An interdisciplinary examination of stress and injury occurence in athletes.

by

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A thesis presented for the degree of Doctor of Philosophy

Cardiff Metropolitan University

August 2019

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Abstract

This thesis examined the multifaceted relationship between stress and sports injury. Study 1 explored the relationships between psychological sources of stress (major life events and personality characteristics) and stress-related physiological markers (heart rate variability, muscle stiffness and postural control) using a prospective, repeated measures design. Two Bayesian networks were used to perform the analysis and provided probabilistic statements regarding the effect of different combinations of variables in the network on injury occurrence. The first network revealed that "High" levels of muscle stiffness resulted in the greatest probability (Pr) of injury (Pr = 0.31). However, there was no meaningful difference between "Low" and "High" levels of negative life events on the probability of sustaining an injury ("Low" Pr = 0.24, "High" Pr = 0.26), despite a large body of research finding evidence to the contrary. The second network explicitly modelled changes between time points and found that the combination of *increases* in muscle stiffness and negative life events resulted in the greatest probability of sustaining an injury (Pr = 0.71). Study 2 addressed a number of research questions that built on those of Study 1, including; whether additional measures such as the stress hormone cortisol was associated with major life events and injury; whether an alternative method of scoring major life events would be related to injury; and how these variables related to both injury occurrence and severity. A subsample from the first study of male football and male rugby players were recruited for the study. Both Bayesian hurdle regression and Bayesian linear regression models were used to analyse the data. Findings revealed that higher levels of both average negative life event score and muscle stiffness increased the probability of injury occurrence and the number of days lost due to injury, although large credible intervals (CrI) were present. The relationship between cortisol and injury was less clear, with each of the two teams involved in the study demonstrating a different response (football, estimate = 0.10, 95% CrI = [-0.43, 0.62]; rugby, estimate = 0.54, 95% CrI = [0.05, 1.05]). The thesis concludes with a discussion of conceptual and theoretical issues, practical implications, strengths and limitations, and directions for future research.

Acknowledgements

I would firstly like to thank Dr Marianne Gittoes, Professor Lynne Evans and Professor Richard Mullen for their continued support and guidance throughout the completion of this thesis. You have been a fantastic team to work with and have pushed me further than I thought would be possible.

I would also like to thank my close family; Mum and Dad, your unwaivering support in whatever I do means so much. David, thank you for being there to listen to all my complaints; and Mary, keep up your excellent job of always keeping Mum and Dad on their toes!

A special thank you to my girlfriend Leah, who for too many reasons to list kept me sane throughout the PhD (although she may debate that sanity…).

Finally, I'd like to thank all the staff and fellow Ph.D. candidates at the Cardiff School of Sport and Health Sciences for being so forthcoming with their time and ideas, and also the participants who made the thesis possible.

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List of acronyms

BAS	Behavioural Activation System
BIS	Behavioural Inhibition System
BMSAIH	Biopsychosocial Model of Stress, Athletic Injury and Health
CPQ	Conditional Probability Query
CrI	Credible Interval
FFFS	Fight-Flight-Freeze System
HRV	Heart Rate Variability
LESCA	Life Event Survery for Collegiate Athletes
NLE	Negative Life Events
Pr	Probability
RMSSD	Root Mean Square Successive Difference
RST	Reinforcement Sensitivity Theory

Chapter 1:

Introduction

Introduction

Despite the numerous physical, mental and social benefits of participating in sport at all levels, one of the more negative and arguably inevitable consequences that can have wide-ranging effects, is injury. Not only are there significant financial (e.g., costs of surgery and rehabilitation), psychological (e.g., increased negative affect, lower self-esteem, and higher levels of depression and anxiety) and physiological (e.g., acute pain from an injury and long term physical damage) consequences for an athlete (Johnston & Carroll, 2000; Mather et al., 2013; Reiner, Niermann, Jekauc, & Woll, 2013). There are also wider economic impacts of injury, with costs associated with accident and emergency, after care and rehabilitation, and days lost in work amounting to tens of millions of pounds each year (Cumps, Verhagen, Armenians, & Meeusen, 2008; Öztürk & Kiliç, 2013; Ryan, Pracht, & Orban, 2019). Perhaps not surprisingly, therefore, there is a considerable body of research that has focused on identifying different factors that are related to injury occurrence and the injury process, in an effort to mitigate against its prevalence and the adverse effects associated with it.

