between 16h and 24h later (Castell *et al.*, 1997; Kratz *et al.*, 2002). In contrast, lymphocyte count is significantly increased immediately and 24h after a 7km hill running race finishing with 14 minutes of downhill running requiring sufficient eccentric muscular contraction to result in significant muscle damage (Simpson *et al.*, 2005). Downhill running leads to increases in shock accelerations at the shank and sacrum alongside increases in normal impact force peaks (Mizrahi, Verbitsky and Isakov, 2000; Gottschall and Kram, 2005), suggesting attenuation of accelerations may contribute to the immune response to acute exercise. Isolated acute vibration exposure can induce transient changes in CD4 and CD8 T-cell count (Noguchi and Ando, 2002) further suggesting that the addition of vibration to exercise significantly alters the immune response to exercise. This is particularly important as cyclists are exposed to potentially harmful levels of hand-arm and whole body vibration (HAV, WBV respectively) which must be attenuated before reaching the central nervous system (Chiementin *et al.*, 2013; Macdermid, Fink and Stannard, 2014; Duc, Puel and Bertucci, 2016). Although elite enduro athletes train and compete on successive days on a regular basis (see chapter 5), little is known about the recovery of the immune system following prolonged bouts of mountain biking. As the duration of enduro racing is considerably greater than that of the hill race study (Simpson *et al.*, 2005) and the demands of acceleration attenuation are likely different from the marathon running studies (Derrick, Dereu and Mclean, 2002), the immune response to enduro mountain bike racing is likely to be unique. As training load must be prescribed alongside sufficient rest periods to allow the immune system to recover from subsequent bouts (Pedersen and Ullum, 1994; Meeusen *et al.*, 2013), the response of the immune system after an enduro mountain bike competition is worthy of further investigation.

Therefore, the primary aim of this chapter is to investigate redistribution of leukocyte cell subsets in response to a maximal exercise test throughout the training and competitive season, and in response to an international enduro mountain bike race. The secondary aim of this chapter is to assess the

influence of vibration exposure, as measured on the bike, on the redistribution of immune cells following the international mountain bike race.

6.2 Methodology

Participants attended the human performance laboratory at Edinburgh Napier University on three occasions throughout training and competition to undergo maximal exercise testing. Participants also competed in the same international endure race event held on terrain which was previously used for a round of the Enduro World Series. Peripheral blood samples were taken for further analysis by flow cytometry before and after laboratory based exercise and the international race event, all of which is detailed further within this chapter. As the race event was closest to the time of the mid-season test, the mid-season test was used to compare the immune response to maximal exercise to the immune response to international competition. For details of participants and laboratory exercise test protocol please see chapter 3 and for details of the international race event please see chapter 4 sections 4.3.1-4.2.3.

6.2.1 Peripheral blood sample collection

Intravenous blood samples were collected during three laboratory visits (baseline, mid-season, off-season) conducted at time points detailed previously (see Chapter 3 section 3.3) and an international enduro mountain bike race, the details of which are presented in chapter 4. To facilitate the collection of blood samples at the race event, a mobile laboratory was created 200m from the finish line of the international enduro race event in Peebles, Scotland. To minimise the demands placed on the participants (elite athletes) in a racing situation, resting intravenous blood samples were collected at 9am between 2 and 7 days prior to the event (prior to practice commencing). Intravenous blood samples were also collected 1 hour after the event (post-1h) and the morning after the event (post-19h). Pre and post-19h blood samples were collected at Edinburgh Napier University human performance laboratory or a mobile laboratory set up in the Mountain Bike Centre of Scotland near Peebles. The race event was selected due to short travel time (~45 minutes) to the Edinburgh Napier University Sighthill campus, removing the requirement for samples to be frozen prior to subsequent analysis and because the venue had previously been used for two World Enduro Series races. In the laboratory, intravenous blood was collected from participants in

the supine position before (pre), immediately after (post) and 1 hour after exercise (post-1h). Blood was drawn into 6ml vacutainers (Becton-Dickson, Oxford, UK) coated in EDTA. An automated haematology analyser (XS 1000i, Sysmex, Milton Keynes, UK) was used to determine total blood leukocyte and differential counts in duplicate and plasma volume changes were estimated (Dill and Costill, 1974). Serum samples were collected in 6ml serum separating tubes (Becton-Dickson, Oxford, UK) and upon removal, serum samples were frozen at -80ºC for further analysis. Serum cortisol was investigated at all tests and time points while IL-6 was assessed only before (pre) and 1 hour after (post-1h) the mid-season laboratory visit and the race event. Serum cortisol and IL-6 concentrations were measured using commercially available enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, UK) performed in accordance with the manufacturer's instructions. Plates were read on a plate reader (Labtech LW5000, UK) set to a wavelength of 450nm for both cortisol and IL-6. The intra-assay coefficient of variation was 11.4% for IL-6 and 11.0% for cortisol.

6.2.2 Separation of peripheral blood mononuclear cells and flow cytometry

Blood samples (3ml) were mixed with an equal volume of 0.9% sodium chloride solution (NaCl; Baxter, UK) and the 6ml solution was layered carefully on 3ml of lymphoprep solution (Axis-shield, Oslo, Norway) before being centrifuged at 800*g* at room temperature for 30 minutes with the brake off. Following centrifuging, the distinct band formed by the peripheral blood mononuclear cells (PBMCs) at the sample/medium interface was carefully removed (see figure 6.1). PBMCs were washed twice for 10 minutes at 250*g,* firstly in 0.9% NaCl solution (Baxter, UK) and then in phosphate buffer solution (PBS; 0.01 M phosphate buffer, 0.0027 potassium chloride and 0.137 M sodium chloride, pH 7.4).

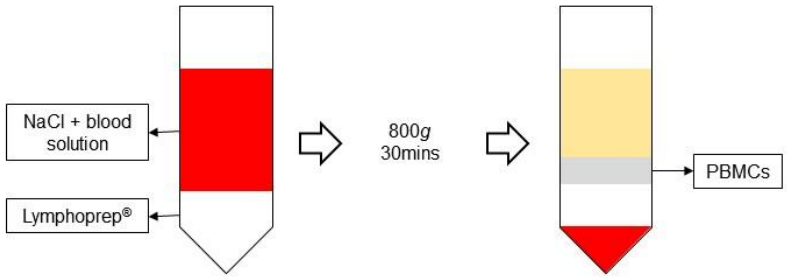


Figure 6.1: Peripheral blood mononuclear cell (PBMC) separation from whole blood.

PBMCs were counted using a haemocytometer in combination with Nigrosin stain. Aliquots of $0.5x10^6$ PBMC's were incubated for 30 minutes at room temperature with 5µl of each pre diluted monoclonal antibody solution as shown in table 6.1 in a three colour (NK cell) or seven colour (T-cell) format. The T-cell tube also contained 50µl Brilliant Stain Buffer (BD biosciences, Oxford, UK). All monoclonal antibodies were manufactured by BD biosciences, Oxford, UK. Cells were washed twice for 5 minutes at 250*g* in 500µl PBS and resuspended in 200µl PBS before analysis by flow cytometry.

Table 6.1: List of fluorescent monoclonal antibodies for cell phenotyping by flow cytometry.

Specificity	Clone	Isotype	Fluorochrome	Optimal dilution (mAb/PBS)	Panel
CD3	SK7	IgG_1	BV786	1/1	NK cell, T-cell
CD4	RPA-T4	IgG_1	V450	Stock	T-cell
CD8	SK1	IgG_1	V500-C	Stock	T-cell
CD27	L128	IgG_1	BV650	Stock	T-cell
CD28	CD28.2	IgG_1	PE-Cy 7	1/1	T-cell
CD45RA	HI100	IgG_{2b}	PerCP-Cy 5.5	1/1	T-cell
CD62L	SK11	IgG_{2a}	BB515	Stock	T-cell
CD56	B159	IgG_1	PE-Cy 7	Stock	NK cell
CD57	HNK-1	IgM	FITC	Stock	NK cell

6.2.3 Flow cytometry and gating

Cell phenotyping was completed using FACSDiva software (BD biosciences, San Jose, CA, USA) in combination with a FACSCelesta flow cytometer (BD biosciences, San Jose, CA, USA) using a three laser configuration. The three argon ion lasers emitted light at a fixed wavelength/power of 488nm/20mW (blue), 561nm/50mW (yellow-green) and 405nm/50mW (violet). Details of detection filters for relative fluorochromes are listed in table 6.2 below. Electronic gates were used on the flow cytometer to differentiate cell populations, firstly using a dot plot of forward scatter (FSC) against side scatter (SSC). As each cell passes the first laser (488/30nm), the FSC detector measures the diffraction of the light beam to determine the size of the cell whilst the SSC detector measures the refraction/reflection of the light to determine the granularity of the cell. This enables the detection of different cell subsets based on size and granularity.

Table 6.2: Details of detection filters used during flow cytometry. BP = bandpass, LP = low pass.

Detection wavelength/ BP filter (nm)	LP mirror (nm)	Fluorochrome
780/60	750	BV786
450/40	N/A	V450
525/50	505	V500-C
670/30	655	BV650
780/60	750	PE-Cy 7
695/40	670	PerCP-Cy 5.5
530/30	505	BB515, FITC

6.2.4 Lymphocyte detection

An electronic gate was placed around the lymphocyte population which was identified using the FSC/SSC mode as shown in figure 6.2. SSC against BV786 fluorescence was used to determine $CD3^+$ (T-cell) and $CD3^-$ (NK cell) populations. Further cell subsets were analysed by the different fluorochromes listed in table 6.1 and details of T-cell population identification and gating is shown in figure 6.2 below. 10,000 gated CD3+ (T-cell) or CD3- (NK cell) events were recorded for analysis of all samples. Total counts of lymphocyte subsets were then obtained by multiplying the population percentage values from the flow cytometer by the total lymphocyte counts from the haematology analyser.

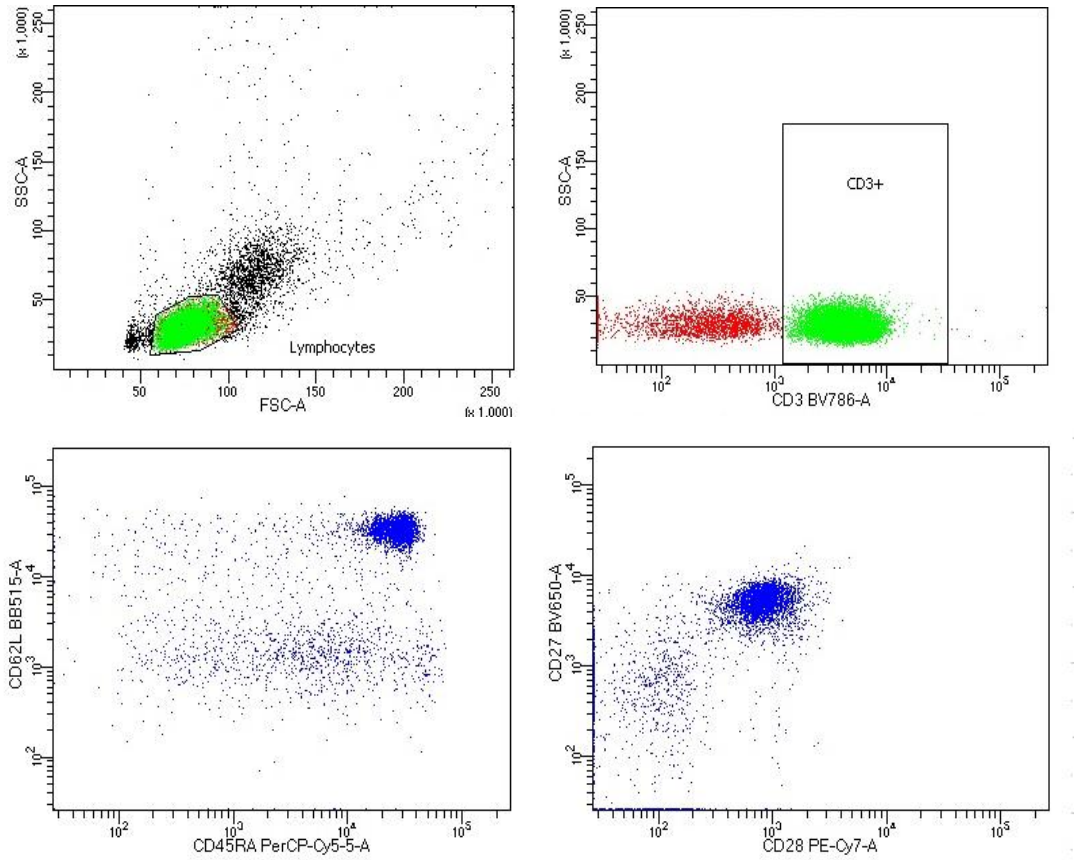


Figure 6.2: Representative lymphocyte and lymphocyte subset identification and gating.

Table 6.3: Cell surface markers used for T-cell and NK cell subset identification

Subset		Identification	References
T-cell (CD3+/CD4+ or CD3+/CD8+)	Naive	$CD62L^+/CD45RA^+$	(Krüger and Mooren, 2007; Simpson, 2011)
	Central memory	$CD62L^+/CD45RA^-$	
	Effector memory	$CD62L^-/CD45RA^-$	
	Senescent	$CD62L^-/CD45RA^+$	
	Early	$CD27^+/CD28^+$	(Azuma, Phillips and Lanier, 1993; Hamann *et al.*, 1997)
	Intermediate	$CD27^+/CD28^-$	
	Late	$CD27^-/CD28^-$	
NK cell (CD3-/CD56+)	Cytotoxic	$CD56^{dim}$	(Cooper *et al.*, 2013)
	Regulatory	$CD56^{bright}$	
	Early	$CD56^+/CD57^-$	(Nielsen *et al.*, 2013)
	Late	$CD56^+/CD57^+$	

Note: for more details of cell subsets and respective functions, please see chapter 2 section 2.4.1.

6.2.5 Incidence of illness

To assess incidence of illness, several paper copies of the Wisconsin upper respiratory symptom survey (WURSS-44) were provided at baseline laboratory testing for completion by athletes in the event of illness. This questionnaire has previously been shown to be a valid and reliable means of assessing the symptoms associated with the common cold (Barrett *et al.*, 2005). The WURSS-44 has also previously been utilised in the assessment of incidence of illness in athletic populations (Nieman *et al.*, 2014).

6.2.6 Data analysis

Blood samples could not be collected for the female participant resulting in removal from further analysis. In addition, one participant did not complete a mid-season test, and a peripheral blood sample could not be collected from two participants at the post-1h time point at baseline testing. To account for these missing samples, generalised estimating equations (GEE) were used to determine the effect of test (baseline, mid-season, off-season), time point (pre, post, post-1h), and interaction between test and time point (test*time point) on total cell count for each leukocyte subset. For each leukocyte subset, a model was created using each available working correlation matrix and the model

with the lowest corrected quasi likelihood under independence model criterion was selected (Marchiori *et al.*, 2015). A one-way ANOVA was used to investigate any differences in ingress and egress (percentage change or change in total cell count) at each laboratory test (baseline, mid-season, off-season). A one-way ANOVA was used to investigate differences in blood sample data at each time point (pre, post, post-19h) at the race event. In the event of a significant effect of time point, Bonferroni *post hoc* tests were employed to identify where differences occurred. To investigate differences between test (race, mid-season) and time point (pre, post-1h), a two-way repeated measures ANOVA (test*time point) was used. If the results of the two-way repeated measures ANOVA or GEE revealed a significant interaction (test*time point), Bonferroni post-hoc analysis was utilised to identify differences in test or time point. All data is reported as mean ± SD unless otherwise stated.

Multiple linear regression was utilised to investigate the influence of whole body vibration exposure (WBV) on cell subset redistribution from pre to post-1h time points ($\%\Delta cells_{pre\text{-}post1h}$) at the international race event while controlling for Lucia's training impulse of the race event (LuTRIMP). LuTRIMP was selected for analysis due to its superior ability to predict changes in performance parameters in chapter 5. Following multicollinearity checks to ensure Pearson's bivariate correlation values between LuTRIMP and WBV were <0.8, a simple linear regression was performed (model1 = $\%\Delta cells_{pre\text{-}post1h}$ ~ LuTRIMP) to control for LuTRIMP. A second model was then created to include WBV (model 2 = $\%\Delta cells_{pre\text{-}post1h}$ ~ LuTRIMP + WBV) where standardized beta estimates (β) were generated using lm.beta() function (Field, Miles and Field, 2012) to calculate the contribution of each component to the overall predictive ability of model 2. The ability of WBV to predict changes in $\%\Delta cells_{pre\text{-}post1h}$ was then considered as the change in R^2 (ΔR^2) between models calculated using the anova() function (R package: car v3.0-2; Fox, 2002)). WBV was therefore able to significantly predict variation in $\%\Delta cells_{pre\text{-}post1h}$ when the change in *F*-ratio from model 1 to model 2 was significant (p <0.05).

6.3 Results

6.3.1 Laboratory tests

No significant interaction was shown between time point and test for total cell count of any leukocyte cell subsets as shown in table 6.4 below. A significant increase in cell count post exercise compared to pre and post-1h values was shown for all cell subsets with the exception of CD4 senescent, CD4 Effector memory, CD8 Late, and CD4 late cell subsets (data not shown). A significant effect of test was shown for total $CD4^+$ T-cell count, whereby baseline total cell count was significantly lower than mid-season and off-season total cell count. An overview of cell subset redistribution including results of generalised estimating equation analysis is shown in table 6.4. A significant effect of test was shown for cortisol concentration, though post-hoc analysis revealed no simple main effects (p>0.05). Similarly, a significant effect of time point on cortisol concentration was shown however no significant differences were shown during post-hoc analysis (p>0.05). A significant interaction between test and time point was also revealed for cortisol concentration where post hoc analysis showed a significant elevation in cortisol concentration at post-1h mid-season compared to post-1h off-season (see Table 6.4). A significant interaction between test and time point was shown for CD4:CD8 ratio, however no significant differences were shown during post-hoc analysis.

Table 6.4: Overview of leukocyte circulating numbers of cell subset and cortisol concentration pre, post and post-1h maximal exercise test (n=8). Generalised estimating equations (GEE) were used to determine the effect of test (baseline, mid-season, off-season), time point (pre, post, post-1h), and interaction between test and time point (test*time point) on total cell count for each leukocyte subset. * denotes significant difference from baseline values and # denotes significant difference from mid-season post-1h measurement ($p < 0.05$, Bonferroni corrected).

Variable	Test	Time point			Effect of time point			Effect of Test			Interaction Time point*Test		
		Pre	Post	Post-1h	Wald-Chi squared	df	*p*	Wald-Chi squared	df	*p*	Wald-Chi squared	df	*p*
Lymphocytes (cells.µl^{-1})	Baseline	1371 ± 150	4303 ± 1429	1288 ± 306	90.19	2	.000	2.90	2	.066	1.30	4	.861
	Mid-season	1571 ± 307	4834 ± 872	1390 ± 265									
	Off-season	1636 ± 316	4717 ± 1395	1466 ± 270									
Neutrophils (cells.µl^{-1})	Baseline	2609 ± 788	4558 ± 1704	2985 ± 472	110.00	2	.000	3.12	2	.235	7.05	4	.133
	Mid-season	2962 ± 943	4929 ± 1690	4018 ± 1126									
	Off-season	2824 ± 582	4554 ± 1086	3415 ± 919									
CD8^{+} T-cells (cells.µl^{-1})	Baseline	353 ± 46	884 ± 200	313 ± 77	92.54	2	.000	2.27	2	.321	2.62	4	.624
	Mid-season	407 ± 152	1044 ± 311	332 ± 122									
	Off-season	411 ± 116	966 ± 281	373 ± 120									
CD4^{+} T-cells (cells.µl^{-1})	Baseline	580 ± 165	1020 ± 370	584 ± 233	30.01	2	.000	13.161	2	.002	8.28	4	.082
	Mid-season*	715 ± 206	1342 ± 485	663 ± 241									
	Off-season*	745 ± 231	1247 ± 461	698 ± 184									
NK cells (cells.µl^{-1})	Baseline	192 ± 73	1791 ± 1076	191 ± 98	46.23	2	.000	0.27	2	.873	4.21	4	.378
	Mid-season	194 ± 61	1737 ± 763	114 ± 50									
	Off-season	224 ± 83	1764 ± 933	146 ± 41									

Table 6.4 (continued): Overview of leukocyte circulating numbers of cell subset and cortisol concentration pre, post and post-1h maximal exercise test (n=8). Generalised estimating equations (GEE) were used to determine the effect of test (baseline, mid-season, off-season), time point (pre, post, post-1h), and interaction between test and time point (test*time point) on total cell count for each leukocyte subset. * denotes significant difference from baseline values and # denotes significant difference from mid-season post-1h measurement ($p < 0.05$, Bonferroni corrected).

Variable	Test	Time point			Effect of time point			Effect of Test			Interaction Time point*Test		
		Pre	Post	Post-1h	Wald-Chi squared	df	*p*	Wald-Chi squared	df	*p*	Wald-Chi squared	df	*p*
CD4:CD8 ratio	Baseline	1.71 ± 0.67	1.20 ± 0.51	1.96 ± 0.81	67.91	2	.000	3.22	2	.200	12.14	4	.016
	Mid-season	1.93 ± 0.89	1.44 ± 0.83	2.21 ± 1.16									
	Off-season	1.95 ± 0.91	1.41 ± 0.74	2.12 ± 1.12									
Neutrophil: lymphocyte ratio	Baseline	1.94 ± 0.78	1.18 ± 0.70	2.47 ± 0.87	40.61	2	.000	2.60	2	.272	7.09	4	.131
	Mid-season	1.99 ± 0.90	1.08 ± 0.56	2.98 ± 1.07									
	Off-season	1.83 ± 0.71	1.08 ± 0.51	2.41 ± 0.8									
Cortisol ($\mu g.dL^{-1}$)	Baseline	7.9 ± 2.3	10.6 ± 2.1	10.6 ± 1.9	12.79	2	.002	7.12	2	.028	29.52	4	.000
	Mid-season	8.2 ± 3.4	9.6 ± 3.0	9.8 ± 3.4									
	Off-season	8.3 ± 2.2	10.0 ± 3.1	7.8 ± 3.6#									

No significant difference was observed in cell subset ingress (pre - post) expressed as percentage change or change in total cell count in any leukocyte cell subset populations. Ingress (percentage change) of lymphocytes, $CD8^+$ T-cells, $CD4^+$ T-cells, and NK cells is shown in figure 6.3 below (change in total cell count data not shown).

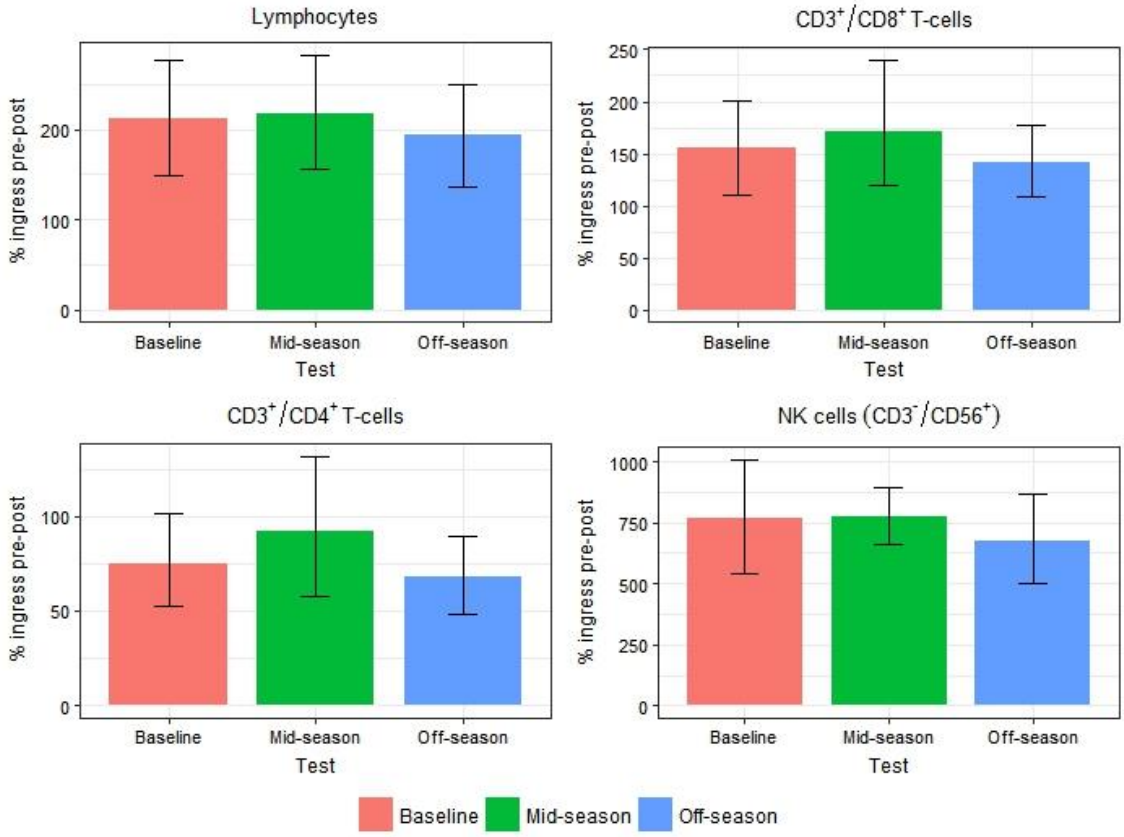


Figure 6.3: Mean ± SD ingress (% change pre to post total cell count) for lymphocyte subsets (n=8). A one-way ANOVA was used to investigate any differences in ingress (percentage change pre to post) at each laboratory test (baseline, mid-season, off-season).

Cell subset egress (post - post-1h) expressed as percentage change or change in total cell count remained unchanged for all leukocyte cell subset populations at all tests. Egress (percentage change) of lymphocytes, $CD8^+$ T-cells, $CD4^+$ T-cells, and NK cells is shown in figure 6.4 below (change in total cell count data not shown).

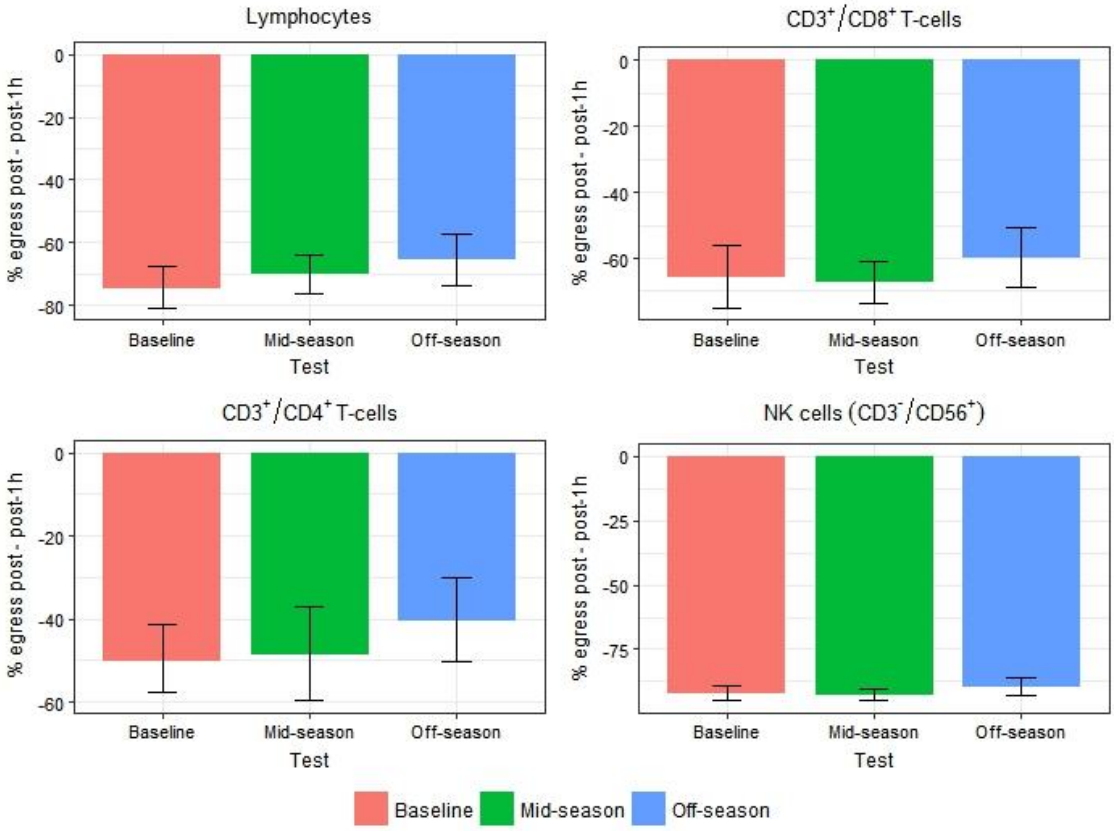


Figure 6.4: Mean ± SD egress (% change post to post-1h total cell count) for white blood cell subsets (n=8). A one-way ANOVA was used to investigate any differences in egress (percentage change post to post-1h) at each laboratory test (baseline, mid-season, off-season).

6.3.2 International race event

A significant increase in neutrophil total cell count was observed from pre to post-1h time points before returning to pre values 19 hours after the international enduro race (see figure 6.5). As detailed in figure 6.5, Lymphocyte, $CD8^+$ T-cell, and $CD4^+$ T-cell total cell count did not change significantly from pre exercise values (p >0.05).

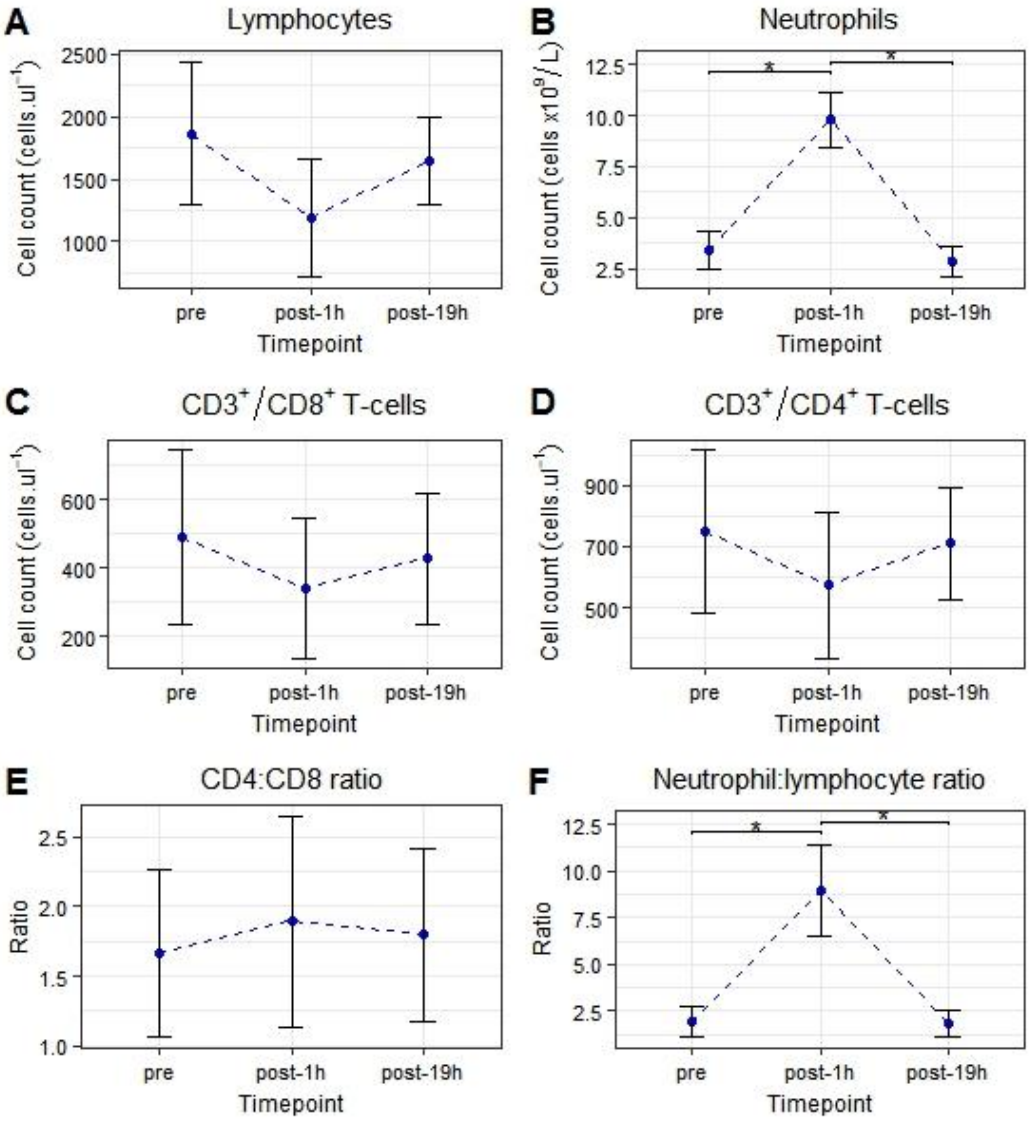


Figure 6.5: Mean ± SD (n=7) total lymphocyte (A), neutrophil (B), $CD8^{+-}$ T-cell (C), $CD4^+$ T-cell (D) counts and CD4:CD8 (E) and neutrophil:lymphocyte (F) ratio, before (pre), 1hour after (post-1h) and 19 hours after (post-19h). A one-way ANOVA was used to investigate differences in blood sample data at each time point. * denotes significant difference between time points (p <0.05, Bonferroni corrected) in the presence of a significant effect of time point (p<0.05).

A significant reduction in $CD8^+$ effector memory T-cell total cell count was observed from pre to post-1h time point ($F(2,17) = 5.14$, $p = 0.018$) as shown in figure 6.6. $CD8^+$ Intermediate T-cell total cell count was significantly reduced at post-1h time point compared to pre and post-19h time points ($F(2,17) = 10.25$, $p = 0.001$). Similarly, total cell count for NK cells and $CD56^{dim}$ NK cells were significantly reduced 1 hour after the race event compared to pre and post-19h measures ($F(2,17) = 12.6$, $p<0.001$ and $F(2,17) = 13.71$, $p<0.001$, respectively). For details of markers used to define cell subset please refer to table 6.2 in section 6.2.4 above and for further details of functions of these subsets refer to chapter 2 section 2.4.1.

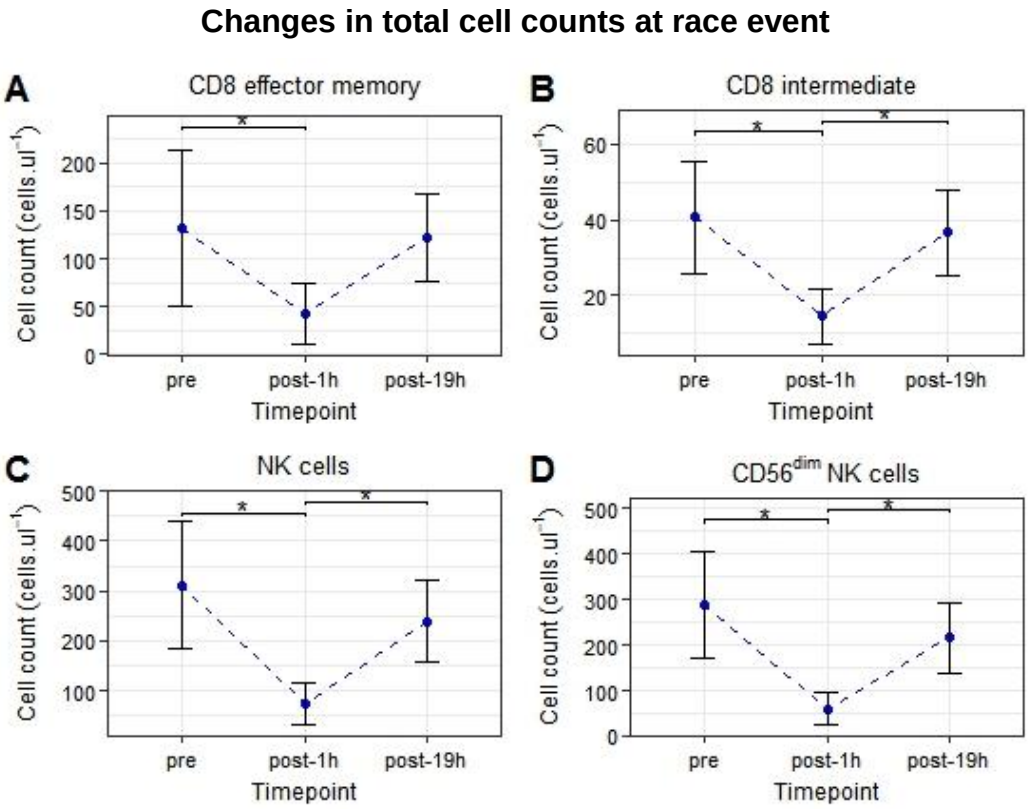


Figure 6.6: Mean ± SD $CD8^+$ Effector memory T-cells (A), $CD8^+$ Intermediate T-cells (B), NK cells (C), and $CD56^{dim}$ NK cells (D)) counts before (pre), 1hour after (post-1h) and 19 hours after (post-19h). A one-way ANOVA was used to investigate differences in blood sample data at each time point. * denotes significant difference between time points (p <0.05, Bonferroni corrected) in the presence of a significant effect of time point (p<0.05).

IL-6 concentration was significantly increased 1 hour after the international race event compared to pre and post-19h values ($F(2,17) = 10.40$, $p = 0.001$). No significant changes in cortisol concentration were observed at any time points at the international race event. See figure 6.7 for further detail of IL-6 and cortisol concentration.

Changes in IL-6 and Cortisol concentration at race event

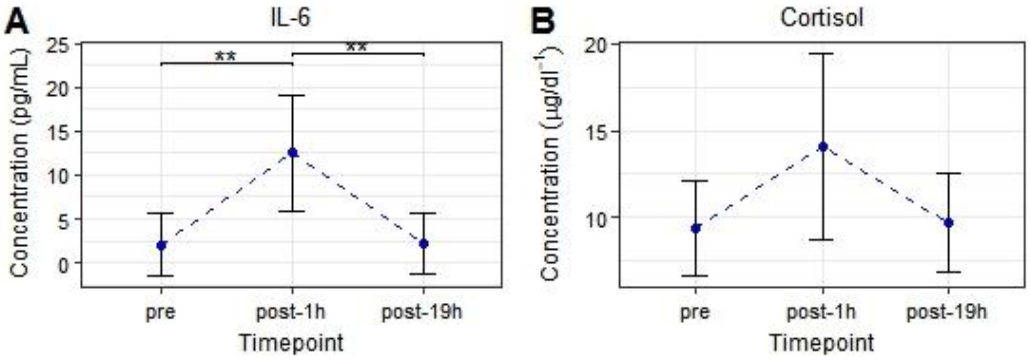


Figure 6.7: IL-6 (A) and cortisol (B) concentration before (pre), 1hour after (post-1h) and 19 hours after (post-19h) international race event (n=7). A one-way ANOVA was used to investigate differences in blood sample data at each time point. ** denotes significant difference between time points (p <0.01, Bonferroni corrected) in the presence of a significant effect of time point (p<0.05).

6.3.3 Incidence of illness

No participants returned a completed version of the WURSS-44 form provided at baseline testing. When asked to consider incidence of illness from memory, participants reported no significant bouts of illness resulting in missed days of training.

6.3.4 WBV and redistribution of lymphocyte subpopulations

TRIMP and WBV were not significantly correlated (R = -0.5, p = >.2) and simple linear regression showed no significant relationship between TRIMP and $\%\Delta cells_{pre\text{-}post1h}$ for any cell subset. After controlling for TRIMP, a significant negative relationship was shown between $CD4^+$ senescent T-cells $\%\Delta cells_{pre\text{-}post1h}$ and WBV (ΔR^2 = -0.673, $F_{(3,1)}$ = 12.12, p = 0.04). β values from model 2 (TRIMP β = -0.10, WBV β = -0.96) show the superior ability of WBV to predict redistribution of $CD4^+$ senescent T-cells $\%\Delta cells_{pre\text{-}post1h.}$ No other significant relationship was found between WBV and $\%\Delta cells_{pre\text{-}post1h}$ after controlling for TRIMP in other cell subsets, cortisol or IL-6.

6.3.5 Comparison of laboratory test and international race event

A significant interaction was observed for test*timepoint for neutrophil count ($F(1,6) = 28.24$, $p = 0.002$) where post hoc analysis revealed a significant increase following the race event but not the laboratory test as shown in figure 6.8 overleaf. A significant interaction for test*timepoint was shown for $CD8^+$ effector memory T-cell ($F(1,6) = 12.03$, $p = .013$) and $CD8^+$ Intermediate T-cell ($F(1,6) = 7.57$, $p = 0.033$) counts were significantly reduced 1 hour following exercise (pre-post1h) following both international race event and the laboratory exercise test. However, post hoc tests revealed this reduction was greater following the international race event compared to the laboratory exercise test in both subsets as shown in figure 6.8. No significant interaction was observed for test*timepoint for CD4:CD8 ratio. However, a significant interaction of test*timepoint was shown for neutrophil:lymphocyte ratio ($F(1,6) = 42.48$, $p = 0.004$) with a significant increase in ratio after the race event but not the laboratory test (see figure 6.8). A significant interaction for test*time point was discovered for $CD56^+$ NK cell ($F(1,6) = 20.56$, $p = 0.004$) and $CD56^{dim}$ NK cell ($F(1,6) = 19.68$, $p = 0.004$) total cell count. Post hoc analysis showed significantly reduced counts for both subsets following both the international race event and the laboratory exercise test (see figure 6.8). However, total cell count for $CD56^+$ NK cells and $CD56^{dim}$ NK cells were significantly higher before the international race event compared to the laboratory based exercise test (see figure 6.8). A significant interaction (test*timepoint) was shown for $CD56^{dim}/CD57^-$ NK cell total cell count ($F(1,6) = 19.27$, $p = 0.005$) and post hoc analysis showed cell count was reduced 1 hour after the international race event but not 1 hour after the laboratory exercise test. Total cell count for $CD56^{dim}/CD57^-$ NK cell was also significantly higher before the international race event when compared to total cell count before the laboratory exercise test; see figure 6.8 for further details.