Typically, research in the sports sciences has addressed the injury problem from a somewhat narrow perspective, with the sub-disciplines of psychology, physiology and biomechanics focusing on factors specific to their respective fields. Often, however, these disciplines have shown little regard for how different factors across disciplines may interact to exacerbate the risk of injury. While the mono-disciplinary approach was arguably necessary in the early stages of sports injury research, to advance our understanding, there is a need to address the problem from a more holistic perspective in order to identify potential interactions between factors that permeate across disciplines. One factor with strong links to injury from several perspectives is stress. Fletcher and Scott (2010) conceptualised stress as a complex multifaceted phenomenon that incorporates both stressors (e.g., environmental demands encountered by an individual) and strain (e.g., an individual's negative psychological, physical and behavioural responses to stressors). Indeed, stress plays an integral role in sport, and is necessary to cause the adaptations athletes need to improve their performance. However, although stress plays an important role in the process of adaptation that athletes need to improve performance, excessive levels of both physiological stress caused by high training volume with inadequate recovery, and psychological duress caused by major life events, have been found to increase the risk of injury (Galambos, Terry, Moyle, & Locke, 2005; Post et al., 2017).

In an attempt to explain the role of stress in injury occurrence, and address the growing injury problem, Andersen and Williams (1988) proposed a model of stress and athletic injury. Revised by Williams and Andersen (1998), the model remains one of the most widely cited sport injury models in the sport psychology literature. To elaborate, the model includes several psychological factors (e.g., personality characteristics, major life events and coping resources) that are thought to influence an athlete's response to a stressful situation, with certain combinations of factors more likely to increase the risk of athletic injury. However, the model has recently been criticised for focusing only on the cognitive factors that are related to injury, and not including other non-psychological variables that research has demonstrated are also important for the stress-injury relationship (Ivarsson et al., 2017). In addition, Appaneal and Perna (2014) provided an independent extension to the Williams and Andersen (1998) model, outlining how additional training-related stress would synergistically interact with psychological stress to increase the risk of injury, however the proposed mechanisms in Appaneal and Perna (2014) have yet to be examined. Further, there have also been calls to move away from the mono-disciplinary approach of identifying isolated risk factors that contribute to injury occurrence that has been typical within the sub disciplines of sport and exercise sciences (Bittencourt et al., 2016). Indeed, there is a growing recognition that the body and mind are inextricably linked, and that a more holistic approach needs to be taken to examining factors associated with injury. As such, in order to advance our understanding of the complex interactions between different sources of stress and their effect on injury occurrence, there is a clear need to address the stress-injury relationship from an interdisciplinary perspective

Purpose of the thesis

The central purpose of the thesis was to explore the multifaceted nature of different stress-related risk factors associated with injury occurrence from an interdisciplinary perspective. Specifically, the thesis aimed to: (a) identify and evaluate the relationship between psychological and physiological markers of stress in the prediction of injury occurrence; (b) examine the relationship between the markers of stress and injury in a prospective, repeated measures study with a large cohort of athletes; and (c) evaluate the relationship between the markers of stress and injury using an analysis method that captures the complex nature of injury occurrence.

Structure of the thesis

The thesis comprises six chapters and contains two empirical studies. Following this introduction, Chapter 2 provides a literature review of the current empirical research. The thesis comprises six chapters and contains two empirical studies. Following this introduction, Chapter 2 provides a literature review of the current empirical research. Specifically, this chapter aimed to describe and critically evaluate Williams and Andersen's (1998) model of stress and injury and the associated literature; discuss recent theories and frameworks that build upon the limitations of Williams and Andersen's (1998) model to address the stress-injury relationship from an interdisciplinary perspective; and explain the rationale that underpins the current programme of research

Chapter 3 provides a rationale for the inclusion of the specific measures and variables that were used in the present programme of research. The reliability and validity of specific markers were evaluated using the relevant research literature, and where appropriate, pilot studies conducted to address specific concerns regarding the choice of a measure. In particular, the chosen measures had to be both robust and reliable within a field-based data collection, in addition to being feasible to use within the time-frame that was available to collect data from a large number of participants across repeated time-points. The reason for these criteria were twofold: firstly, to safeguard the quality of the data collected during the study, and secondly, to develop a collection protocol that was feasible for both the research and participant. As a result of the pilot studies and literature review, several modifications were made to the chosen measures to improve their reliability and validity within the prospective repeated measures design of the current programme of research.

Chapter 4 reports a study (Study 1) designed to capture markers of stress using the measures outlined in Chapter 3 in a large cohort of athletes in a prospective, repeated measures study. A total of 351 healthy athletes were recruited and completed a battery of measures at approximately 4-monthly intervals over a 12-month period. At each data collection point, athletes completed the set of measures identified in Chapter 3 to assess major life events, the reinforcement sensitivity theory of personality, muscle stiffness, heart rate variability and postural stability, in addition to reporting any injuries they had sustained since the last data collection. Two Bayesian networks were used to examine the relationships between variables and model the changes between data collection points in the study. Findings revealed muscle stiffness to have the strongest relationship with injury occurrence, with high levels of stiffness increasing the probability of sustaining an injury. In contrast, negative life events (NLE) did not increase the probability of injury occurrence. In contrast, when examining changes between time points, increases in NLE between time points was found to increase the probability of injury, and the combination of increases in NLE and muscle stiffness resulted in the greatest probability of sustaining an injury. Findings from the study demonstrated the importance of both a repeated measures design and interdisciplinary perspective in furthering our understanding of the relationship between stress-related markers and injury occurrence.

The study detailed in Chapter 5 provides a more fine-grained examination of stress related factors and injury and addresses additional questions that arose from Study 1. Specifically, (a) whether the stress hormone cortisol was also associated with major life events and injury; (b) whether an alternative method of scoring the major life events would be related to injury; and (c) how these measures related to both injury occurrence *and* severity. A sub-group of male football and rugby players ($n = 51$) were chosen due to the nature of the high intensity training they completed on a regular basis. In addition to the measures outlined in Study 1, participants also provided a sample of saliva before and after high intensity training sessions, which was used to determine the changes in concentration of cortisol. The NLE scoring was modified from the original procedure of using the sum of the negative life events by dividing each participant's score by the number of events they reported, to reflect an average NLE score. A combination of Bayesian hurdle regression and Bayesian linear regression models were used to explore the relationship between average NLE, muscle stiffness, cortisol and both injury occurrence and severity. Findings revealed high levels of both average NLE and muscle stiffness increased the probability of injury occurrence and increased injury severity; however the estimates had large credible intervals implying uncertainty regarding the observed relationships. The relationship between cortisol and injury was less clear, with each team demonstrating a different response.

The final Chapter (6) draws together the major findings presented in the thesis; discusses the major conceptual and measurement issues in the thesis; highlights the practical implications for athletes, coaches, practitioners and researchers; outlines the programme's strengths and limitations; and provides directions for future research.

Chapter 2:

Literature Review

Literature review

Injury remains an unfortunate bi-product of participation in sport and despite efforts to reduce injury rates through the identification and modification of risk factors associated with injury, the incidence of sport related injuries remains high (Aman, Forssblad, & Henriksson-Larsén, 2016; Bueno et al., 2018). For example, Sheu, Chen, and Hedegaard (2016) reported an age-adjusted injury rate of 31.1 per 1000 people with individuals between five and 24 years of age the most likely to sustain an injury. Rosa et al. (2014) found almost half (49.91%) of university level athletes sustained at least one injury during their career, and Palmer-Green and Elliott (2014) observed 39% of the British team at the 2014 Sochi winter Olympics sustained at least one injury. The detrimental effects of sustaining an injury for individual include lowered self-esteem, increased depression and an increased risk of sustaining subsequent injuries (Brewer, 2012; Leddy, Lambert, & Ogles, 1994).