Comparison of race event and laboratory data

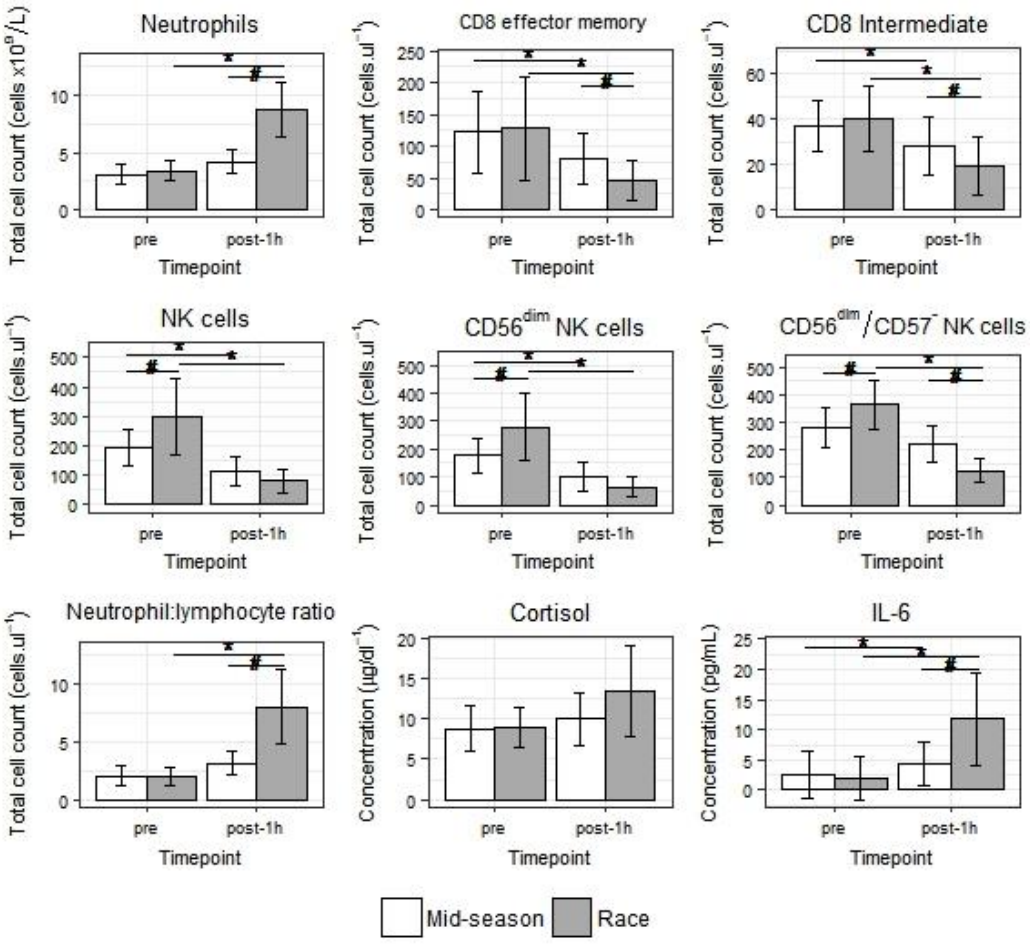


Figure 6.8: Mean ± SD total cell count and concentration (Cortisol and IL-6) for mid-season laboratory test ('mid-season') and international enduro race ('race'). A two-way repeated measures ANOVA (test*time point) was used to investigate differences between test (race, mid-season) and time point (pre, post-1h). * denotes significant difference from 'pre' values and # denotes 'race' significantly different from 'mid-season' in the presence of a significant interaction (test*time point), all $p<0.05$ (Bonferroni corrected).

6.4 Discussion

The purpose of this study was to investigate the redistribution of leukocyte cell subsets, IL-6, and cortisol in response to a laboratory based maximal exercise test at different time points throughout a season of training and competition and at an international race event in elite mountain bike athletes. This study also aimed to investigate the effect of vibration on cell subset mobilisation 1 hour after an international mountain bike race. The results of this study show that redistribution of leukocyte cell subsets remains unchanged in response to a laboratory based maximal exercise throughout both the training and competitive seasons. There was a significant redistribution of cytotoxic lymphocyte cell subsets of both the innate and adaptive components of the immune system one hour after the international race event before all cell counts returned to pre values by the morning after the event. There did, however, appear to be a differential immune response to the international race compared to the laboratory based maximal incremental testing, as $\%\Delta_{pre\text{-}post1h}$ in cell subsets with potent effector functions was significantly greater at the race event, suggesting the race event offers a greater challenge to the immune system than a laboratory based maximal exercise test. Whole body vibration was shown to be a superior predictor of $\%\Delta_{pre\text{-}post1h}$ $CD4^+$ senescent T-cells when compared to LuTRIMP on its own, suggesting whole body vibration is potentially a key component of training load measurement in mountain bike applications.

The present study is the first to assess the redistribution of lymphocyte cell subsets in response to the same exercise stressor at different time points throughout training and competition seasons. The current results showed no changes in resting cell count of lymphocyte subsets with the exception of CD4+ T-cells, which were reduced at all baseline time points (pre, post and post-1h) in comparison to mid- and off-season tests. An increase in CD4+ T-cells likely benefits the host by improved cellular immunity during periods of increased infection (Walsh *et al.*, 2011). Mechanisms for the increase in CD4 cells during mid-season and off-season tests are unclear, possibly because changes in training load (which may alter CD4+ T-cell

count; LaPerriere *et al.*, 1994) are not accurately reflected in currently available measures of training load in this cohort of enduro athletes, as discussed in chapter 5. No change in CD4:CD8 ratio was observed in the current study and mean values were all >1, suggesting the absence of viral reactivation resulting in CD8+ T-cell proliferation (Gleeson, 2007; Turner *et al.*, 2010). The current data also supports previous findings of no change in lymphocyte, neutrophil, and CD8+ T-cell count at rest in elite road cyclists undergoing heavy training (Ferrari, Gobatto and Manchado-Gobatto, 2013). The present findings also contrast previously observed increases in resting proportion of highly differentiated $CD4^+$ T-cell subsets in club level triathletes undergoing heavy training (Cosgrove *et al.*, 2012). Maintenance of resting leukocyte cell subset population shown in the elite athletes of this study may preserve host immunity, further supporting previous research showing a reduced risk of infection in elite athletes in comparison to highly active populations (Malm, 2006; Cosgrove *et al.*, 2012; Mårtensson, Nordebo and Malm, 2014). In the study of club level triathletes, training load significantly increased ~30% above baseline in the three month period preceding the Ironman event (Cosgrove *et al.*, 2012). In contrast, the training load of the current cohort of elite athletes did not increase significantly between training phases and only decreased during the regeneration phase. VO_{2peak} values were almost identical between cohorts, suggesting baseline fitness does not explain the difference in response to intensified training load (Kendall *et al.*, 1990). Instead, it appears that the 30% increase in weekly training load in the club-level athletes was sufficient to cause significant alterations in components of cellular immunity while the elite athletes of the current study were able to maintain cellular immunity, potentially due to a relatively constant training load. This supports previous work showing a 10% increase in risk of illness with every 10% increase in weekly training load (Hellard *et al.*, 2015). Appropriate management of training load and in particular avoiding increases in weekly training load >10% therefore appears to be a key component of maintaining immune health in athletes (Walsh, 2018).

The redistribution of leukocyte cell subsets after (post) and 1 hour after (post-1h) exercise in the current study is similar to that presented

previously, with the largest magnitude of ingress (pre-post) observed in $CD8^+$ and NK cells (Nieman *et al.*, 1993; Simpson *et al.*, 2008). NK cells and cytotoxic T-cell subsets expressing potent effector functions are preferentially mobilised in response to psychological and physiological stressors via β_2-adrenergic receptor stimulation (Atanackovic *et al.*, 2006; Simpson, Florida-James, Cosgrove, *et al.*, 2007; Simpson *et al.*, 2008). No significant changes were observed between laboratory tests in the current study, showing that the relative physiological and psychological stress of the maximal exercise test remained constant throughout the season. Mobilisation of NK cells and cytotoxic T-cells is largely reliant on catecholamine signalling via the β_2-adrenergic receptor (Graff *et al.*, 2018), expression of which has previously been shown to reduce during periods of heavy training in moderately trained individuals (Schaller *et al.*, 1999). However, despite large volumes of training combined with the potential for increased travel (Waterhouse, Reilly and Edwards, 2004) and competition (Fortes *et al.*, 2017) related stress, the elite athletes in the current study did not display any changes in lymphocyte subset redistribution in response to exercise. This finding supports the theory of the immune response being trainable, suggesting elite athletes do not experience significant immune disruption in response to the stimulus of training (Soppi *et al.*, 1982; Kendall *et al.*, 1990). Lastly, no change in leukocyte cell subset redistribution in response to the laboratory based exercise tests suggests the relative stress of the exercise remained constant at each laboratory visit and presented no major changes in haemodynamic forces as a result of changes in cardiac output (Nieman *et al.*, 1994; Dhabhar *et al.*, 2012). Together, these findings suggest that elite athletes are able to cope with the demands of physiological stress and training load throughout the training and competitive season, supporting the S-curve hypothesis of Malm (2006).

The reduction in peripheral blood cytotoxic NK cell subsets and T-cell subsets 1 hour after the international mountain bike race may offer a protective or suppressive immune effect as the exact location during recovery from exercise is not currently known in humans. $CD8^+$ Effector

memory T-cells do not express the CD62L homing ligand suggesting these cells are not returning to sites of likely infection such as the secondary lymphoid tissues (Shephard, 2003). $CD8^+$ intermediate and effector memory T-cells, and $CD56^{dim}$ NK cells exhibit potent cytotoxic functions as a common feature. Therefore, in the current study these cells appear to be preferentially redistributed away from the peripheral blood during recovery from exercise due to their potent cytotoxic capacity, and for T-cells, depending on the retention of proliferative capacity. This recruitment away from the peripheral blood may serve to increase immunosurveillance in other tissues, however the destinations of these cells in humans requires further investigation (Krüger and Mooren, 2007). Cells expressing CD3, including all T-cell subpopulations, have previously been shown to relocate to the Peyer's patches, lungs, and bone marrow in response to acute exercise in mice (Krüger *et al.*, 2008). This is proposed to be a positive response that serves to increase immune vigilance at potential sites of infection following exercise (Krüger and Mooren, 2007; Campbell and Turner, 2018). Furthermore, no clinical link has been made between exercise induced lymphopenia and risk of infection. It is therefore suggested that the reduction in cytotoxic lymphocyte cell subsets observed in the current study is not detrimental to the athlete and may instead offer increased protection against infection during recovery from racing (Campbell and Turner, 2018). A substantial increase in neutrophil count one hour after the race is likely due to cortisol induced release of neutrophil reservoirs from the bone marrow, however values are slightly less than the increases observed following marathon running (Kratz *et al.*, 2002; Suzuki *et al.*, 2003). Increased circulating neutrophils is proposed to be a mechanism of protection against infection or injury associated with exercise, and further to repair damaged tissue during the recovery from exercise (Neubauer *et al.*, 2013). Neutrophils can also contribute to the activation, orientation and expression of lymphocyte immune responses, including influencing NK and T-cell activity (Mantovani *et al.*, 2011). Thus, a transient increase such as that observed here, is proposed to be beneficial to the host by means of increasing orchestration of immune response to potential challenges.

All cell subset counts were comparable to resting values on the morning following the event, demonstrating that one day of international competition has an acute effect on the redistribution of leukocyte cell subsets in elite enduro mountain bikers. These results are similar to data from hill and marathon runners (Kratz *et al.*, 2002; Simpson, Guy, *et al.*, 2006) and is particularly interesting as the enduro athletes of the current study are often required to compete or train on multiple subsequent days. A return to baseline values of all cell subset counts is of benefit to the cellular immunity of the host and therefore suggests these athletes are able to recover prior to subsequent days of competition. However, these findings must be interpreted with caution as the resting cell count does not reflect potential changes in cellular function (Santos *et al.*, 2013) and subsequent response to exercise on the following day, which may differ as shown previously (Simpson, Guy, *et al.*, 2006). Accordingly, investigation of cell function and immune response to exercise on the subsequent day requires further research. These factors considered, the current data suggest that elite athletes are able to successfully cope with the demands of a year of racing and training whilst avoiding chronic alterations in resting or post-exercise lymphocyte subset cell count (Meeusen *et al.*, 2013; Simpson *et al.*, 2015; Peake *et al.*, 2017).

As the race event was of considerably longer duration compared to the laboratory test, it is to be expected that neutrophil response is significantly greater an hour after the race event (Peake *et al.*, 2017). Similarly, a decrease of CD8+ effector memory and intermediate T-cell subsets from post to post-1h is dependent on exercise duration and intensity, as reflected by a greater reduction at the race compared to the laboratory test (Simpson *et al.*, 2008). Interestingly, the reduction of the NK cell subsets from pre to post-1h shown here is also greater, but pre values are significantly increased prior to the race compared to the same time point at the laboratory test. NK cells are the leukocyte cell subset which is most responsive to psychological and physiological stress. As resting blood samples are taken prior to exercise and thus in the absence of physiological stress, it is suggested that the psychological stress

associated with the upcoming race may be the driver of increased resting counts of NK cells (Atanackovic *et al.*, 2006). This has previously been suggested as a protective mechanism where psychological stress acts to 'prime' the immune system before the stressful event has even happened (Dhabhar *et al.*, 2012; Aschbacher *et al.*, 2013). This priming has not been shown in an elite population prior to competition, however it appears to be a healthy response to psychological stress prior to competition as all athletes went on to finish in the top 10 in the elite category. In addition to alterations in leukocyte cell subset counts, IL-6 concentration was significantly increased 1 hour after both the laboratory based maximal exercise test and the international race event in agreement with previous findings (Nehlsen-Cannarella *et al.*, 1997). IL-6 concentration is much greater following the race event which may serve as means to increase delivery of energy substrate to working muscle via upregulation of hepatic glucose production and increase of fat oxidation (Van Hall *et al.*, 2003). Cortisol concentration showed a non-significant increase following the race event, however the cortisol stress response is often highly individualised (Kirschbaum, Prussner and Stone, 1995) and as such changes may not be reflected in group mean data presented here.

While the demands of this race event on the aerobic and anaerobic system have been documented previously (see chapter 4), the immune response to WBV exposure during mountain biking was not clear. The results of the current study show no relationship between traditional means of measuring training load (LuTRIMP) and leukocyte subset distribution one hour after the race. Conversely, in the current study WBV increases the percentage reduction in $CD4^+$ senescent T-cells in the peripheral blood compartment one hour after the race event. Furthermore, a significant increase in neutrophil count, neutrophil:lymphocyte ratio, and IL-6 concentration alongside significant reductions in absolute count of cytotoxic cells shows an inflammatory environment is created during the recovery from the race event (Suzuki, 2018). The results of this study also suggest a link between WBV and immune response, however more research is required to detail this relationship fully. One study has previously shown peripheral blood

count of $CD4^+$ T-cells to be reduced in response to acute vibration and further showed that the magnitude of response depended on the frequency of vibration implemented (Noguchi and Ando, 2002). No change in $CD4^+$ T-cells was observed in the current study which may be a result of transient changes in $CD4^+$ T-cell count occurring prior to the 1 hour post blood sample utilised in the current study. The findings of the current study must be interpreted with caution due to WBV measurements which do not meet strict IS regulations (ISO, 2017). Together, these findings suggest there is an inflammatory response associated with WBV exposure during mountain biking; however further research is required to corroborate this assertion (Berenbaum, 2013). Further work should aim to detail the vibration exposure during mountain biking in accordance with ISO guidelines, and further to detail the immune response to vibration with regard to pathologies associated with vibration such as OA (Bovenzi, 1998).

In conclusion, the elite athletes in the current study are able to cope with the demands of training and competition without significant alterations in redistribution of leukocyte subsets. The international race event resulted in a transient relocation of cytotoxic cell subsets of both the innate and adaptive immune system, though values returned to resting levels by the following morning. Prior to international competition, NK cell subset count is increased and appears to prime the immune system prior to the race event, proposed to be a result of psychological stress associated with competition. A significant relationship between WBV and redistribution of $CD4^+$ S T-cell suggests a link between vibration and immune response, though more work is required in the accurate assessment of vibration exposure in mountain biking.

Chapter 7: Hand-arm vibration exposure in elite enduro mountain biking

7.1 Introduction

The findings of chapters 4 and 6 suggest that racing performance and the recovery of the immune system following elite enduro racing is influenced by the whole body vibration (WBV) exposure associated with the race. Measurements of WBV within this thesis have used a GPS accelerometer incorporating a 100Hz triaxial accelerometer attached to the bicycle seat tube, chosen for its low interference with the athlete. This was the best equipment available in terms of recording frequency for mountain bike field applications at the beginning of this research project. These specifications do not meet the ISO guidelines for measurement of WBV, where a minimum recording frequency of 160Hz is required and the accelerometer must be placed between the seat and the ischial tuberosities of the athlete. While previous methodology provides a suitable estimate of WBV for the purpose of this thesis, this means that WBV exposure values reported in this thesis cannot be compared to exposure values of other activities or professions. Further, WBV measurement guidelines are primarily designed around assessing vibration exposure in a seated position, meaning optimal analysis of WBV relies on the participant remaining in contact with the seat throughout the measurement (ISO, 2017).

Mountain bike athletes adopt a standing position when descending on challenging terrain, remaining in contact with the handlebars and pedals, thus making analysis of WBV in accordance with ISO guidelines very complicated in a field setting. Mountain bike footwear and pedals are designed to spread the pressure exerted from the pedal across the entirety of the foot and footwear often incorporates materials designed to mitigate vibration (e.g. Mi6 rubber, Five Ten, USA). As a result, pain or discomfort in the feet is relatively rare in mountain biking populations (Aitken, Biant and Court-Brown, 2011). In contrast, handlebar grips feature only a thin layer (~5mm) of rubber at the interface of the handlebar with the hand, and gloves (if worn) are primarily designed to be as thin as possible to preserve dexterity. It is therefore unsurprising that mountain bike riders frequently report pain and discomfort in the hands/forearms, generally referred to as '*arm pump'* when riding off road, particularly in competition (Bell, 2018). A

severe but temporary palsy of the intrinsic ulnar supplied hand muscles and associated motor weakness following cycling has also been widely reported but it is not known if this is a result of exposure to harmful levels of vibration (Capitani and Beer, 2002; Patterson, Jaggars and Boyer, 2003; Dettori and Norvell, 2006). This suggests that measurement of hand-arm vibration during competitive mountain biking may offer a more valuable insight to the influence of vibration on performance and recovery compared to measurement of WBV. Rapid technological advances have also led to the availability of accelerometers capable of measuring vibration in accordance with ISO guidelines for hand-arm vibration without interfering with elite riders in a racing scenario (BSI, 2015). This enables standardised assessment of hand arm vibration exposure in mountain biking and allows comparisons to be made with other well documented sources of vibration, such as the workplace (Griffin, 1990).

Exposure to hand-arm vibration in the workplace is tightly controlled due to evidence linking excessive exposure to musculoskeletal, neuromuscular, vascular and other types of pathologies (Griffin, 1990). Hand-arm vibration syndrome (HAVS) is a recognised industrial disease induced by excessive exposure to vibration through occupational tasks involving vibrating machinery (Bovenzi, 1998). HAVS comprises a range of disorders affecting the peripheral circulatory system, peripheral nervous system and muscular skeletal system of the hand and arm. As a progressive and irreversible condition, the ability to predict a rate of progression and take timely preventative action through exposure reduction or complete elimination of hazardous exposure is highly desirable. Despite strict enforcement of vibration exposure guidelines in the work place, professional sports have received less attention in this context despite evidence of potentially harmful vibration exposure. However, vibration data has been considered in relation to overuse injury prevention in sports (Spörri *et al.*, 2017). There have also been significant competitive wins in road cycling where increased performance has been associated with vibration management. These include the Paris-Roubaix Gilbert Duclos-Lassalle win in 1992 and the more recent win of Peter Sagan in 2018. The Paris-Roubaix event is

a road race of which a significant proportion covers cobbled terrain leading to increased vibration exposure to the rider than tarmac featured in traditional road racing (Duc, Puel and Bertucci, 2016; Taylor, Edgar and Raine, 2018). Both winning bicycles were fitted with shock absorbing devices designed to reduce vibration induced from the cobbles encountered throughout this race. Mountain bike manufacturers have also been working to develop bicycles with increased suspension travel incorporating components such as foam filled handlebars and suspension handlebar grips designed to reduce vibration transferred to the rider (Rev Grips, 2019). Vibration exposure is therefore recognised as an important issue within the industry and among athletes yet no research, which meets the ISO guidelines, is available regarding vibration exposure in mountain biking.