In an attempt to mitigate against the undesirable consequences of injury, Van Mechelen, Hlobil, & Kemper (1992) proposed a four-step model for the sequence of injury prevention, which described the necessary steps to address the growing injury problem. Steps one and two of the model involve identifying the nature of the problem through incidence rates and identifying the factors and mechanisms through which injuries may occur. Indeed, a large body of research has identified several risk factors, both internal (e.g. anatomical, biomechanical, psychological) and external (e.g., equipment, playing surface, playing conditions), which are thought to contribute to injury occurrence (Bahr & Krosshaug, 2005; Wiese-Bjornstal, 2009). Based on these factors, several models of injury causation have been proposed in an attempt to understand the multifaceted nature of injury. For example, Bahr and Krosshaug (2005) proposed a model of injury causation that identified how both internal and external factors contributed to an athlete's susceptibility to sustaining an injury. The model was criticised however, for being too linear and not reflecting the true nature of exposure to risk factors and injury occurrence (Meeuwisse, Tyreman, Hagel, & Emery, 2007). Consequently, Meeuwisse et al. (2007) proposed a dynamic, recursive model of sport injury that improved Bahr and Krosshaug's (2005) model, by including a cyclical element, whereby an athlete could be exposed to risk factors and go through a period of adaptation. A recovery phase was also included in the model that linked back to characteristics of the predisposed athlete; demonstrating how an athlete's injury history can contribute to their set of risk factors, and how the injury cycle can start over again following recovery from injury. While both Bahr and Krosshaug (2005) and Meeuwisse et al. (2007) provided useful frameworks to inform sport injury research, their use has been limited within the literature. In contrast, one of the most influential models in sport injury research over the last three decades has been Williams and Andersen's (1998) model of stress and injury (Figure 1).

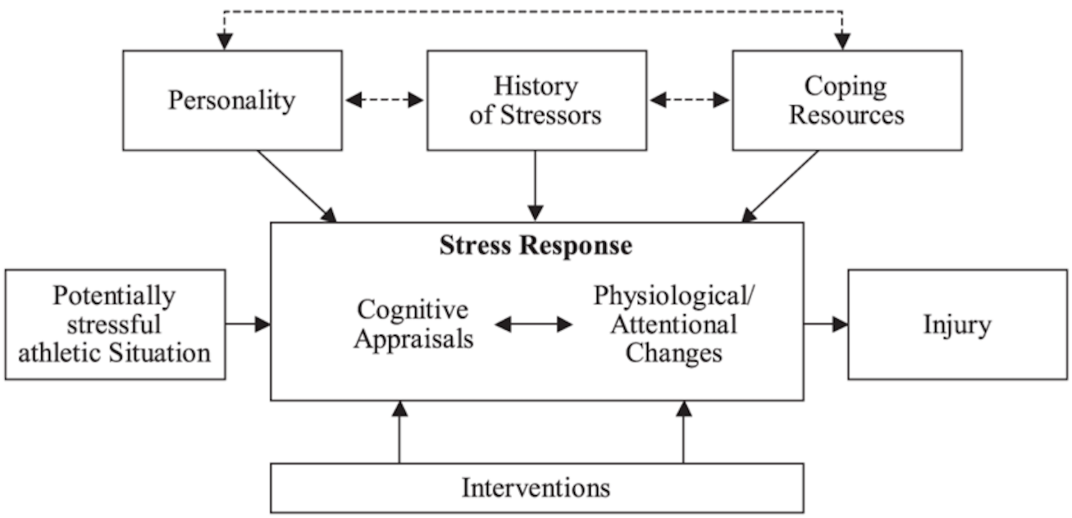

Figure 1. Stress and injury model (Williams & Andersen, 1998).

Williams and Andersen's (1998) model proposed that when faced with a potentially demanding athletic situation, the athlete's personality traits (e.g., hardiness, locus of control, sense of coherence, competitive trait anxiety, achievement motivation and sensation seeking), history of stressors (e.g., daily hassles, major life events, previous injuries), and coping resources (e.g., general coping behaviours, social support, psychological skills) contribute to their stress response, either interactively or in isolation. The stress response is the central core of the model and reflects a bi-directional relationship between the athlete's cognitive appraisal of, and physiological response to, a potentially demanding athletic situation. The model predicts that an athlete who faces a potentially demanding athletic situation may perceive the situation as stressful if they have a history of many stressors, personality traits that intensify the stress response and few coping resources to deal with the situation. Consequently, a heightened stress response causing the athlete to exhibit increased physiological activation (e.g., increased muscle tension) or attentional disruption (e.g., peripheral narrowing), is thought to be the mechanism through which injuries occur. Despite the stress response being the central core of the model, personality traits, history of stressors and coping resources have received the most attention in the research literature (Johnson, Tranaeus, & Ivarsson, 2014). Further, of the psychosocial variables in the model, major life events appears to most consistently predict injury occurrence (Gunnoe, Horodyski, Tennant, & Murphey, 2001; Ivarsson & Johnson, 2010; Ivarsson et al., 2017; Maddison & Prapavessis, 2005; Passer & Seese, 1983; Williams & Andersen, 2007).

Psychosocial variables

History of stressors. Research into the relationship between the history of stressors and injury has largely focused on major life events. The early research in life event stress evolved from the work of Holmes and Rahe (1967) who developed the Social Readjustment Rating Scale, which was used to identify and rank the magnitude of life events. The first evidence of a relationship between major life events and sports injury was reported by Holmes (1970), who found that 50% of the athletes that experienced high life stress sustained an injury over the preceding 12 months, compared to 9% and 25% of athletes with low and moderate life stress respectively. This initial evidence of a relationship between life event stress and injury has been largely supported in subsequent research across a range of different sports (Gunnoe et al., 2001; Ivarsson & Johnson, 2010; Maddison & Prapavessis, 2005; Petrie, 1992; Rogers & Landers, 2005). Indeed, in their review of the sport injury literature, Williams and Andersen (2007) reported that of the 40 studies that had examined the relationship between major life events and injury, approximately 85 % had found some correlation between life event stress and injury (Williams & Andersen, 2007).

While the early research in life event stress did not differentiate between the type of stress being experienced (i.e., whether the source of the stress was perceived as positive or negative), Sarason, Johnson, and Siegel (1978) contended that the effects of life events perceived as negative may differ from those events viewed positively. Consequently, Sarason et al. (1978) developed the Life Experience Survey (LES) which asked participants to indicate whether they perceived each life event as negative or positive, and whether the life event had no effect (score of 0), little effect, (-1 or +1 score depending on whether the event was negative or positive), moderate effect (-2 or +2) or a great effect (-3 or +3). Negative, positive and total life events (absolute sum of negative and positive event) could then be determined. As expected, Sarason et al. (1978) found negative life events to have a greater effect on health-related dependent variables compared to positive or total life events. Using a modified version of the LES, Passer and Seese (1983) were subsequently able to identify that in a sample of university football players, those who reported high negative life events were at the greatest risk of sustaining an injury. Indeed, in the research that followed, life events with *negative* valance were found to most frequently predict injury occurrence (Andersen & Williams, 1999; Ivarsson & Johnson, 2010; Ivarsson et al., 2017; Williams & Andersen, 2007). However, both positive and total life event stress have also been found to be related to injury. For example, Petrie (1993) reported that positive life events were the only life event stressor to predict injury. To explain this finding, Petrie (1993) suggested that events such as being promoted to captain of the team or receiving an athletic scholarship may be initially perceived as positive, however such events may put more pressure on the individual causing them to perceive athletic situations as more stressful, and thus be at greater risk of injury. Despite these suggestions, negative life events have received the most attention in the literature and remain one of the factors most strongly related to sport injury.

In addition to major life events, previous injury was also identified as a contributing factor to the history of stressors in Williams and Andersen's (1998) model. Like major life events, research has consistently found previous injury to increase the risk of future injuries (Hägglund, Waldén, & Ekstrand, 2006; Kucera, Marshall, Kirkendall, Marchak, & Garrett, 2005; Williams & Andersen, 2007). These findings can be partly explained by the physical effects of sustaining an injury; with athletes not completing the full rehabilitation programme before returning to sport, or the rehabilitation programme not preparing them fully for return to sport (Clement, Granquist, & Arvinen-Barrow, 2013; Kraemer, Denegar, & Flanagan, 2009). Without a comprehensive rehabilitation programme that fully prepares an athlete for their return to sport, significant deficits in strength, muscular activation, power, postural stability, lower extremity mechanics, and psychological preparedness may exist, and the risk of re-injury is greatly increased (Bien & Dubuque, 2015). However, sustaining an injury is also likely to be a major life event in itself, and the psychological issues an athlete may face when returning from a serious injury are well documented (Brewer, 2012). For example, fear of re-injury may result in the athlete not performing the movement or skill at the required level and/or in the required way (Hsu, Meierbachtol, George, & Chmielewski, 2017). An athlete who is fearful of re-injury may also try to "protect" the injured limb by favouring the uninjured limb. The result is that the previously healthy limb is now at greater risk of injury due to increased load (Fulton et al., 2014). Taken together, it is clear to see how the physical and psychological effects of an injury may increase the risk of subsequent injuries, particularly if a full recovery is not made before returning to sport.