Previous research has assessed the relative difference of bicycle components on the vibration induced in the hands and body of road cyclists. Lépine, Champoux and Drouet (2015) assessed the relative contribution of vibration through measurement in three locations. These included the vibration transmitted through the handlebars, saddle and brake hoods. Results showed that the handlebar and fork were the main contributors of vibration induced at the hands, whilst the frame and wheels were the main components associated with vibration induced at the buttocks of the cyclist (Lépine, Champoux and Drouet, 2015). Gomes and Savionek (2014) conducted hand-arm vibration exposure assessment on a range of pavement surfaces including asphalt, precast concrete and interlocking concrete blocks. Using an accelerometer attached to the handle bars, they determined the daily vibration exposure using a two-hour duration to represent the average time of a commuter cyclist's journey. Terrain was shown to be a key factor of vibration exposure with interlocking concrete blocks presenting significantly higher values than asphalt or precast concrete. Parkin and Eegenie Sainte (2014) provided a study of comfort and health factors including the nature of vibration from riding in different circumstances in the City of London. Findings showed that

several cyclists experience discomfort or pain after cycling, proposed to be related to vibration exposure during cycling.

Munera *et al.* (2014) summarised the different standards and guidelines associated with the evaluation of vibration and exposure limits whilst cycling. Focussing on performance athletes, they considered the application of European Directive 2002/44/EC (EC, 2002) in defining the limits of exposure and action 'triggers' for safe exposure management in sport. The current research considers the application of such exposure management to mountain bike enduro race events. Therefore, daily vibration exposure is considered in the present study with reference to the exposure action value (EAV = 2.5 ms^{-2}) and the exposure limit value (ELV = 5.0 ms^{-2}). In a limited number of studies on road cycling, harmful levels of hand-arm vibration have been reported when riding on cobbled surfaces where exposure limit values (ELV) values are exceeded in less than 20 minutes (Chiementin *et al.*, 2013; Duc, Puel and Bertucci, 2016; Taylor, Edgar and Raine, 2018). This is particularly concerning as riders competing in races such as the Paris-Roubaix spend ~90 minutes riding on cobblestones and are therefore subjected to harmful levels of hand-arm vibration. Despite the broad range of research concerning road or commuter cycling, to the researchers' best knowledge, there has been no attention given to the hand-arm and hand-transmitted vibration that mountain bike enduro athletes are exposed to. Additionally, studies that have explored magnitude of vibration experienced by downhill (Hurst *et al.*, 2013) and cross-country riders (Macdermid, Fink and Stannard, 2014; Macdermid *et al.*, 2015) were limited by the fact that they did not meet the analysis requirements of hand-arm vibration exposure in compliance with of the international standard BS EN ISO 5349-1:2001. In particular, there has been limited attention to measurement of the appropriate frequency range and the application of the appropriate weighting filters within the previous work.

Previously in this thesis (chapter 4), results showed that faster riders experienced greater vibration exposure values (r.m.s., ms^{-2}) over the

duration of an international enduro race stage, though no further detailed vibration analysis was possible. The extreme terrain, high velocities and prolonged duration warrant further investigation of hand-arm vibration in enduro mountain bike competition. As vibration exposure has been associated with the immune response to exercise in the previous chapter (section 6.3.4), a means to accurately measure vibration exposure may facilitate a more accurate measurement of training load in mountain biking applications. Therefore, the aim of this study was to assess the hand-arm vibration exposure associated with enduro mountain bike competition in accordance with ISO guidelines (BSI, 2001).

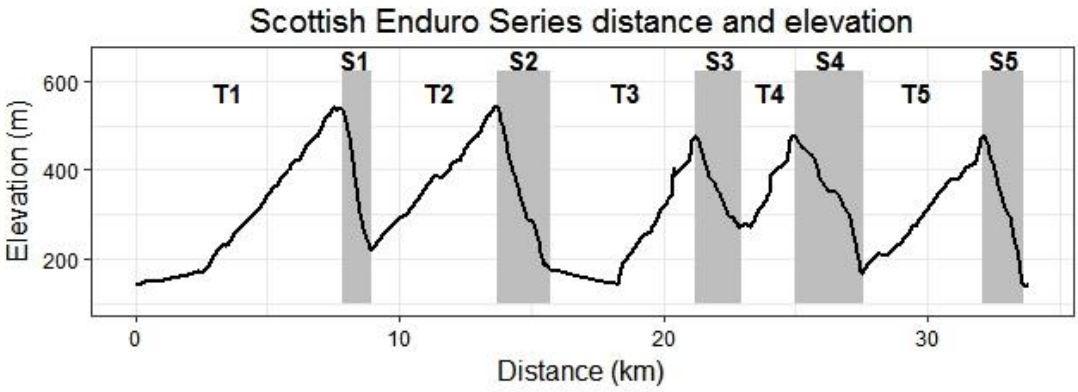
7.2 Methodology

7.2.1 Participants

Two male elite enduro athletes (athlete no. 1 age = 24 years; athlete no. 2 age = 31 years) who were either currently or recently professional athletes and previously placed in the top 10 overall positions at an Enduro World Series race were recruited for this study. Ethical approval for this study was granted by the Edinburgh Napier ethics committee in accordance with the World Medical Association Declaration of Helsinki (World Medical Association, 2001). Written and verbal consent was obtained from both participants prior to commencement of data collection.

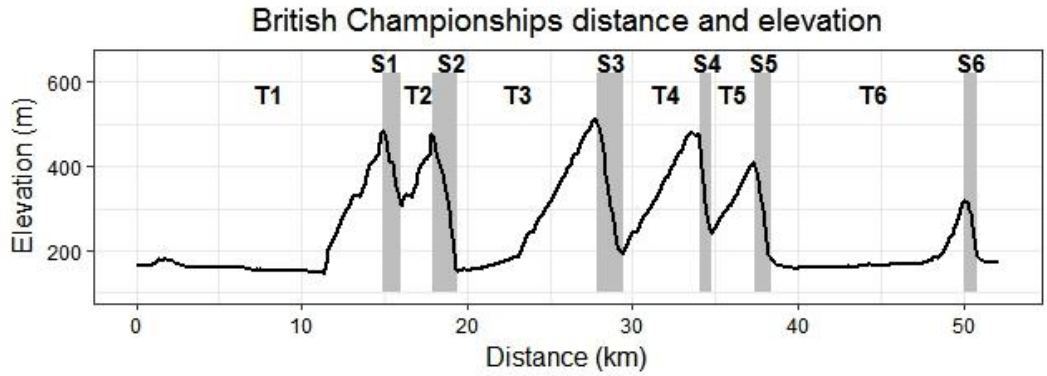
7.2.2 Race track and bicycle details

Vibration exposure data was collected during two national level enduro races; a round of the Scottish Enduro Series (SES) and the British Enduro Championship Race from the same year (BC). Elevation and distance profiles of each race event are provided in Figure 7.1 and 7.2. Data concerning the elevation, distances covered and gradients for the BC ad SES stages are provided in Tables 7.1 and 7.2. The athletes rode their own bicycles (all size large) which were set up to personal preference as detailed in Table 7.1. Athlete 1 (A1) rode a bicycle with 584mm outer diameter rims (650b) front and rear in both events while athlete 2 (A2) rode a 650b bicycle during SES and a bicycle with 622mm outer diameter rims (29er) front and rear during BC. The SES race consisted of five race stages over a distance of 33.8km with a total elevation gain of 1579m. The BC race consisted of six race stages within a 52.2km course featuring 1493m elevation gain.



Section	Distance (km)	ΔElevation (m)	Gradient (%)
Entire course	33.8	1579	-
S1	1.12	-297	-26.5
S2	1.05	-221	-21.1
S3	1.58	-198	-12.6
S4	2.52	-308	-12.2
S5	1.43	-331	-23.1

Figure 7.1: Distance, elevation and gradient details for SES race event including specific details of the entire course, individual race stages (S) and transition stages (T).



Section	Distance (km)	Elevation (m)	Gradient (%)
Entire course	52.2	1493	-
S1	0.99	-157	-15.9
S2	1.38	-298	-21.5
S3	1.40	-292	-20.9
S4	0.72	-215	-29.9
S5	0.76	-153	-20.2
S6	0.60	-114	-19.1

Figure 7.2: Distance, elevation and gradient details for BC race event including specific details of the entire course and individual race stages (S) and transition stages (T).

Table 7.1: Details of participants, bicycle components and set-up. Note: Total mass (kg) refers to the mass of the athlete wearing cycling equipment, total cycling mass refers to the total weight of the athlete and bicycle.

	Scottish Enduro Series		British Championships	
Participant	**1**	**2**	**1**	**2**
Height (cm)	181	182.3	181	182.3
Total mass (kg)	78.9	80.4	77.5	81.5
Bike mass (kg)	15.2	15.5	14.8	15.9
Total cycling mass (kg)	94.1	95.9	92.3	97.4
Tyre pressure (front/rear; psi)	22/27	18/20	22/26	20/20
Fork pressure (psi)	75	77	75	70
Fork suspension travel (mm)	170	160	170	160
Wheel size	650b	650b	650b	29
Frame	Ibis Mojo HD4	Ibis Mojo HD4	Ibis Mojo HD4	Ibis RipMo
Fork	Fox 36			
Shock	Fox Float X2			
Handlebars	Joystick Analog Carbon			
Stem	Joystick Analog 50mm			

Note: Fork, shock, handlebars and stem were the same for all tests.

7.2.3 Accelerometer and mounting position

A proprietary three axis accelerometer and data logger (Axivity AX-3) was selected as a robust and compact measurement device with suitable overall dimensions and data storage capability. The device sample rate was 3.2 kHz with a range of ±16*g*. It is essential that human vibration exposure is quantified by the vibration conditions at the interface between the environment and the human body; not by the vibration at any other arbitrary position on the body or in the vibration environment (Griffin, 1990).

However, due to the need to avoid potential interference with the riders hand grip and control ergonomics under racing conditions, a compact, lightweight and generic handle bar mount adaptor was utilised. Due to the low mass of the combined mount and accelerometer (26.432g < 5% of the handle bar, refer to BS EN ISO 5349-2:2001, Clause 6.1.5), it was deemed not to affect the vibration characteristics of the handlebars. The accelerometer mount was positioned in close proximity to the handle bar grip. The bespoke accelerometer mount was constructed from a stereolithography file using a 3D printer (Makerbot Replicator 2) and was printed from acrylonitrile butadiene styrene (ABS) thermoplastic polymer. Figure 7.3 shows the adaptor dimensions. Figure 7.4 shows the position of the accelerometer mount on the handlebar.

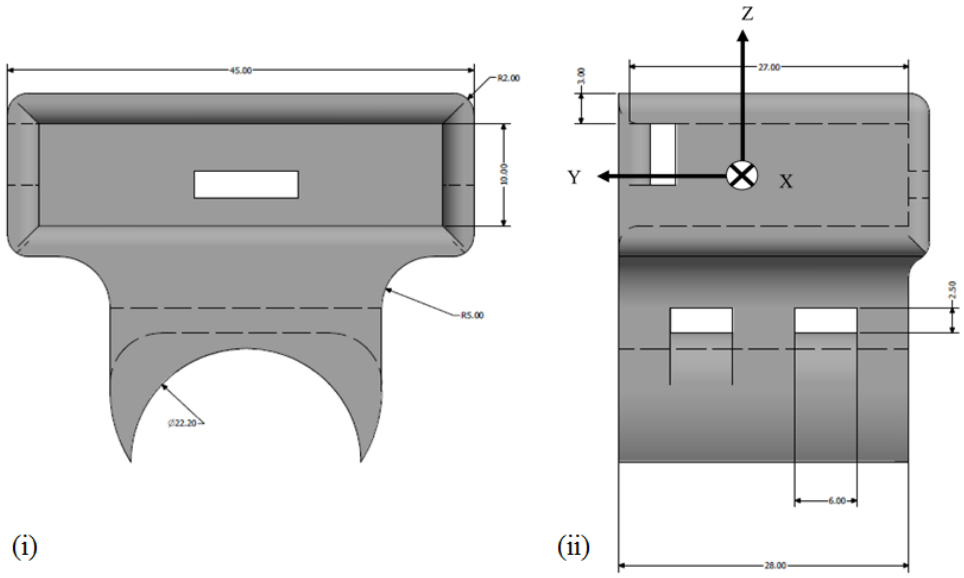


Figure 7.3: (i) Front and (ii) end elevation of handle bar accelerometer mount showing apertures for fixing ties and orientation of measurement axes.

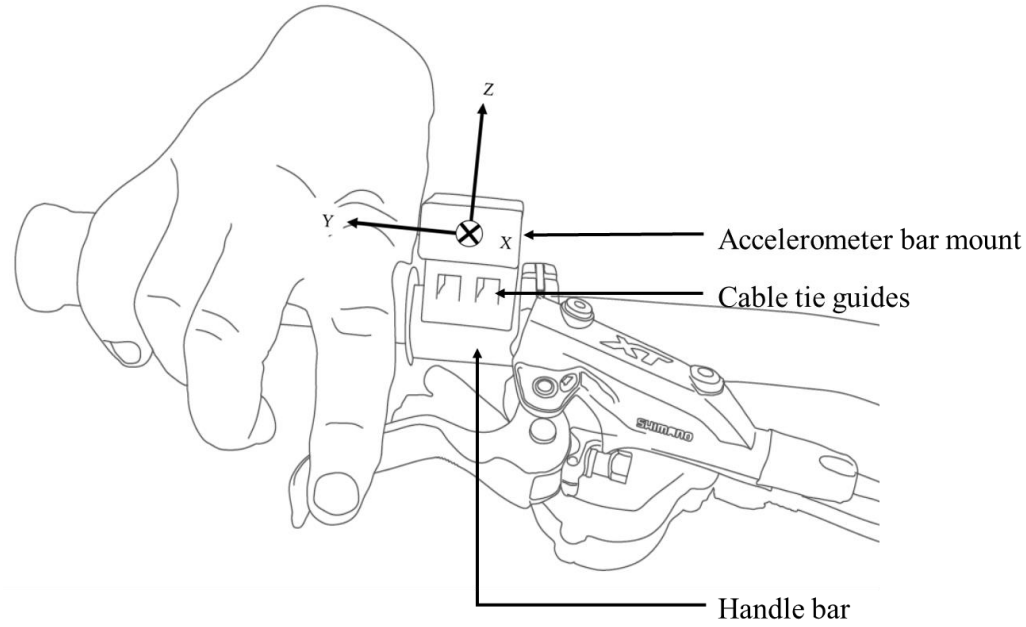


Figure 7.4: *In-situ* handle bar accelerometer mount showing proximity to hand grip.

7.2.4 Signal processing and analysis

Digital signal processing was undertaken using Matlab 2018b. Toolbox add-ons included the Control System Toolbox (Version 10.2), Digital Signal Toolbox (Version 9.4) and Signal Processing Toolbox (Version 7.4). Digital filters (W_h) were constructed in accordance with ISO 5349 (BSI, 2001) using continuous time transfer functions. Each racing stage of the race was considered as a discrete operation and as a partial vibration exposure ($A_i(8)$). Transition stages were not included in the present analysis. However, despite riders not racing, these stages may also contribute to additional partial vibration exposure over the duration of the race. The r.m.s. acceleration values (Equation 1) were calculated for each rider on each race stage (Scottish Enduro Series, Stage 1-5 and British Championship Stage 1-6). The exposure time for each stage was calculated in accordance with the official event times provided by the race organiser. The partial exposure time for each race stage was then combined to calculate the 8-hour energy equivalent vibration total value (see equation 2 and 3 below). This value can then be considered to be the race vibration exposure value. The daily vibration exposure for the rider would include all race stages, transition stages and all riding throughout

the entire day. Due to the data storage requirements of recording a rider's entire daily vibration exposure, race stage vibration exposure has been considered for the present study.

The r.m.s. acceleration value was calculated using:

$$a_{hv} = \sqrt{a_{hwx}^2 + a_{hwy}^2 + a_{hwz}^2}$$

Equation 1

where a_{hv} is the total vibration value (frequency-weighted acceleration sum), a_{hwx}, a_{hwy} and ah_{wz} are the single axes acceleration values for the axes denoted *x*, *y* and *z*. To facilitate comparison between the different stages and evaluate the individual contribution, each stage was considered as a partial stage vibration exposure calculated as:

$$A_{i,stage}(8) = a_{hvi}\sqrt{\frac{T_i}{T_0}}$$

Equation 2

The race exposure (considering racing stages only) has been calculated in the similar manner to the calculation of a daily vibration (BSI, 2015) considering the summation of the partial exposure values as:

$$A_{race}(8) = \sqrt{\sum_{i=1}^{n} A_i^2(8)}$$

Equation 3

Frequency weighted partial vibration exposure values (r.m.s., ms^{-2}) are calculated by applying the W_h weighting filter (BSI, 2001). The human sensitivity to vibration depends on (i) the frequency, (ii) the direction of vibration, both translational and rotational and (iii) the posture of the human (Giubilato and Petrone, 2012). Frequency weighting curves consider these aspects of human sensitivity. The frequency-weighting and band-limiting

filter reflected the assumed importance of the different frequencies in causing injury to the hand and arms. Band-limiting high-pass and low-pass filters are used to restrict the measured value of vibration frequencies. These filters were realised using digital methods and applied using a Matlab 2018b programme. The characteristics of the W_h filter are provided in Annex A of BS EN 5349-1:2001 (BSI, 2001).

7.3 Results

The athletes successfully finished both race events and provided complete data sets. Athlete 1 finished in first position overall in both races and athlete 2 finished in third position at SES and seventh position at BC, highlighting the elite status of these athletes. The athletes provided permission for these details to be included as it is realised that they could potentially be identifiable from these data. Details of overall race and individual stage performance are provided in Table 7.2 below.

Table 7.2: Time and percentage back from winning time for each athlete on each stage and overall for both events.

Stage	**Time (s)**		**% from winning time**	
Athlete	**1**	**2**	**1**	**2**
SES Overall	1282.8	1319.0	-	2.8
SES 1	195.47	202.27	-	3.5
SES 2	266.35	273.32	-	2.6
SES 3	259.95	270.20	-	3.9
SES 4	327.67	327.67	-	-
SES 5	233.33	245.58	-	5.3
BC Overall	928.5	1041.0	-	12.1
BC1	138.90	159.91	-	15.1
BC 2	186.80	214.58	-	14.9
BC 3	242.17	266.98	-	10.2
BC 4	141.11	158.21	+0.4	12.5
BC 5	127.44	140.44	-	10.2
BC 6	92.08	100.84	+0.5	10.1

Table 7.3 provides the overall stage time and vibration exposure (*r.m.s*) for the duration of the stage including the mean (x'), standard deviation (σ), root-mean-quad (*r.m.q.*) and partial vibration exposure ($A_{i,stage}$ *(8)*). The race vibration exposure for both athletes in both the British Championship and Enduro Series races was in excess of the ELV (5.0 ms^{-2}) in accordance with EC Directive 2002/44/EC. The greatest race vibration exposure value

was experienced by A1 at SES ($A_{race}(8)$ = 6.97 ms^{-2}) while the lowest vibration exposure was A2 at BC ($A_{race}(8)$ = 5.47 ms^{-2}). Both athletes experienced lower vibration exposure at BC compared to SES. The faster rider (A1) also presented larger stage and overall race vibration exposure values throughout both races and all stages with the exception of BC stage 2. The root mean quad (*r.m.q.*) considers the *r.m.s*. acceleration raised to the fourth power and ensures that consideration is given to the peaks in the acceleration levels. For example, the results for BC A1 Stage 2 show that the course has more peak acceleration values despite the r.m.s. value being lower that the other stages in the race. The authors propose the use of the r.m.q., alternatively known as the vibration dose value (VDV) and commonly used in whole body vibration analyses, as an indicator of the peak vibrations (or shock) experienced by the rider.

Table 7.3: Summary of vibration analysis results from British championship (BC) and Scottish Enduro Series (SES). Table

Athlete/ race		t (s)	x' (ms^{-2})	σ (ms^{-2})	*r.m.s.* (ms^{-2})	*r.m.q.* ($ms^{-1.75}$)	$A_i(8)$ (ms^{-2})	$A_i^2(8)$ (ms^{-2})
	S1	138.90	27.05	17.73	32.34	42.35	2.25	5.04
	S2	186.80	21.87	18.32	30.61	43.18	2.46	6.08
A1	S3	242.17	25.82	17.51	31.20	41.38	2.86	8.18
BC	S4	141.11	26.61	17.51	31.85	41.55	2.23	4.97
	S5	127.44	26.72	17.19	31.77	41.29	2.11	4.47
	S6	92.08	26.48	17.25	31.60	41.07	1.79	3.19
Total race		**-**	**-**	**-**	**-**	**-**	**-**	**5.65**
	S1	159.91	23.28	15.55	27.99	36.85	2.09	4.35
	S2	214.58	25.51	17.16	30.75	40.38	2.65	7.04
A2	S3	266.98	23.22	16.10	28.25	37.76	2.72	7.40
BC	S4	158.21	23.62	15.71	28.37	37.46	2.10	4.42
	S5	140.44	23.78	15.73	28.51	37.15	1.99	3.96
	S6	100.84	23.37	15.54	28.06	37.12	1.66	2.76
Total race		**-**	**-**	**-**	**-**	**-**	**-**	**5.47**
	S1	195.47	26.24	17.87	31.75	41.79	2.62	6.84
A1	S2	266.35	23.56	16.52	28.77	38.98	2.77	7.66
SES	S3	259.95	27.75	18.59	33.40	43.57	3.17	10.07
	S4	327.67	24.79	16.35	29.69	39.18	3.17	10.03
	S5	233.33	27.38	19.20	33.44	44.43	3.01	9.06
Total race		**-**	**-**	**-**	**-**	**-**	**-**	**6.61**
	S1	202.27	22.86	15.87	27.83	36.99	2.33	5.44
A2	S2	273.32	20.42	14.16	24.85	33.37	2.42	5.86
SES	S3	270.20	22.99	15.32	27.63	36.20	2.68	7.16
	S4	327.67	20.58	13.44	24.58	32.30	2.62	6.87
	S5	245.58	23.08	15.66	27.89	36.85	2.58	6.63
Total race		**-**	**-**	**-**	**-**	**-**	**-**	**5.65**

Legend: *t* = time (s), **x'** = mean acceleration (ms^{-2}), **σ** = standard deviation of acceleration (ms^{-2}), r.m.s. = root mean square (ms^{-2}), r.m.q = root mean square raised to the fourth power ($ms^{-1.75}$), $A_i(8)$ = partial vibration exposure of stage (S) or total race (ms^{-2}), A = athlete.