Personality characteristics. In addition to history of stressors, personality traits have been identified as factors that moderate the stress-injury relationship. For example, both high trait anxiety and high competitive trait anxiety have been reported to increase the risk of injury (Lavallée & Flint, 1996; Petrie, 1993). Ivarsson and Johnson (2010) reported that somatic trait anxiety ($p = 0.025$), psychic trait anxiety ($p = 0.044$), stress susceptibility ($p = 0.016$), and trait irritability ($p = 0.023$) were significant predictors of injury within a group of male soccer players ($n = 48$). A particular strength of this study was the prospective design; however, the use of a single sport and small sample size limit the generalizability of the findings. Other personality characteristics identified in Williams and Andersen's (1998) model have received less attention in the literature, and findings are inconclusive with regard to predicting injury occurrence (Junge, 2000). For example, Pargman and Lunt (1989) reported that external locus of control was associated with increased injury rate in American football players. In contrast, Kolt and Kirkby (1996) found that internal locus of control predicted injury in elite, but not non-elite, gymnasts.

In their recent meta review, Ivarsson et al. (2017) found that personality characteristics generally have a limited direct relationship with injury prediction; however, research indicates that the presence of personality characteristics in combination with other factors such as a major life event is likely to increase the risk of injury in athletes (Petrie, 1993).

Coping resources. The final psychosocial variable proposed in Williams and Andersen's (1998) model is coping resources. The model predicts that athletes with well-developed psychological coping skills and a strong social support network will appraise situations as less stressful, reducing their likelihood of injury. As a result, coping resources are proposed to moderate the effect of major life events on sports injury, with high levels of coping resources potentially buffering the effect of negative life events. Several studies have supported the proposed relationship between life event stress, coping and injury. Several studies have found support for the proposed relationship between life event stress, coping and injury. For example, both Hardy (1992) and Petrie (1993) found social support to have a buffering effect on the relationship between negative life events and injury. In contrast, Rider and Hicks (1995) found no significant relationship between coping skills and sports injury (r = -0.11).

While several of the psychosocial characteristics proposed in Williams and Andersen's (1998) model have received significant attention in the literature, there remains a number of unanswered questions surrounding the contribution of characteristics that have received less consideration (e.g., hardiness, sense of coherence and achievement motivation). To elaborate, inconclusive findings for certain personality characteristics suggests these characteristics interact with injury occurrence is likely to be complex, and dependent on a number of other factors associated with the individual athlete (Junge, 2000). For example, a number of physical and/or environmental factors are also likely to influence injury occurrence and may moderate the effects of various psychosocial factors. Examining how psychosocial characteristics contribute to injury when considered in a wider context that includes training-related factors and physiological characteristics may therefore help to address some of the inconsistencies within the literature.

The stress response

Central to Williams and Andersen's (1998) model is the "stress response", which reflects a bi-directional relationship between an athlete's cognitive appraisal of, and physiological response to, a potentially demanding athletic situation. For example, an athlete will make an appraisal of the demands of a particular training session or competition, evaluate their ability to meet those demands, and the potential consequences of success or failure. The model predicts that athlete's with a history of many stressors, personality traits that intensify the stress response and few coping resources to deal with the situation are at risk of sustaining an injury due to the "increased physiological arousal and/or attentional deficits" caused by the stress response (Williams and Andersen, 1998, p. 7). Specifically, Williams and Andersen (1998) suggested several mechanisms through which an elevated stress response might exert its effect, for example, increased muscle tension, attentional narrowing and increased distractibility. Compared to the psychological factors outlined in the previous section, few researchers have investigated these proposed mechanisms and the research that has been conducted has mainly focused on the attentional stress response mechanisms proposed by Williams and Andersen. For example, Andersen and Williams (1999) measured injury occurrence, negative life event stress and performance on a perception task under both normal and stressful conditions and found that individuals with high negative life events and who experienced greater peripheral narrowing under stress sustained more injuries than individuals with the opposite profile The findings were later supported by Rogers and Landers (2005) who reported that peripheral narrowing during stress mediated 8.1% of the relationship between major life events and injury. The remaining studies examined attentional deficits using the ImPACT (ImPACT Applications, Inc., Pittsburgh, PA) test battery to examine the relationship between verbal memory, visual memory, processing speed, reaction time and injury. Wilkerson (2012) reported that increased reaction time at the start of the season predicted injury in a sample of 76 football players, whereas Swanik, Covassin, Stearne, and Schatz (2007) employing a retrospective design found individuals who had reported a previous anterior cruciate ligament injury demonstrated increased reaction time compared to healthy matched controls.