Figure 7.5 and Figure 7.6 show the time domain data for the maximum and minimum partial vibration (stage) exposures. Figure 7.5 shows a peak value of the total vibration (frequency weighted acceleration sum) of 144.14 ms^{-2}. Figure 7.6 shows a peak value of the total vibration (a_{hv}) of 126.15 ms^{-2}. Furthermore, BC Stage 6 also shows a considerable amount of shock impacts with high VDV of 37.12 $ms^{-1.75}$ in comparison with the other stages in race.

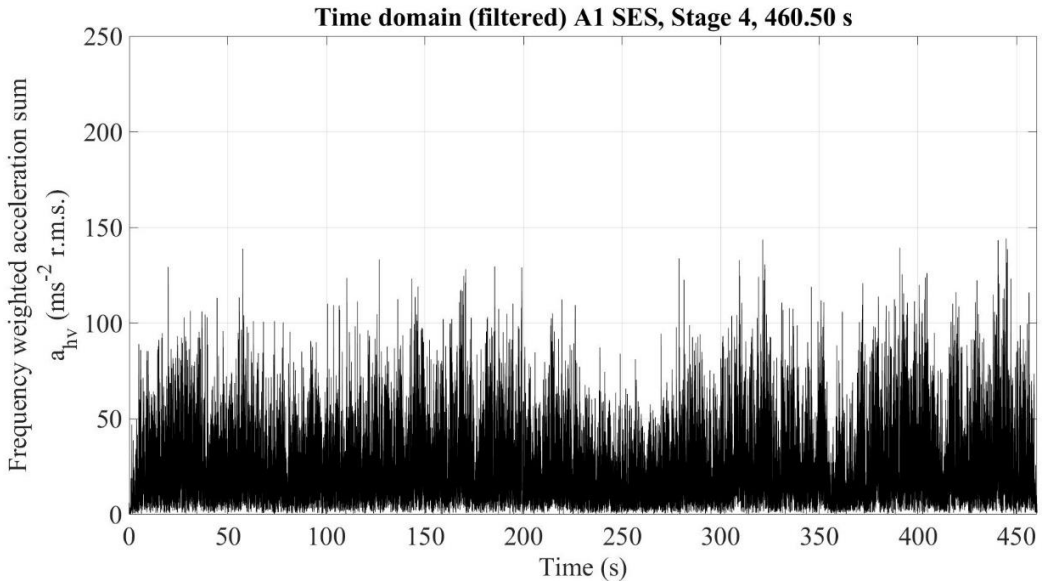


Figure 7.5: Time domain data showing magnitude and time of vibrations (SES A1, Stage 4).

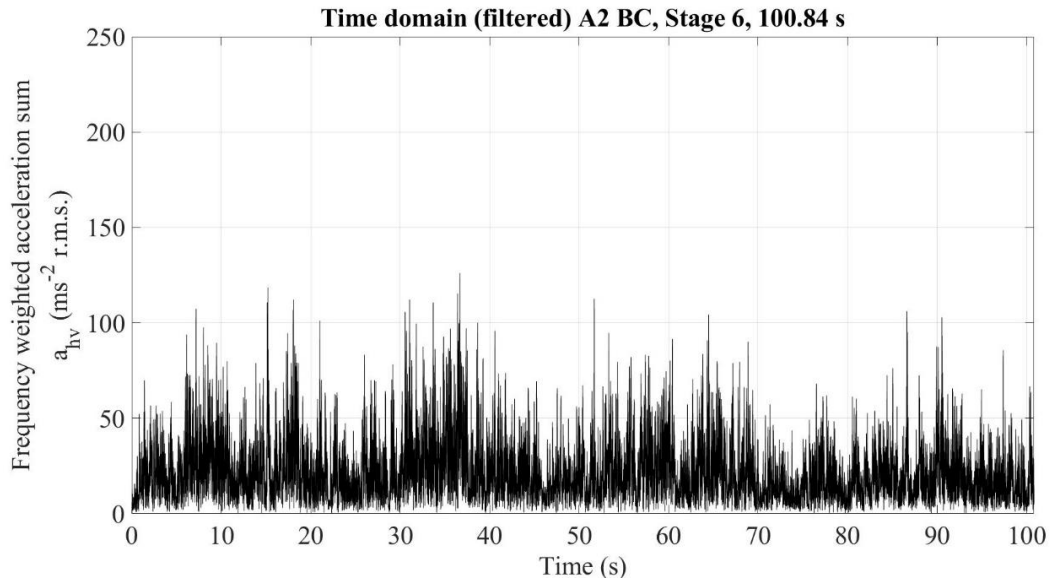


Figure 7.6: Time domain data showing magnitude and time of vibrations (BC A2, Stage 6).

Figure 7.7 and Figure 7.8 show the frequency domain data for the two stages in the SES and BC races. The race stage (A1, SES, Stage 4) with the higher partial (stage) vibration exposure shows a reduced magnitude of vibration in comparison with the lower partial (stage) vibration exposure. Power spectral density has been used to compare the power in each of the example vibration signals.

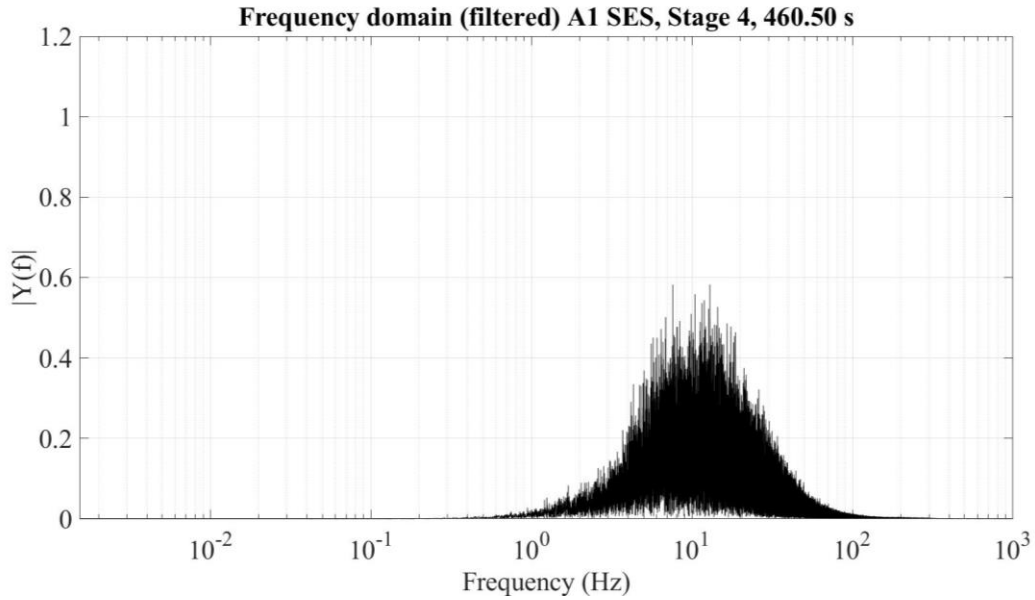


Figure 7.7: Frequency domain data showing the dominant frequencies and magnitudes (SES A1, Stage 4).

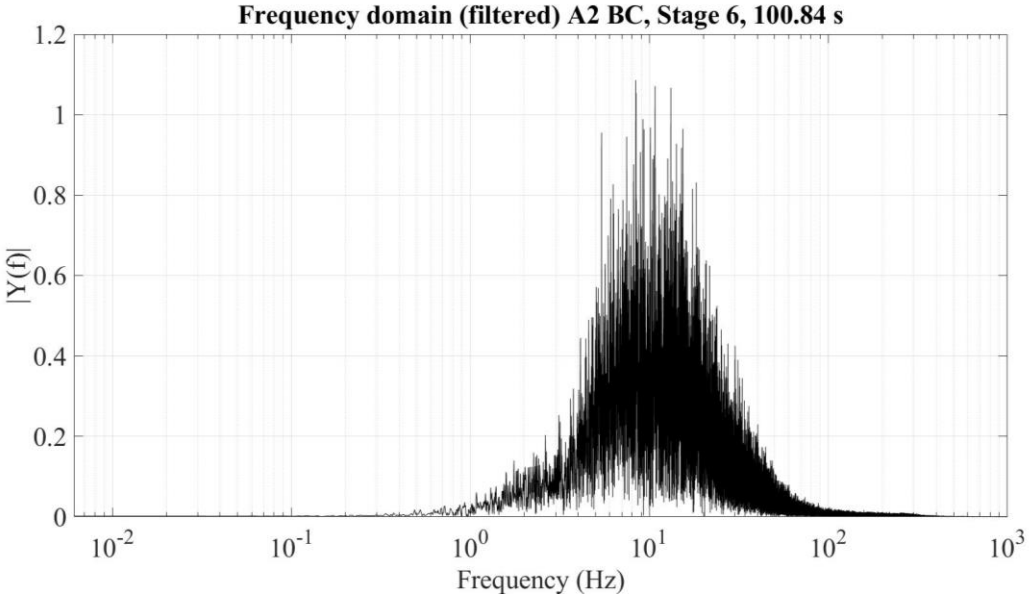


Figure 7.8: Frequency domain data showing the dominant frequencies and magnitudes (BC A2, Stage 6).

Figure 7.9 shows a power spectral analysis of the SES Stage 4 (A1). Considering the power over the frequency range 6.3 Hz to 1259 Hz, 25.19 dBW was recorded. If power of the range of 6.3 Hz to 80 Hz is considered only, 24.61 dBW as recorded. The results demonstrate that the majority of the power in the vibration signal is in a low frequency range (6.6 to 80 Hz). Two peak frequencies are shown at 25 Hz and 43.7 Hz. Figure 7.10 shows a power spectral analysis of the BC Stage 6 (A2). The power in the vibration signal was 25.13 dBW (6.3 Hz to 1259 Hz) and 24.05 dBW (6.3 to 80 Hz). These results also show that despite the overall reduction in the partial (stage) vibration exposure, the power transferred to the hand-arm system remains similar. Two peak frequencies are shown at 18.7 Hz and 50 Hz.

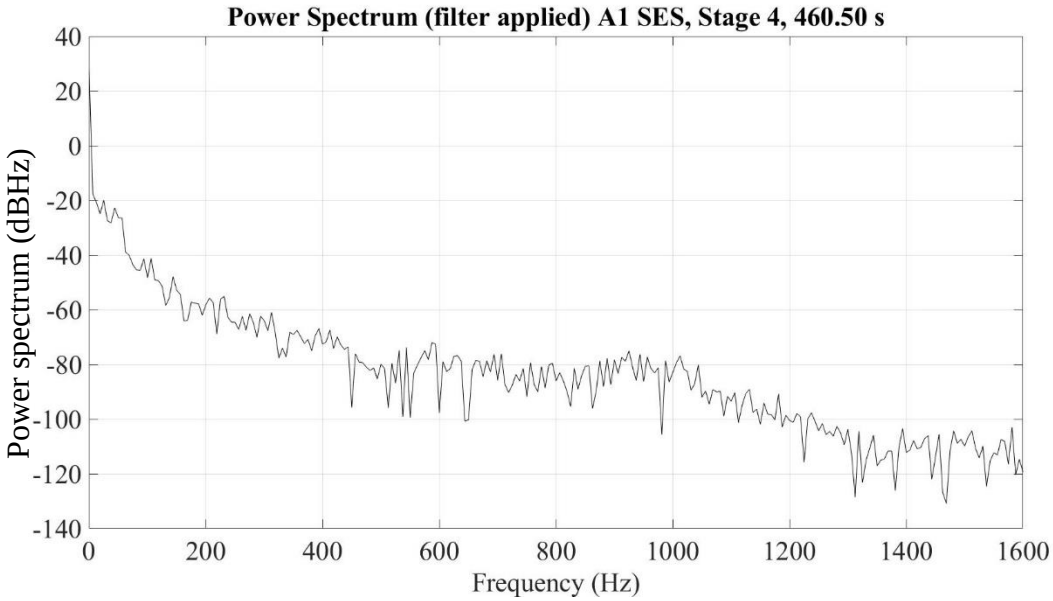


Figure 7.9: Power spectral analysis for SES A1, Stage 4.

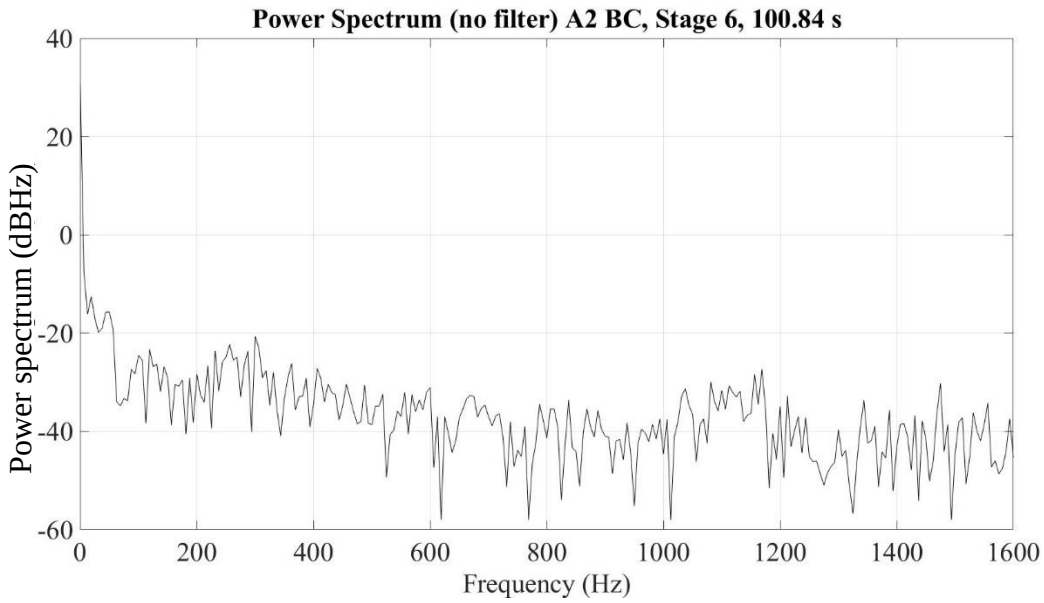


Figure 7.10: Power spectral analysis for BC A2, Stage 6.

7.4 Discussion

The results presented in this study suggest that elite enduro mountain bike athletes are exposed to potentially harmful levels of hand-arm vibration during the race stages of an enduro event. As the total race vibration exposure (A(8)) is exceeded at each event for both athletes, prolonged or repeated exposure to such levels of vibration could lead to the development of vibration related pathologies. Under the Control of Vibration at Work Regulations adopted in industrial sectors, the employers of professional athletes have an obligation to ensure they take immediate action to reduce riders' exposure to below the limit value. Furthermore, they should introduce a programme of controls or new equipment to eliminate risk, or to reduce exposure to as low as reasonably practicable. As the competitive season spans March to November and athletes potentially train on similar terrain at similar velocities it appears that prolonged exposure is a likely scenario, however more work is required to investigate this suggestion. The findings of this study are aligned with those of Duc et al (2016) who showed that ELV for hand arm vibration was exceeded during a cobbled road cycling event. However, the vibration exposure values presented here are significantly greater than those observed in cycling on a range of surfaces on a commuting bicycle (Taylor, Edgar and Raine, 2018). This suggests that mountain bike athletes are at an increased risk of exposure to potentially harmful levels of hand arm vibration, particularly when taking a prospective longer-term view of chronic exposure which requires further investigation. Chronic exposure to excessive levels of hand arm vibration similar to that presented in the current study may result in the development of osteoarthritis (OA) though a direct link has yet to be formed (Hoogendoorn *et al.*, 2015). In addition to OA, excessive vibration exposure during mountain biking which is shown for the first time in the current study may offer an explanation for the incidence of handlebar palsy in cycling populations (Capitani and Beer, 2002; Patterson, Jaggars and Boyer, 2003). Hours of vibration exposure has been correlated with reduced conductivity of the ulnar nerve in forestry workers, attributed to compression of the ulnar nerve in a similar fashion to

that reported in sufferers of handlebar palsy (Koskimies *et al.*, 1990). The current study may therefore provide evidence to explain the prevalence of handlebar palsy in cycling populations, however further work is required to explore the possibility of a dose-response relationship in cycling. These findings may offer a novel insight to the physiological response to vibration and the development of pathologies such as OA and handlebar palsy and highlight the growing necessity to monitor vibration exposure in cycling applications (Taylor, Edgar and Raine, 2018). As with almost every other physiological response to training stimuli (Goutianos, 2016), it is likely that the physiological responses to vibration, such as increased oxygen uptake or reductions in maximal voluntary contraction, are trainable factors (Bongiovanni, Hagbarth and Stjernberg, 1990; Sperlich *et al.*, 2009). Such adaptations are proposed to reduce the stress placed on the body and in turn may reduce the risk of developing vibration related pathologies. However, considerable further research is required to explore the relationship between chronic vibration exposure and development of pathologies.

The findings presented here also support previous work suggesting that faster riders encounter greater exposure to hand arm vibration (Duc, Puel and Bertucci, 2016; Kirkwood *et al.*, 2017). As prolonged vibration exposure can reduce motor output in maximal voluntary contractions, the data presented here may also offer an explanation for previous findings of ~30% reductions in grip strength during downhill mountain biking dependant on the number of impacts experienced by the rider on the day before (Florida-James, Ball and Westbury, 2010). This may have negative implications for performance both by reducing the riders grip on the handlebar that may result in loss of control and reduced ability to operate the brakes. Effective braking is an essential component of performance, as shown by experienced riders producing more braking power for shorter periods of time than inexperienced riders (Lopes and McCormack, 2017; Miller *et al.*, 2018). Therefore, it is likely that reductions in grip strength due to vibration may compromise this ability meaning the athlete has to reduce velocity during the technical terrain typically associated with race

stages in enduro, resulting in reduced performance and potentially resulting in '*arm pump*'. Overall, it appears than employing strategies to mitigate vibration exposure during enduro mountain biking will benefit performance.

Previous studies have shown different components, frames and tyre pressure to have different vibration transmission properties (Lépine, Champoux and Drouet, 2015; Macdermid *et al.*, 2015). Industry led attempts to design products focussing on the attenuation of vibration transferred to the rider are supported by the current findings, however it is not known if these proprietary components are effective in mitigating vibration. Therefore, further work is required to explore the vibration transmission of different components with the aim to find ways to reduce vibration exposure in mountain biking. Additionally, due to the rising popularity of mountain biking as a recreational sport, future studies should assess the vibration exposure in recreational settings. Many of the vibration exposure values for the individual race stages analysed here exceed the EAV level suggesting further investigation in downhill mountain biking (one timed race run) are warranted. Furthermore, the races analysed in the present study have a shorter duration (~16-25 minutes overall) when compared to EWS events (up to 60 minutes for winning rider) thus suggesting further investigation is required to measure vibration exposure during international competition.

As the addition of vibration to cycling at fixed power output reduces time to exhaustion and increases oxygen uptake but not heart rate (Samuelson, Jorfeldt and Ahlborg, 1989b; Rønnestad *et al.*, 2018), the current findings suggest that vibration exposure is a key component of physiological workload during elite enduro mountain bike racing. Current estimates of training load are based on measurement of heart rate and power output which do reflect vibration exposure and are thus unlikely to truly represent the physiological load of mountain biking (Rønnestad *et al.*, 2018). It is therefore proposed that future attempts to define training load in off-road cycling disciplines include a measurement of vibration exposure in addition

to heart rate and/or power output. Such a measurement should be completed for every training ride in order to account for differences in terrain, equipment, velocity, and movement of the rider as all of these factors can influence vibration exposure (Hurst *et al.*, 2012; Lépine, Champoux and Drouet, 2015; Kirkwood *et al.*, 2017).

In conclusion, elite enduro mountain bike athletes are exposed to potentially harmful vibration exposure values during the race stages of national enduro events. Further work is required to explore the extent of potential long-term health effects and the influence of vibration exposure on performance, physiological load and recovery from racing and training in enduro mountain biking. Consideration must be given to the use of wearable devices to monitor hand-arm and human transmitted vibration exposure during training and competition. Including training load measures associated with hand-arm vibration exposure may contribute to improved event performance.

Chapter 8: General Discussion

This chapter will provide an overview of the main findings of each study within this thesis followed by a general discussion of the key findings and the implications for athletes. The limitations of the findings included within this thesis are discussed prior to recommendations of future research in this area.

The main aim of this thesis, as stated in chapter 2, was to detail the demands of elite enduro mountain bike training and competition, and the influence of these demands on the immune system and autonomic nervous system. The individual aims for each chapter and main findings of this thesis were as follows:

Chapter 4

Aim 1: To investigate the current physiological characteristics of international elite enduro athletes;

Aim 2: To investigate the physiological demands of current international elite enduro racing including transition stages and whole body vibration exposure; and

Aim 3: To investigate relationships between physiological characteristics and enduro race performance.

Key findings:

International enduro mountain bike racing features high intensity race stages where mean HR exceeds 90% of recorded laboratory maximum HR (HR_{max}), linked by transition stages of longer duration but lower intensity (<75% HR_{max}). Challenging technical terrain also exposes riders to greater accelerations measured at the bicycle when compared to non-technical terrain, accounting for sustained heart rate observed in the absence of pedalling. Accordingly, elite enduro athletes have a large submaximal workload capacity to facilitate completion of transition stages primarily below heart rate at fixed blood lactate concentration 2mmol.L^{-1} (HR_{FBLC2}) and a large maximal aerobic capacity to facilitate high intensity workload of race stages and recovery thereafter (Kirkwood *et al.*, 2017). Absolute and relative measures of peak oxygen uptake, power output at fixed blood

lactate concentrations (2 and 4mmol.L^{-1}) and power output at respiratory exchange ratio (RER) of 1 were identified as prerequisites of enduro performance. Whole body vibration (WBV) exposure during the race event suggested potentially harmful levels of vibration exposure, howveer more work is required.

Chapter 5

Aim 1: To determine the training characteristics of elite mountain bike athletes throughout training and competition.

Aim 2: To assess how these training characteristics relate to the demands of enduro and further to identify the training load measure that shows the strongest relationship with changes in key physiological characteristics defined in Chapter 4.

Aim 3: To assess resting cardiac autonomic system parameters in relation to performance and changes in training load in a free running field setting.

Key findings:

Elite enduro athletes completed on average ~650h/year of training, a majority of which (81%) was mountain biking and training intensity distribution was comparable to other endurance sports (Neal, Hunter and Galloway, 2011; Tønnessen *et al.*, 2015). Descent per week completed when mountain biking was correlated significantly with rank within study, demonstrating the impact of sport-specific training practices on performance. Mean Lucia's TRIMP (LuTRIMP) per week was significantly correlated with change in power at FBLC2, FBLC4 and RER ≥ 1, showing superior ability to estimate training load in enduro athletes compared to other training load models. An upper limit of ~800 A.U. LuTRIMP per week was identified by signs of non-functional overreaching (NfOR) displayed in one athlete which is significantly less than values of LuTRIMP reported in physiologically comparable road cyclists (Sanders *et al.*, 2017). This discrepancy is proposed to be due to increases in fatigue caused by vibration exposure during MTB vs road cycling (Sperlich *et al.*, 2009; Macdermid, Fink and Stannard, 2015). No measure of resting heart rate was able to reflect changes in training load when using methodologies

employed by many athletes and coaches, thus suggesting it is not a suitable measure of training load in enduro MTB athletes.

Chapter 6

Aim 1: to investigate redistribution of leukocyte cell subsets in response to a maximal exercise test throughout the training and competitive season, and in response to an international enduro mountain bike race.

Aim 2: To assess the influence of vibration exposure, as measured on the bike, on the redistribution of immune cells following the international mountain bike race.

Key findings:

Redistribution of leukocyte subsets in response to a maximal laboratory test remained unchanged throughout a season of training and racing. This finding is positive and suggests elite athletes are able to deal with the stress of the laboratory tests without significantly altering disruption to the immune system. This finding may be attributed to several years of adaptation leading to a training effect on the immune system in elite athletes. This is particularly evident when comparing the relatively constant training load of the current elite athletes throughout training phases to the rapid increase in training load observed in non-elite athletes with less training history performing at a lower level (Cosgrove *et al.*, 2012; Hellard *et al.*, 2015). Cytotoxic cell subsets (Neutrophils, CD8 effector memory, CD8 intermediate, CD56+ NK cells, and $CD56^{dim}$ NK cells) of the innate and adaptive immune system were redistributed from the peripheral blood 1 hour after the international race event, however values returned to resting levels by the following morning. This is also a positive finding as it suggests elite enduro athletes are capable of recovering quickly following a race event, allowing training or successive days of racing to commence with reduced risk of immune suppression (Pedersen, Rohde and Ostrowski, 1998; Simpson *et al.*, 2015). Interestingly, whole body vibration exposure was correlated with change in CD4 senescent T-cell count pre-post1h. This is a novel finding and may suggest a link between vibration exposure and immune response (Felson, 2000; Shen and House, 2017).

Chapter 7

Aim 1: To assess the hand-arm vibration exposure associated with enduro mountain bike competition in accordance with ISO guidelines (BSI, 2001).

Key findings:

This is the first study to assess hand arm vibration exposure in mountain biking in accordance with ISO guidelines (BSI, 2001). Results show that elite enduro mountain bike athletes are routinely exposed to potentially harmful doses of hand-arm vibration during the race stages at national level enduro races. The magnitude of the vibration exposure observed is associated with hand arm vibration syndrome (HAVS), an irreversible condition which comprises a range of disorders affecting the peripheral circulatory system, peripheral nervous system and muscular skeletal system of the hand and arm (Bovenzi, 1998).

The results of this thesis show that elite enduro mountain bike athletes have a large aerobic and anaerobic capacity and are capable of completing large training loads without sustained disturbance to the immune system or autonomic nervous system. Lucia's training impulse (LuTRIMP) was shown as a superior (but not perfect) training load model in the measurement of training load in enduro mountain bike populations in chapter 5. However, vibration exposure has been shown as a crucial component of performance and training load missing from current training models such as LuTRIMP in enduro mountain bike racing. This culminated in the realisation of potentially damaging hand arm vibration exposure values recorded by elite enduro athletes over one day of competitive riding. This thesis has shown the demands of international enduro racing to be unique both in terms of the physiological demands of racing and the physiological characteristics of athletes when compared to the traditional disciplines of XCO and DH. Enduro mountain bike racing requires athletes to complete the time limited and primarily ascending transition sections at the lowest intensity possible in order to preserve precious carbohydrate stores for use during the high intensity race stages. During the race stages

heart rate is consistently elevated >90% HR_{max} regardless of terrain while greater magnitude of accelerations must be mitigated by the athlete on technical terrain compared to non-technical terrain. This is crucial as the key section of track in downhill and enduro racing has shown to be the most technical (Florida-James, Ball and Westbury, 2010; Hadden and Florida-James, 2011; Kirkwood *et al.*, 2017) and thus suggests ability to mitigate terrain induced accelerations must be developed in coordination with ability to pilot the bicycle. Vibration exposure such as that associated with navigating technical terrain has been shown increase oxygen uptake (Rønnestad *et al.*, 2018). The large aerobic capacity seen in elite enduro athletes is therefore likely a response partly to meet the demands of vibration-induced increases in oxygen consumption. This is proposed to preserve the athlete's ability to navigate technical terrain at high velocities, in turn improving performance (Kirkwood *et al.*, 2017). This assumption is further corroborated by significant strong relationships observed between faster overall race time and increased grip strength and oxygen uptake.

Athletes meet the demands of the discipline by completing a majority of training by mountain bike with a large proportion (~87.5%) of training load at HR corresponding to intensity below fixed blood lactate concentration 2mmol.L^{-1} (FBLC2). A smaller proportion of training time was designated to workload intensity between FBLC2 and fixed blood lactate concentration 4mmol.L^{-1} (FBLC4; ~7.5%) and above FBLC4 (~5%), corresponding with values presented in other elite endurance sports (Solli, Tønnessen and Sandbakk, 2017). As a group, the proportionate distribution of intensity (%time) during training approximately matches that of a day of international enduro racing as shown in chapter 4, proposed to be beneficial to performance (Seiler and Tønnessen, 2009). However, the fastest rider in the current study (world number 3) was closest to replicating the demands of racing during training phases by completing the largest volume over HR equivalent to FBLC4 (~1.25 h/wk; Buchheit and Laursen, 2013). This highlights the importance of high intensity discipline specific training in preparation for enduro racing, where a majority of the time aggregated towards the overall result is spent >90% maximum HR (Hassenfratz,

Ravier and Grappe, 2012; Kirkwood *et al.*, 2017). Within the current study, faster riders also completed greater ascent and descent per week while mountain biking, thus replicating the demands of competition and increasing training specificity (Reilly, Morris and Whyte, 2009). Increased mountain bike descending time is proposed to offer development of skills specific to downhill riding but also increased conditioning to mitigate the terrain induced accelerations and vibrations (Hurst *et al.*, 2012; Macdermid, Fink and Stannard, 2014). With equipment currently available it is not possible to simultaneously replicate both the dynamic muscle contractions and vibrations associated with downhill riding in a strength and conditioning setting. Thus, the current findings show that the most effective way to optimise training specificity and condition the rider to the demands of downhill terrain is to perform the majority of training on terrain comparable to that of enduro racing (Reilly, Morris and Whyte, 2009).

A dose-response relationship was shown between several measures of weekly training load and changes in parameters of physiological performance. Lucia's TRIMP was shown to offer the strongest relationship with multiple parameters associated with performance in enduro mountain bike racing. However, training load values in the current study are proposed to be nearing the maximum value possible before NfOR occurs, and yet are around half of those presented in road cyclists of similar physiological characteristics (Meeusen *et al.*, 2013; Sanders *et al.*, 2017). This is proposed to be due to the significantly greater mitigation of terrain induced accelerations associated with mountain biking compared to road (Macdermid, Fink and Stannard, 2015) and shows that training load measured by HR in enduro mountain biking is not comparable to that of road cycling. Accordingly, limitations to the HR based training models arise due to their inability to reflect the contribution of vibration exposure to overall workload (Samuelson, Jorfeldt and Ahlborg, 1989b; Sperlich *et al.*, 2009). This has implications for athletes and coaches using measures of training load which are employed in road cycling and illustrates the demand for the development of a mountain bike specific training model.

Previous studies that have found relationships between resting heart rate variables and changes in weekly training load used heart rate recordings of over 2 minutes in duration (Pichot *et al.*, 2000; Manzi, Castagna, *et al.*, 2009; Schmitt *et al.*, 2018). In this study we aimed to replicate the ultra-short heart rate recording duration (55 seconds) widely used by coaches and athletes in the field, previously shown to correlate with results of longer recording durations (Esco, Flatt and Nakamura, 2016; Nakamura *et al.*, 2018). The results of this thesis show that current the practice employed in the field does not reflect changes in training load, despite signs of NfOR (increased training load accompanied by reduction in performance; (Meeusen *et al.*, 2013)). While ultra-short recording durations correlate very well with gold standard ECG in healthy individuals at rest (Esco, Flatt and Nakamura, 2016), changes in cardiac autonomic activity associated with NfOR may only be reflected in longer recording durations (Bellenger *et al.*, 2017; Schmitt *et al.*, 2018). It is therefore suggested that resting heart rate recordings of 2 minutes or longer may offer greater insight to training adaptations in elite athletes (Bellenger *et al.*, 2016). Additionally, it is not possible to control for confounding factors such as light exposure, temperature, hydration status, life stress, and competition anxiety across a competitive season featuring multiple bouts of international travel (Waterhouse, Reilly and Edwards, 2004; Plews, Laursen, Stanley, *et al.*, 2013; D'Ascenzi *et al.*, 2014; Bellenger *et al.*, 2016).

Vibration exposure has also been shown to acutely influence autonomic nervous system (ANS) activity dependant on frequency and duration of vibration exposure (Jiao *et al.*, 2004; Zhang *et al.*, 2018). We used the HR based training load model which appears to represent training load most accurately in order to monitor ANS response, however this model does not account for vibration induce fatigue and thus does not reflect the influence of vibration on ANS activity. Therefore, the development of a mountain bike specific training load model accounting for the influence of vibration may alter the relationship currently observed between training load and ANS response. The current study also demonstrated no link between maximal aerobic capacity and resting heart rate parameters, contrasting

findings in recreational athletes (Buchheit *et al.*, 2010). This notion is corroborated in the current study by a significant relationship between maximal aerobic power and reduced race time but no relationship between performance and resting heart rate measures. Together, this suggests that resting heart rate measures cannot be used as an estimate of performance in a population of elite enduro mountain bike athletes. Therefore, laboratory based assessment of physiological parameters remain the most valuable estimate of performance to the athlete (Paton and Hopkins, 2001; Reilly, Morris and Whyte, 2009). At present, this thesis shows that current practices of ANS monitoring in elite enduro mountain biking have limited practical applications. Future research should aim to investigate the use of longer recording duration and use of a training model which includes vibration exposure, a proposal discussed in greater detail later in this chapter.

Lymphocyte cell subset redistribution in response to laboratory based maximal exercise test did not change in the current study despite the stress of international competition, travel and training load. The influence of exercise intensity and thus demargination of leukocyte cell subsets due to shear stress was held constant (Foster *et al.*, 1986; Arber *et al.*, 1991). Therefore, consistent redistribution of leukocyte subsets observed in the current study suggests limited alteration in the sensitivity or density of β_2-adrenergic receptors and glucocorticoid receptors, however more research is required to confirm this assertion (Duclos, Gouarne and Bonnemaison, 2002; Krüger *et al.*, 2008; Graff *et al.*, 2018). The elite athletes of the current study were subjected to the stress of large training loads alongside travel (Waterhouse, Reilly and Edwards, 2004), competition (Fortes *et al.*, 2017), and daily life (Edwards *et al.*, 2018). Therefore, this finding suggests these elite athletes are managing training load successfully, allowing them to cope with the demands of training and racing without significant alterations to the immune system and its response to exercise. This supports previous evidence of leukocyte redistribution in response to exercise being trainable over time, in turn gradually increasing the training capacity of the athlete (Hellard *et al.*, 2015). The substantial training history

of the athletes included in this study (>10years) likely facilitates the described gradual training effect and together the results of this thesis support the S-curve hypothesis (Malm, 2006).

Circulating numbers of cytotoxic cells of the adaptive and innate immune system were significantly altered one hour after the international enduro race. Leukocyte cell subset redistribution following exercise is an ongoing subject of debate within the literature and as such it is not confirmed as beneficial or detrimental to the host at present (Kakanis *et al.*, 2010; Campbell and Turner, 2018). As incidence of infection increases following large social gatherings (Choudhry *et al.*, 2006) and immunity may be compromised following endurance exercise (Kakanis *et al.*, 2010), it is suggested that appropriate recovery practises are implemented in the hours immediately following the race to limit risk of infection. Human rhinovirus is able to replicate more robustly in temperatures slightly below body temperature (33-35ºC) hence athletes should seek a warm environment or at least wear warm clothing to limit a reduction in nasal cavity temperature where possible (Foxman *et al.*, 2015). In addition, sufficient quantities of carbohydrate and protein must be consumed both to restore muscle glycogen stores and to provide energy to mobilised lymphocyte populations (MacIver *et al.*, 2008; Bishop *et al.*, 2009; Howie *et al.*, 2018). The destination(s) of mobilised lymphocyte subsets which have left the peripheral blood is not known in humans, though the strongest argument in the literature suggest cells are relocated to sites of potential infection such as the lung, Peyer's patches and bone marrow (Krüger *et al.*, 2008). Though the destination of lymphocytes leaving the peripheral blood is not currently known, vibration was shown to be responsible for 67% of variation in the reduction of $CD4^+$ senescent T-cells 1 hour after the enduro race event. However, the relationship between the immune system and vibration exposure requires considerably more work. These data further signify the importance of including vibration in future models of training load in mountain bike applications.

Measurement of hand-arm vibration exposure in mountain bike enduro racing provided novel data showing athletes are exposed to potentially damaging levels of hand-arm vibration within one day of competition (BSI, 2015). Chronic hand-arm vibration exposure of this magnitude is associated with development of OA and ulnar nerve compression in working populations (Koskimies *et al.*, 1990; Bovenzi, 1998). The employers of professional enduro athletes (i.e. bicycle manufacturers) are obligated to reduce vibration as much as reasonably possible under the Control of Vibration at Work Regulations. Some bicycle manufacturers are already designing products aiming mitigate vibration transferred to the rider, however no standardised testing has compared the vibration mitigation properties of these products. Hand-arm vibration exposure of this magnitude is proposed to cause reductions in grip strength which may limit braking ability, thus reducing performance and increasing the likelihood of loss of control (Färkkilä *et al.*, 1980; Florida-James, Ball and Westbury, 2010; Burr *et al.*, 2012; Chiementin *et al.*, 2013). Enduro World Series races comprise of race stages longer than those analysed for hand-arm vibration exposure within this thesis, likely resulting in even greater exposure values. Multiple races per season coupled with the large volume of training completed on downhill terrain suggests that long term monitoring of vibration exposure and subsequent physiological and immunological response is warranted. Further, although vibration exposure is shown to increase oxygen consumption, the physiological demands of this magnitude of vibration exposure and resulting fatigue are not currently known (Samuelson, Jorfeldt and Ahlborg, 1989b; Sperlich *et al.*, 2009). It is proposed that vibration accounts for a majority of the discrepancy in training load values between road cycling and enduro mountain biking populations, however more work is required to develop a training load model incorporating vibration.

8.1 Limitations of work included within this thesis

This thesis is limited by a relatively low number of participants, partly due to the inclusion criteria, particularly with regard to sections concerning the

immune response to exercise and hand arm vibration exposure associated with enduro racing. Another potential limitation of this thesis is the lack of completion of any Wisconsin upper respiratory symptom surveys (WURSS-44, Barrett *et al.*, 2002) which. Incidence of illness would allow calculation of odds ratio in relation to training load increases and upper respiratory tract infection as previously completed in elite athletic populations (Hellard *et al.*, 2015). An electronic equivalent such as a phone application may increase adherence in future longitudinal studies and reduce reliance on recall ability (Smyth and Stone, 2003). Collection of training data in a field setting requires the compliance of the athlete, which ultimately remains outside of the control of the researcher. However, athletes confirmed the presence of all training sessions at each laboratory visit, suggesting missing data is minimal. No data on strength training was returned in the current study, which is proposed as a further limitation ultimately out with the control of the researcher

8.2 Future research directions

Chapter 4 described the demands of an international enduro race, highlighting the influence of transition stages and vibration mitigation on overall race performance. Further, athletes were shown to poses physiological characteristics specific to the discipline of enduro. Future research should aim to investigate:

1. The demands of Enduro World Series racing
2. The demands of enduro racing using mechanical uplift vs pedalling
3. The anaerobic capacity and strength characteristics of elite riders

The results of chapter 5 show that faster riders completed greater ascent and descent per week by mountain bike and further highlighted the importance of high intensity training volume. LuTRIMP was shown as the best training model currently available but values are not comparable to road cycling and resting heart rate measures did not reflect changes in training load. Therefore, future work is warranted to investigate:

1. Development of a training load model incorporating vibration
2. The ANS response to the proposed vibration training model

Leukocyte redistribution was shown to remain constant throughout a year of training and competition in Chapter 6. Cytotoxic cell subsets were redistributed one hour after the race and returned to normal after 19 hours. Reductions in circulating $CD4^{+}$ senescent T-cells were related to magnitude of whole body vibration exposure. These findings warrant further research aiming to:

1. Investigate the influence of vibration on the redistribution of leukocyte cell subsets, particularly synovial CD4+ T-cells, in response to hand-arm vibration exposure.
2. Use vibration exposure associated with mountain biking to investigate the link between hand-arm vibration exposure and the development of vibration related pathologies such as OA and compression of the ulnar nerve.

The data of chapter 7 showed elite enduro riders are exposed to potentially damaging values of hand-arm vibration during one day of competition. This finding encourages further research aiming to:

1. Quantify the vibration transmission properties of individual bicycle components
2. Assess the influence of equipment settings such as tyre pressure and suspension setting on vibration exposure
3. Measure the effectiveness of products designed to reduce vibration transferred to the rider with a view to minimise vibration exposure during mountain biking.

In the future when investigating enduro mountain biking it would be beneficial to widen the eligibility criteria or recruit a larger number of elite participants, perhaps in different geographical locations. Nonetheless, the current thesis shows that vibration contributes significantly to training load and has a significant impact on performance. Future research should aim to collaborate with the industry to investigate means to reduce vibration exposure within training and racing but also at a recreational level (Taylor, Edgar and Raine, 2018). It would be beneficial to all cycling disciplines to develop a measure of training load incorporating vibration. This would require industry collaboration in order to create the necessary equipment to collect sufficient vibration data in a practical manner alongside heart rate and GPS data. This would facilitate the measurement of vibration exposure over long periods of time to assess development of vibration related pathologies but also crashes and subsequent injury as a result of loss of vibration induced loss grip strength.

Further to this, considerably more work is required to investigate the potential physiological and/or immune responses to vibration. Currently, no research has aimed to explore the physiological or immunological response to excessive vibration which leads to the development of vibration related pathologies such as OA. In order to address this, it is suggested that proteomics techniques are employed alongside flow

cytometry analysis of functionally discrete cell populations to investigate changes in physiology associated with vibration exposure. Ideally, investigations would be performed longitudinally, following young riders through a career of mountain biking with all vibration exposure and physiological/immunological responses monitored regularly throughout. Genotyping may allow further identification of dose-response training effects associated with vibration exposure or predisposition to vibration related pathologies. We are only just beginning to understand the prevalence of potentially harmful vibration in cycling and hence a considerable body of further research is required to understand the implications for all cyclists.

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Appendix I: Participant information

Edinburgh Napier UNIVERSITY

Participant Information

The physiological characteristics of elite enduro mountain bike riders: the influence of training status, sleep and recovery

The aim of this study is to understand the stresses of training and racing placed on elite international enduro mountain bike athletes throughout the season. Understanding the demands of enduro will allow coaches and athletes to tailor training load to help athletes achieve optimum performance whilst avoiding a state of overtraining.

This study plans to monitor the body's response to daily training input which requires information of heart rate and duration of all training sessions including resistance training (gym work), road riding, skate park and any other sports. The main researcher, Lewis Kirkwood, is the only person able to view this information. A non-disclosure act will be drafted and presented to the coach and athlete. The main researcher presents no conflict of interest in training an elite enduro mountain bike rider.

The proposed study duration is **12 months** (December 2016 to December 2017 **inclusive**).

The body's response to training and racing will be measured by several methods:

- **Daily** ithlete recording of heart rate upon waking in order to calculate heart rate variability; a measure of autonomic nervous system activity.
- A **daily** response to a short questionnaire of mood, fatigue, sleep, stress and diet (available as an app)
- **Three single day visits over one year** (this is the **second**) to Edinburgh Napier University's human performance laboratory to assess performance and the immune response to exercise (more details available below)
- **Once per week** orthostatic test. You will be asked to lie down for 3 minutes and then stand up for 3 minutes to assess the heart rate and heart rate variability in response to standing up.
- **Once per month** Submaximal Test performed remotely, i.e. no lab visit required. This test should be performed on a turbo trainer. The test can be used as a warm up for a training session.

The **single day** visits to Edinburgh Napier University's human performance will ideally be following a period of regular training, intense training and competitive season subject to discussion. The visit will consist of:

- Height, weight, waist and body composition
- Intravenous blood sample at rest, immediately after exercise and 1h after exercise protocol.
- Graded exercise test to exhaustion to measure blood lactate concentration in response to exercise and peak oxygen uptake. The workload (power) increases gradually until voluntary exhaustion or when cadence above 60rpm cannot be maintained.

Intravenous blood will be analysed for circulating immune cells (natural killer cells and T-lymphocyte subsets) and other markers of stress such as cortisol, epinephrine and norepinephrine. Immediately following exercise circulating levels of immune cells assessed here are increased which is good for fighting infection. However, 1-3h following exercise these levels are then reduced below baseline - referred to as the 'open window' for infection. If insufficient recovery time is allowed circulating levels cannot recover properly, the immune response to exercise is compromised and illness may follow.

In addition to the laboratory visits an **international race** will be used to assess the immune response to an international race. Tweedlove International will be used as the race event. An intravenous blood sample will be taken in a clinical area prior to the event, immediately post event and 18 hours post event and analysed by the same methods as the laboratory visit.

Where possible throughout and after the study athletes will be provided with their own data. All data will be presented as mean and data will be presented anonymously. You have the right to withdraw from the study at any point throughout without having to provide reason or facing penalty for doing so.

If you are interested and would like to participate then please contact Lewis Kirkwood (email: [redacted] Mobile [redacted].

Appendix II: Informed consent

Edinburgh Napier UNIVERSITY

Participant consent form

Date: Laboratory Visit: mid season .

Physiological characteristics of enduro mountain bikers: the influence of sleep, training status and recovery

You are asked to complete the form below regarding your current health. In accordance with the data protection act 1998 all the information you provide will be held securely and treated in the strictest confidence. This information will be viewed only will not be shared with anyone else unless this is: with your agreement, required by law or to protect your vital interests.

If you would like to speak to someone who has knowledge of this project but is independent of the research team then please contact: Dr Mick Rae (email: [redacted] telephone [redacted]

Blood Pressure if applicable: *Systolic* ☐ *Diastolic* ☐

GENERAL HEALTH INFORMATION

Look carefully at the following list and tick which symptoms apply to you. If you feel necessary please discuss with the experimenter whether you should exercise.

Allergies	☐	**Arthritis/swollen/stiff/painful joints**	☐
Asthma	☐	**Chest Pains / Discomfort**	☐
Cold or flu like symptoms (in past week)	☐	**Diabetes**	☐
Epilepsy	☐	**Heart or Lung trouble**	☐
High Blood pressure	☐	**Orthopaedic problems**	☐
Palpitations	☐	**Shortness of breath**	☐
Other ☐	☐	**None of the above**	☐

Please read the following carefully:

If you suffer any unusual or any unexpected symptoms during the activity, please stop immediately. If you experience any such feelings once the experiment/test period is over, please consult the Experimenter or, if they occur after leaving, please consult your own doctor.

Declaration

I…….....volunteer to be /continue to be an experimental participant in this experiment.
I have read and understood the practical descriptor provided and the experimenter has explained to my satisfaction the purpose of the experiment/test and possible risks involved.
I understand that it is my responsibility to advise the experimenter to any changes in my health during the course of the study.
I understand that I may withdraw from the activity at any time and that I am under no obligation to give reasons for withdrawal.
I undertake to obey the laboratory regulations and the instructions of the experimenter regarding safety, subject to my right to withdraw as declared above.

Name of participant: _____

Signature of participant: _____

Signature of researcher: _____

Date: _____

Contact details of the researcher

Name of researcher: Lewis Kirkwood ([redacted])
Address: Sports and Exercise Science, School of Life, Sport and Social Sciences, Edinburgh Napier University, Sighthill Court, Edinburgh, EH11 4BN
Telephone: [redacted]

Appendix III: Par Q & You

Edinburgh Napier UNIVERSITY

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE

Appendix IV: Published article

J Sci Cycling. Vol. 6(2), 13-21
DOI: 10.28985/171231.jsc.10

RESEARCH ARTICLE **Open Access**

Physiological characteristics and performance in elite vs non-elite enduro mountain biking.

Lewis A. Kirkwood [1], Lesley A. Ingram[1], Jamie Cunningham [1], Eva Malone [1] and Geraint D. Florida-James [1]✉

Abstract

Enduro mountain bike racing is composed of several timed predominantly downhill race stages linked by time restricted, non-competitive transition stages. This study aimed to 1) detail and compare the laboratory assessed physiological characteristics of elite and non-elite enduro mountain bike riders, and 2) evaluate the use of 10Hz global positioning systems (GPS) unit including a 100Hz triaxial accelerometer to define the demands of enduro mountain bike racing and identify components of successful performance. Eleven male enduro mountain bike riders completed laboratory protocols for peak aerobic capacity (VO_{2peak}), fixed blood lactate concentrations (FBLC: 2 and 4 mmol.L^{-1}) enduro specific test (EST), and anthropometry measures. Participants were divided into elite (*n*=5) and non-elite (*n*=6) groups for analysis. Nine (*n*=9) elite enduro mountain bike athletes participated in field data collection at an international enduro mountain bike race. Two race stages were used for analysis of velocity, accumulated load, heart rate and time to complete specific sections of track calculated from GPS units placed on the bicycle seat mast and the rider's torso. Elite athletes produced greater power during the EST (475± 15W vs 390 ± 31W) and at VO_{2peak} (417 ± 29W vs 363 5 ± 30W), FBLC 2mmol.L^{-1} (267 ± 39W vs 198 ± 36W), FBLC 4mmol.L^{-1} (318 ± 31W vs 263 ± 25W) when compared to non-elite riders (all $p<0.05$) with no significant differences in anthropometry ($p>0.05$). Accumulated load was significantly greater on the bicycle than the rider on both stages ($p<0.05$) and load in both locations was significantly higher during technical terrain compared to non-technical terrain ($p<0.05$). GPS analysis allowed detailed analysis of performance showing winning performances were characterised by reduced time to complete both technical downhill and non-technical climbing sections during race stages. In conclusion, successful performance in enduro mountain bike racing requires ability to sustain high velocities over technical and non-technical terrain coupled with large aerobic and anaerobic capacities.

Keywords: Mountain biking, enduro, performance, physiology.

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Received: 10 February 2017. Accepted. 5 December 2017.

Introduction

Enduro racing compromises of timed downhill sections of trail and non-competitive transition sections that must be completed within the provided time limit but do not contribute to overall race result. Enduro World Series (EWS) races feature a minimum of two race stages per day with a minimum of four stages per event, a maximum elevation gain of 2000m for a single day event and 3200m for a two-day event. Athletes ascend by pedalling or use of mechanical uplift to meet their time checks at the start of each race stage and overall time spent riding is between 3 and 9 hours per day. According to EWS guidelines race stages should comprise of a maximum of 20% ascending terrain. The remaining 80% will feature terrain similar to that of downhill (DH) racing designed to test the riders' technical ability. The winner of the general classification (GC) is the rider with the lowest combined time to complete all race stages. GC time must exceed 20 minutes per day of competition for the fastest rider overall, averaging 40 minutes for each round for the winner in 2017 with a mean total ride time of approximately 6h 40min per day of competition (Enduro World Series 2017).

Previously, research has concentrated on cross country (XC) and DH disciplines and demonstrated that elite athletes have specific characteristics suited to the demands of their chosen discipline. For example, XC athletes have larger aerobic capacity and produce greater power across bouts ≥15s when compared to DH athletes who produce greater power over durations <15s reflecting the longer duration of XC (~1.5hrs) compared to that of DH (~4mins) (Baron 2001; Novak & Dascombe 2014; Stapelfeldt et al. 2004). The rapid evolution of enduro has led to a large expansion in the number of professional enduro athletes resulting in a gap in knowledge concerning the demands of enduro racing and the physiological characteristics of successful enduro athletes. The prolonged total duration of enduro (3-9hrs), the time constrained uphill transitions (up to 2.5hrs alone) and average heart rate (HR) of >90% maximum (Hassenfratz et al. 2012) for up to 20 minutes per race stage suggests a considerable demand is placed on the aerobic system and thus provides rationale to

assess aerobic exercise capacity. Additionally, (Hassenfratz et al. 2012) showed that blood lactate concentration in a case study of a world-class enduro rider rose from a mean of 3.0 mmol.L^{-1} at the start of a race stage to 15.2 mmol.L^{-1}immediately post stage thus providing rationale to assess blood lactate response to increasing workload. Hassenfratz et al. (2012) also reported that one elite rider produced <50W for 37-56% of the duration of timed stages though average HR was maintained at around 90% of maximum throughout. This discrepancy between power and HR may be due to the workload associated with damping accelerations transferred to the rider from the bicycle as a result of rough terrain (Macdermid et al 2014). Upper body muscular contractions are shown to contribute to this damping effect with greater magnitude of activity on rough terrain when compared with smooth terrain (Hurst et al. 2012). Magnitude and frequency of accelerations experienced by the rider are also influenced by suspension (Levy & Smith, 2005), wheel size (Macdermid et al., 2014), tire size and tire pressure (Macdermid et al. 2015) thus it is feasible these factors also influence physiological workload. Interestingly, when compared to a hardtail bike a full suspension bike reduced vibrations on the downhill section of a cross country course but did not improve performance or reduce physiological workload (Macdermid et al 2016). This suggests that further research is required to include the specific, likely more technical terrain and specialist equipment used by professionals (e.g. 160-200mm travel bikes vs hardtail) associated with other off road cycling disciplines such as downhill and enduro.

GPS devices incorporating accelerometers have been used to create a GPS activity profile in DH mountain biking, though to the best of the authors knowledge the components of successful performance have not yet been fully detailed (Florida-James et al. 2010; Hurst et al. 2013). The validity and reliability of GPS systems to assess activity profile of outdoor sports has been reported extensively (e.g. Aughey, 2011). Further, the validity of GPS units incorporating triaxial accelerometers for measuring physical activity has also been reported (Boyd et al. 2011) and been deemed suitable for use in sports similar to enduro such as DH (Hurst et al. 2013). Rider load was introduced as a term to describe accelerations and accumulated accelerations experienced by the bike rider, detailed previously (Hurst et al. 2013). Abbiss et al. (2013) showed that faster riders overall spent significantly less time in the technical uphill section when compared to slower riders overall at a World Cup XC race. The potential influence of terrain (Hurst et al. 2013) and time to complete designated sections (Abbiss et al. 2013) provides rationale to assess differences in GPS activity profile in relation to performance in elite enduro mountain bike racing.

The aims of this study were therefore to:

1) detail and compare the laboratory assessed physiological characteristics of elite and non-elite enduro mountain bike riders
2) Evaluate the field use of GPS/accelerometer units to define the demands of different terrain in enduro mountain bike racing and identify components of successful elite performance

Methodology

Laboratory data collection

Participants

Eleven (n=11) male enduro mountain bike riders (age= 24 ± 5 years, height=181 ± 5cm, mass=72 ± 6kg) participated in the laboratory testing. Participants were divided into elite (n=5; Top 40 EWS result) and non-elite (n=6; national level rider) groups for analysis. Ethical approval for the study was granted from the ethics committee of [name deleted to uphold integrity of review process], adhering to the ethical standard of this journal (Harriss & Atkinson, 2013). Subsequently, both oral and written consent was obtained from all participants.

Laboratory protocols

Participants were required to visit the human performance laboratories on two occasions, with at least 48hrs between visits. During the first visit, power at fixed blood lactate concentration of 2mmol.L^{-1} and 4 mmol.L^{-1} (FBLC 2 and 4 mmol.L^{-1} respectively) and peak oxygen uptake (VO_{2peak}) were assessed. On the second visit body composition and power output was measured. Participants were fully informed of the details of all protocols prior to each test. FBLC, VO_{2peak} and power were all assessed on a cycle ergometer (Racer Mate Pro, Velotron, USA) fitted with the participant's own clip-in pedals.

Lactate threshold protocol

FBLC was assessed using an incremental exercise test on cycle ergometer (Racer Mate Pro, Velotron, USA). Blood samples taken from the ear lobe were analysed for blood lactate concentration using the Lactate Pro Meter (Arkray LT-1710, Japan). The initial workload was set at 110W increasing 40W every 3 minutes. Samples were taken within the last 30s of workload until lactate concentration exceeded 4mmol.L^{-1} at which point the test was terminated. Lactate concentration was then plotted against power output in order to determine power output at 2 and 4mmol.L^{-1}.

VO_2peak protocol

Fifteen minutes of active recovery at a self-selected intensity (HR<120bpm) followed the lactate threshold test. VO_{2peak} was assessed by a ramp test to exhaustion where online gas analysis was used to determine oxygen uptake (Jaeger Masterscreen CPX, Germany; Hans Rudolph V2, Germany). The initial workload was set at 160W and increased 20Wmin^{-1} until volitional exhaustion or when cadence dropped below 60rpm. VO_{2peak} was taken as the highest 8-breath average from raw breath-by-breath data. Rating of perceived exertion (RPE) (Borg 1970) was assessed at every stage and HR (Polar, Finland) was recorded at 5s intervals throughout the two tests.

Anthropometry

The ISAK (International Society for the Advancement of Kinanthropometry, 2010) restricted profile was used to collect anthropometric data on the second visit to the laboratory. In addition, corrected measurements of calf, thigh, and upper arm girth were used to calculate estimated muscle mass (Martin et al. 1990). Body mass was measured using scales (Seca 761, Germany) and height was measured using a stadiometer (Holtain Limited Harpenden Portable, UK).

Enduro Specific Test protocol

The Enduro Specific Test (EST) is designed to test power with respect to enduro racing and is based on the Downhill Specific Test (DST; Florida-James, 2010). The cycle ergometer used for the test (Racer Mate Pro, Velotron, USA) was fitted with a Wingate specific chainring (85-tooth chain ring, Velotron, USA) and torque factor applied was 0.05 kilogram-force per kilogram body weight (kgf/kg). Resistance was constant for the duration of both stages and power data was recorded using Velotron Wingate software (Wingate Software, Computrainer, USA, 2012) at 10Hz. The EST was designed to replicate two competitive enduro stages separated by a pedal powered transition. Participants continued to pedal in the transition at low cadence. The first stage was followed by 150s active rest at self-selected intensity. Participants then completed 15mins fixed workload set at 80% of the individual power output at FBLC 4mmol.L^{-1} recorded in the previous laboratory visit. This was in an effort to replicate the physiological demand of a transition stage in enduro racing, which normally involves a climb back up a hill/mountain to the start of next stage. Stage 2 of the EST followed after a second period of 150s active rest at a self-selected intensity. See figure 1 below for further detail on the EST structure and specific sprint durations. Sprint and rest durations for the EST were informed by the power data of an elite athlete collected using a power meter at a national race presented by Hassenfratz et al. (2012).

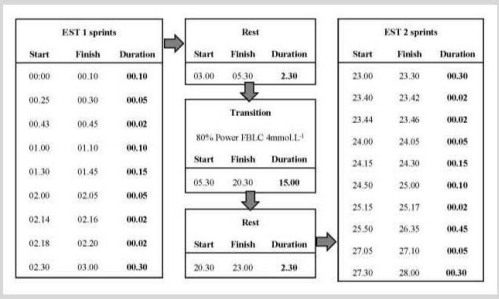
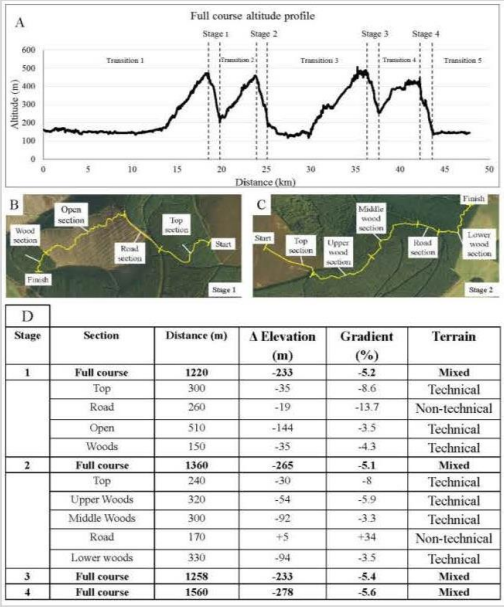


Figure 1: EST protocol including duration of sprints in EST 1 and EST 2. Time presented as min.s; FBLC 4mmol.L^{-1} = power at fixed blood lactate concentration of 4mmol.L^{-1}. 'Start' indicates start of sprint effort and 'finish' indicates end of sprint effort.



D

Stage	Section	Distance (m)	Δ Elevation (m)	Gradient (%)	Terrain
1	**Full course**	**1220**	**-233**	**-5.2**	**Mixed**
	Top	300	-35	-8.6	Technical
	Road	260	-19	-13.7	Non-technical
	Open	510	-144	-3.5	Technical
	Woods	150	-35	-4.3	Technical
2	**Full course**	**1360**	**-265**	**-5.1**	**Mixed**
	Top	240	-30	-8	Technical
	Upper Woods	320	-54	-5.9	Technical
	Middle Woods	300	-92	-3.3	Technical
	Road	170	+5	+34	Non-technical
	Lower woods	330	-94	-3.5	Technical
3	**Full course**	**1258**	**-233**	**-5.4**	**Mixed**
4	**Full course**	**1560**	**-278**	**-5.6**	**Mixed**

Figure 2: Full course profile of altitude and distance for all stages and transitions (A) and GPS trace of stage 1 and 2 (B & C respectively) overlaid on Google Earth including separate sections created for detailed analysis. Details of length (m), elevation change (m), gradient and terrain type for full course stage and stage sections are also detailed here (D).

Field data collection

Participants

Nine (n=9) elite enduro mountain bike athletes were recruited for field data collection. All nine participants had finished an Enduro Worlds Series (EWS) race in the top 40 positions. Complete field data sets were obtained for four (n=4) riders as a result of issues with compatibility of GPS/Accelerometer units (see results section for more information).

Race event

A two-day international enduro race in the United Kingdom was chosen for the race analysis. The race day consisted of four timed stages of which two were used for analysis. The course was 48km in total length with a total ascent of 1094m. For details of stage, stage sections, distance, elevation and terrain please refer to figure 2 below.

GPS and accelerometer methodology

A GPS device featuring 100Hz triaxial accelerometer (Catapult MinimaxX S4 or Catapult Optimeye S5, Catapult Innovations, Melbourne, Australia) was fixed to the seat mast of each participants bicycle in the orientation specified by the manufacturer. A second GPS device was placed in a specifically designed neoprene harness (Catapult Innovations, Melbourne, Australia) as described previously (Hurst et al. 2013). Three (n=3) participants used two MinimaxX S4 units,

five (n=5) participants used two Optimeye S5 units and one (n=1) used an Optimeye S5 in the neoprene harness and a MinimaxX S4 attached to the bicycle. All participants wore a wireless HR monitor strap (Wearlink, Polar, Finland) encoded to their individual GPS units recording HR at 1Hz. The Catapult MinimaxxX S4 and Optimeye S5 use GPS signal to record position and subsequently velocity and altitude at sample rate of 10Hz however the Catapult Optimeye S5 also recognises Global Navigation Satellite System (GLONASS) signals. Both devices incorporate a 100Hz triaxial accelerometer which is used to calculate rider load and accumulated rider load, the validity and reliability of these parameters have been reported elsewhere (Boyd et al. 2011; Van Iterson et al. 2016). The GPS devices were switched on and checked for sufficient GPS signal 10minutes prior to leaving the race headquarters and the race start (Catapult Innovations, Melbourne, Australia). Upon return to the event headquarters the GPS unit was connected to a laptop and the data downloaded for later analyses.

Figure 3. Percentage time spent in velocity zones in technical and non-technical terrain. Data presented as mean ± SD. * denotes significant difference between technical and non-technical terrain (p<0.05).

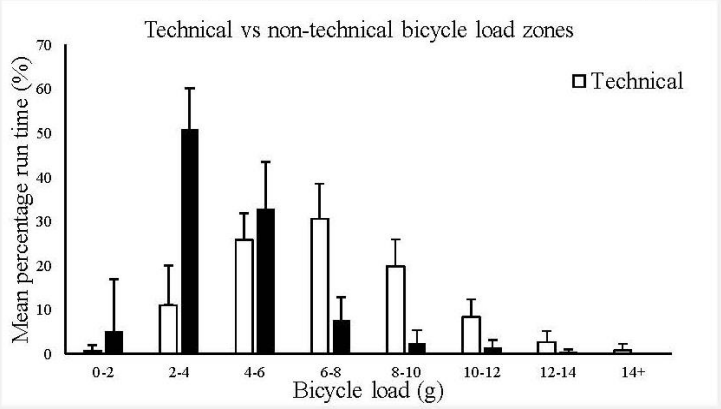
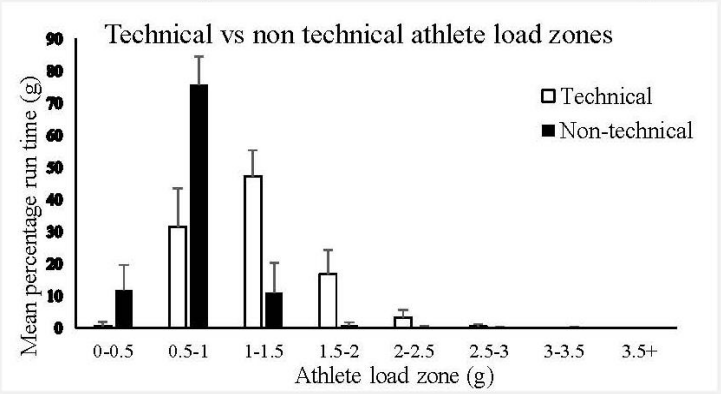


Figure 4. Percentage time spent in velocity zones in technical and non-technical terrain. Data presented as mean ± SD. * denotes significant difference between technical and non-technical terrain (p<0.05).



GPS analysis

GPS data was analysed on Catapult Sprint 5.1 software (Catapult Innovations, Melbourne, Australia). Race stages were identified where velocity moved above 0ms^{-1} at the stage start and back to 0ms^{-1} at the stage finish in conjunction with the map feature. Mean and peak HR data is displayed for each race stage. Stages were divided into sections detailed in figure 2 above. Terrain was either technical (single track with rocks and roots) or non-technical (gravel road) as noted below.

GPS data (10Hz sample rate) was used to calculate overall run time (s) in agreement with event organiser's time, section time (s) and percentage time spent in velocity zones (0-10, 10-20, 20-30, 30-35, 35-40, 40-45, 45-60 (km.h^{-1})). Rider load has been previously defined by Hurst et al., 2013 and is calculated using the formula available in the Catapult Sprint software manual (Catapult Innovations, 2013, page 80). Rider load for the Catapult unit placed on the athlete will be referred to as athlete load whilst rider load for the Catapult unit on the bicycle will be referred to as bicycle load from here on. Accelerometer data (100Hz sample rate) was used to calculate percentage time spent in bicycle load zones (0-2, 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, >14 (g)), athlete load zones (0-0.5,0.5-1,1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-3.5, >3.5(g)), and accumulated rider load (bicycle and athlete) for each track and terrain type (technical and non-technical) as defined in figure 2.

Statistical analysis

Laboratory

All data analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Difference between physiology data of the elite and non-elite groups was assessed using an independent sample T-test. Significance was accepted at the 95% confidence interval (p=0.05). All data presented as mean ± standard deviation (SD). For ramp test data, power is presented as relative

(watts/kilogram body weight, W/kg) and absolute (watts, W).

Field

Mean differences for stage time, accumulated rider load, accumulated bicycle load, mean heart rate and peak heart rate between stages 1 and 2 were analysed by paired sample T-tests. Between terrain differences for velocity, athlete load and bicycle load data were assessed using two-way repeated measures analysis of variance (ANOVA). Greenhouse Geisser correction was used to adjust the degrees of freedom if the assumption of homogeneity was violated. In the case of a significant main effect, significant simple main effects were also calculated and reported. All data presented as mean ± standard deviation (SD). Relationship between run time and mean bicycle load were investigated using Pearson's correlation coefficient. The magnitude of correlation coefficients was considered as trivial (r<0.1), small (0.1<r<0.3), moderate(0.3<r<0.5), large (0.5<r0.7), very large (0.7<r<0.9), almost perfect (r>0.9) or perfect (r=1; Hopkins, 2002). Significance was accepted at the 95% confidence interval (p=0.05) throughout.

Results

Laboratory testing

The physiological data obtained during the laboratory tests indicated that elite riders produced greater power output at VO_{2peak}, at an RER of 1, FBLC 2mmol.L^{-1} and FBLC 4mmol.L^{-1} (see Table 1).

The results of the Enduro Specific Test (EST) shown in table 1 demonstrated that the elite athletes are capable of producing greater power in seven out of the eight power indices measured in this specific test. Overall relative peak power (W/kg) was the only power parameter which elites did not produce more power than non-elite riders, suggesting that maintaining near maximal power across repeated sprints efforts is more important than the magnitude of maximal power produced in a single sprint effort. No significant differences were found in body mass (kg), skeletal muscle mass (kg), sum of 6 skinfolds, sum of 8 skinfolds, percentage muscle mass or thigh girth 1cm distal of the gluteal fold between groups (p>0.05) shown below in table 1.

Table 1. Results of laboratory tests. All data presented as mean ± SD for each group.*denotes significant difference between elite and non-elite groups (p<0.05).

Parameter (unit)	Non-Elite	Elite
VO_{2peak} (ml.kg.min^{-1})	63.6 ± 6.1	65.8 ± 3.7
Power VO_{2peak} (W)	363 5 ± 30	417 ± 29*
Relative Power VO_{2peak} (W/kg)	5.2 ± 0.4	5.6 ± 0.3
Power RER = 1 (W)	282 ± 27	343 ± 25*
Power FBLC 4mmol.L^{-1} (W)	263 ± 25	318 ± 31*
Power FBLC 2mmol.L^{-1} (W)	198 ± 36	267 ± 39*
Relative power FBLC 4mmol.L^{-1} (W/kg)	3.8 ± 0.3	4.3 ± 0.3*
Relative power FBLC 2mmol.L^{-1} (W/kg)	2.9 ± 0.5	3.6 ± 0.4*
EST 1		
Mean Power (W)	390 ± 31	475 ± 15*
Mean Power (W/kg)	5.6 ± 0.1	6.3 ± 0.1*
EST 2		
Mean Power (W)	387 ± 30	469 ± 12*
Mean Power (W/kg)	5.6 ± 0.2	6.3 ± 0.4*
Complete EST		
Mean power (W)	388 ± 29	466.3 ± 21*
Mean power (W/kg)	5.5 ± 0.3	6.2 ± 0.5*
Peak power (W)	541 ± 66	658 ± 57*
Peak power (W/kg)	7.7 ± 0.5	8.8 ± 1.2
Body Composition		
Body mass	69.6 ± 5.6	75.1 ± 5.1
Skeletal muscle mass (kg)	41.3 ± 4.4	44.2 ± 4.2
Skeletal muscle mass (%)	59.5 ± 4.3	58.8 ± 2.4
Thigh girth 1cm distal gluteal fold (cm)	55.3 ± 2.8	57.4 ± 1.3
Sum of 6 skinfolds (mm)	46.9 ± 8.0	45.4 ± 11.3
Sum of 8 skinfolds (mm)	61.1 ± 10.2	57.8 ± 13.1

Field results

All participants completed the race successfully. GPS data for one participant did not log and hence his data were removed from further analysis. The GPS system on the Catapult MinimaxX S4 unit failed to record complete files in the dense woodland surrounding the race tracks and thus could not be used for analysis. Thus only four (n = 4) sets of Catapult MinimaxX S5 data is presented. Mean time to complete stage 2 was significantly longer than stage 1 and bicycle load was substantially reduced in comparison to athlete load on both stages. Respective values of both athlete load and bicycle load were significantly greater on stage 2 than stage 1 (see table 2) though mean HR was similar for both stages.

There was a significant interaction between terrain and velocity zone, F(3.15, 22.01) = 6.429, p= 0.002. Analysis of simple main effects showed significant differences between technical and non-technical terrain in the 0-10, 10-20 and 20-30 km.h^{-1} zones as shown in figure 3 below.

There was a significant interaction between terrain and bicycle loading (F(2.38, 16.65) = 35.29, p = 0.000). Analysis of simple main effects showed significant differences in percentage time in bicycle load zone between terrain for all zones except 0-2 and 4-6g (see figure 4).

There was also a significant interaction between terrain and athlete loading (F(2.17, 15.18) = 81.82, p = 0.000). Analysis of simple main effects showed significant differences in percentage run time in athlete load zone between terrain for all athlete load zones except 3-3.5 and 3.5+ g as shown in figure 5 below.

On stage 1 Rider B was fastest by 2.9s (see table 2). This was achieved during both the top section and road section (see figure 3). Rider C recorded the quickest

Table 2: Mean time (s), athlete load (A.U.), bicycle load (A.U.), mean heart rate (HR) for full course of stages 1 and 2 (n=4) and individual time to complete stage sections (s). * indicates significant mean difference between full course stage 1 & 2, #indicates significant difference between athlete load and bicycle load ($p < 0.05$).

Stage (full course)	Time (s)	Athlete load (A.U.)	Bicycle load (A.U.)	Mean HR (%max)	Maximum HR (%max)
1	171.1 ± 15.5	49.4 ± 3.7	281.0 ± 7.7#	87 ± 4	94 ± 1
2	212.7± 6.43	60.9 ± 4.6*	352.7 ± 8.8*#	87 ± 3	95 ± 4

Stage 1 – time to complete section (s)

Rider	Top (T)	Road (NT)	Open (T)	Wood (T)	Total
A	44.5	26.9	73.9	21.0	166.3
B	42.3	25.5	71.3	20.9	160.0
C	43.3	27.9	71.0	20.8	162.9
D	57.8	38.6	76.6	21.2	194.2

Stage 2 - time to complete section (s)

Rider	Top (T)	Upper wood (T)	Middle wood (T)	Road (NT)	Lower wood (T)	Total
A	24.6	43.7	49.5	22.6	70.7	212.1
B	24.5	48.4	51.0	21.1	66.5	211.5
C	24.8	45.6	48.3	21.0	64.1	203.8
D	24.7	47.3	52.0	24.6	70.7	219.2

speeds in the open and wood section, however the advantage was marginal in comparison to time lost previously and thus did not result in a winning time.

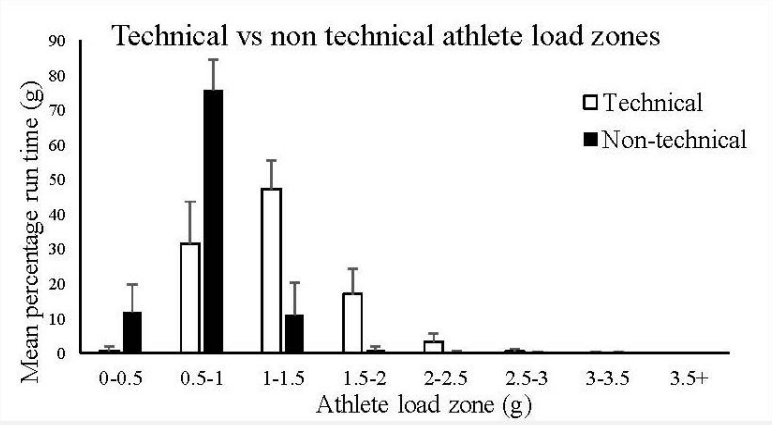
On stage 2 Rider C had the fastest time of 203.8s; 7.7s quicker than second fastest rider B (see table 2). Rider C won the stage by going fastest on the middle wood and road section before extending his advantage in the lower wood section going 2.4s faster than second placed rider B and 6.6s faster than third placed rider A. The winning rider gained time in the most technical sections and did not relinquish any time in the non-technical pedalling sections. In both stages, the winning rider was consistently in the top 2 fastest times for each section with the exception of the top section of stage 2.

There was a significant almost perfect negative correlation between mean bicycle load and overall stage time during stage 1 and stage 2 as shown in figure 6 below.

Discussion

This study aimed to define the physiological characteristics of elite and non-elite enduro mountain bike athletes and to identify the demands of an international enduro race. The main findings of this study demonstrated that elite enduro athletes have a large aerobic capacity and the ability to produce greater power at submaximal workloads, when compared to non-elite counterparts. This reflects the prolonged overall duration of enduro events and suggests the elite riders are working at a relatively lower workload than non-elite riders during time limited transition stages. Elite and non-elite athletes exhibited similar VO_{2peak} values, however elites produced significantly greater absolute peak power suggesting peak power output contributes to successful enduro racing performance. Furthermore, elite riders produced greater relative and absolute power at FBLC (2mmol.L^{-1} and 4mmol.L^{-1}) in comparison to non-elite counterparts, suggesting that the elite athletes are capable of completing the transition stages of an enduro event at a submaximal workload and

Figure 5. Percentage time spent in athlete load zones during technical and non-technical terrain. Data presented as mean ± SD (n= 4).



thus contributing to successful performance. Additionally, blood lactate clearance appears to be crucial during the race stages as Hassenfratz et al. (2012) showed an average post stage blood lactate concentration of 15.2 mmol.L^{-1}. The absence of a peak lactate value from the laboratory in this study is a limitation and should be considered in future research in enduro mountain biking performance. The importance of submaximal workload capacity was further enhanced by larger wattages demonstrated at RER=1 in the elite group, thus suggesting elite athletes can work at greater wattages before shifting to carbohydrate as the predominant fuel source, which should translate into improved performance. EST data shows that elite riders are able to repeatedly produce more power over shorter durations suggesting improved recovery between maximal efforts. However, as previous work has shown that pedalling is not crucial to descending performance in XC (Miller et al. 2016) and DH (Hurst & Atkins 2006) further research in the field is required to explore fully the relationship with power output and performance in enduro.

Periods of near-maximal, intermittent power output during enduro race stages has previously been documented (Hassenfratz et al. 2012). The data presented here shows that the elite group have a superior ability to recover and reproduce short bursts of near-maximal power output when compared to non-elite athletes, suggesting increased intermittent power output is crucial for successful enduro performance. In comparison to other disciplines, elite and non-elite enduro athletes showed relative VO_{2peak} values only marginally lower than reported of national level (Novak & Dascombe 2014) XC athletes. Elite enduro athletes also demonstrated comparable absolute and relative power at VO_{2peak} and FBLC 4mmol.L^{-1} to national level XC athletes (Impellizzeri et al. 2005) suggesting the physiological profile of elite enduro athletes shows more similarities with XC athletes than DH athletes. In summary, elite enduro athletes can produce more power at VO_{2peak} combined with improved ability to repeatedly produce near maximal power output when compared to non-elite athletes.

The aim of this field study was to evaluate the use of GPS and accelerometer technology to identify the difference in terrain profiles during an international race and to determine components of successful enduro mountain bike race performance based on this data. The main findings were 1) terrain significantly altered the activity profile, 2) winning performance was determined by time to complete technical and non-technical sections and 3) reduced run time was significantly negatively correlated with mean bicycle load. Athlete and bicycle load profile showed that technical terrain resulted in significantly increased time in higher load zones when compared to non-technical terrain despite a higher average velocity over non-technical terrain. The GPS accelerometer technology demonstrated a sensitivity that was able to detect changes in terrain and accumulated workload in enduro mountain bike racing, showing this could potentially be used for monitoring athlete performance and load in the future.

The GPS unit attached to the bicycle recorded significantly greater accumulated load when compared to the accumulated load measured at the torso, supported by previous work showing that accelerations are attenuated significantly between the bicycle and the rider's torso by the rider's limbs (Macdermid et al. 2014). Reduction in accelerations at the torso are deemed necessary to maintain balance and coordination, both of which are critical to maintain control of the bicycle (Mester et al. 1999). Given the difference between accumulated load at the bicycle and torso observed here, the dissipation of accelerations between bike and torso is proposed to contribute to the overall workload of piloting the bicycle over technical terrain more so than non-technical terrain, similar to the findings of Macdermid et al. (2015). Therefore it is feasible (in addition to analysis of power output) that GPS accelerometer technology allows quantification of workload components contributing to sustained elevation of HR during negotiation of technical terrain (Hassenfratz et al. 2012; Hurst & Atkins 2006). However, this workload may also influenced by suspension type, set up, and rider technique ; the assessment of which was beyond the scope of this study and is thus a limitation of the data presented (Macdermid et al. 2017). In addition, the trend for elite riders to have a greater mass and predicted muscle mass may be a requirement to withstand higher mean load associated with reduced run time shown here.

Values of accumulated athlete load presented here are slightly lower than values presented in elite DH riders, likely due to reduced velocity of enduro riders (19-23kmh-1) compared to DH riders (25-30kmh^{-1}) (Hurst et al. 2013) and can also be influenced by terrain type, course design or the increased suspension travel of a DH bike (200mm) compared to the enduro bicycles (140-160mm) used here. As enduro athletes compete on several stages in one day potentially for multiple successive days, the implications of the accumulating workload may be significant over the course of a race event. In the present study faster athletes spend a greater percentage of run time in higher bike load zones highlighting the increased demand placed on faster athletes. This highlights the importance of specific conditioning programmes designed to prepare the athlete to cope with the increased overall workload demands. During EWS races where stages and transitions can be more than 4-5 times greater in length (Enduro Mountain Bike Association 2016) than seen in this current study, accompanied by greater velocities even more load could be placed on the athletes, thus warranting further research. As one international level enduro athlete has been shown to produce 457-821W for 20% of the duration of a 15 minute race stage, it is suggested that a high capacity for propulsive workload is also likely to be crucial for long stage durations (Hassenfratz et al., 2012). Future work should also aim to quantify the workload of the transition stages in enduro racing in addition to the demands of the race

stages. This would be particularly relevant at EWS races where transitions can be ~2hours long with overall durations of up to 9 hours and thus place significant demands on the athlete in addition to the workload of the race stages.

The relationship between skill level in piloting the bike over technical terrain and the consequent overall loading on the athlete requires further research. The present study demonstrated that faster riders moved quicker through technical sections, thus suggesting a greater level of skill in comparison to slower riders. However this relationship is multifactorial and not reliant on load alone, therefore future research should focus on the influence of bike setup including factors such as wheel size and suspension type and set up, to determine the associated impact on performance.

Individual analysis shows that stage winning performance featured consistently competitive times through both the most technical sections of the race stage and the non-technical uphill/mixed gradient sections. This suggests that skill development in piloting the bike is a crucial component that should be part of training programme but also indicates that elite enduro athletes must also be able to produce large bouts of power to cover uphill and flat sections of smoother terrain within a stage. Additionally, the ability to negotiate technical terrain immediately following large anaerobic bursts both within the stages and during the transition phases between stages is essential for successful competition. This is supported by individual analysis showing that the winning rider on stage 1 completed the uphill road section 2.4 seconds quicker than any other rider and was able to maintain this advantage over subsequent technical sections. The physiological characteristics measured within this study appear to corroborate this assertion, as the elite enduro athletes displayed values of peak aerobic power output similar to those of national level XC racers (Novak & Dascombe 2014).

In conclusion, successful performance in enduro mountain biking requires a large aerobic and anaerobic capacity coupled with adequate skill and technique to ensure high velocities can be sustained over differing types of terrain. Athletes and coaches should consider suitable conditioning to prepare the athlete for the accumulated load associated with enduro racing. Use of a GPS accelerometer based activity profile allows more detailed analysis of workload than traditional means with the ability to describe components of successful performances in enduro mountain bike racing.

Practical Applications

Defining the physiological characteristics of elite enduro mountain bike athletes allows coaches and athletes to tailor their training plans accordingly following needs analysis. This study shows that both technical ability and physical fitness are crucial for performance and therefore enduro athletes cannot rely on one component alone for successful performance. GPS/accelerometer units may be used to further quantify intensity and performance in enduro mountain biking to manage athlete load across a season of racing and competition.

Acknowledgement

We would like to thank all of the athletes who participated in this research project and the event organiser for their time and effort.

Conflict of interest

The authors have no personal or financial relationships with other people or organisations that could have inappropriately influenced this work.

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