Although these studies provide support for a relationship between attentional deficits and injury as proposed by Williams and Andersen (1998), the ecological validity of the studies can be questioned. All of the studies identified by Ivarsson et al. (2017) used a laboratory-based design that is unlikely to be reflective of the environment that an athlete will be training and competing in. Furthermore, Swanik et al. (2007) used a retrospective design that has limited value for assessing the predictive power of attentional deficits. Wider criticisms can also be made about the particular mechanism of peripheral narrowing that is proposed to increase the risk of injury. Indeed, the peripheral narrowing mechanism fails to account for overuse injuries, which represent approximately 30% of the total number of injuries that are sustained annually (Yang et al., 2012). To elaborate the mechanisms for overuse injuries are typically caused by maladaptation to, or inadequate recovery from, high intensity training, and are more likely to be explained by the physiological changes proposed in the model such as increased muscle stiffness (Bahr & Krosshaug, 2005). However, these mechanisms have received little attention in the literature to date. Furthermore, other research has found some degree of peripheral narrowing to be beneficial to performance (Eysenck, Derakshan, Santos, & Calvo, 2007; Hanoch & Vitouch, 2004; Hertwig & Todd, 2005). For example, under stressful conditions, peripheral narrowing may limit the amount of information perceived, which may facilitate improved decision making and physiological mobilisation of the body to react to task relevant cues. Consequently, an athlete may be able to avoid potentially harmful situations under stress by using peripheral narrowing to focus on only the most relevant information and respond quickly to information they perceive (Hanoch & Vitouch, 2004; Öhman, Flykt, & Esteves, 2001). As a result, mechanisms, particularly those that are related to the physiological changes proposed in Williams and Andersen's (1998) model merit further attention.

Model critique

While Williams and Andersen's (1998) model has been the most widely cited model of injury in the literature, it has a number of limitations. A major criticism of the model is the narrow focus on cognitive aspects of stress and the stress response (Ivarsson et al., 2017). The model does not include any physiological or environmental factors that are also likely to contribute to injury occurrence that are central to other models of injury such as those proposed by Bahr and Krosshaug (2005) and Meeuwisse et al. (2007). A more complete model of injury occurrence needs to acknowledge the contribution of both psychosocial variables and other physiological or environmental factors known to relate to injury. An example of such a model, which included a combination of biomechanical factors such as; mechanical load, on field behaviour and skill, load tolerance, and various psychological factors including competitive motivation, cognition, risk assessment and perception of load, was proposed by McIntosh (2005). Unfortunately, the model has not been adopted as a framework for research, possibly due to the emphasis on biomechanics, which makes it less accessible for researchers from other sports science disciplines.

In addition to the limitations of Williams and Andersen's (1998) model itself, there has been some wider criticism of the literature that has investigated the different parts of the model; specifically, with regard to the populations studied and methodologies employed. Much of the research that has supported Williams and Andersen's (1998) model has used athletes from team sports, such as American football (Cryan & Alles, 1983; Gunnoe et al., 2001), soccer (Ivarsson & Johnson, 2010; Steffen, Pensgaard, & Bahr, 2009) and rugby (Maddison & Prapavessis, 2005). Furthermore, many of these studies have only included male athletes, with relatively few studies including female athletes (Ivarsson et al., 2017). Support for the model would undoubtedly be strengthened if samples were more representative of the sporting population at large. A further criticism that relates to methodology is the use of one time point for data collection purposes (Johnson et al., 2014). An athlete's injury risk is likely to be dynamic and will vary over the course of a season depending on a number of different factors, such as the stage of the season (e.g., early, middle or late), number of competitions and the amount of recovery between training sessions/competitions. By only measuring risk factors at one point in time, important information is missed regarding how *changes* in those risk factors may relate to injury occurrence. A further issue with a single measurement is that the time interval between the measurement and actual injury occurrence is not considered. For example, if risk factors are measured in pre-season, but an injury does not occur until much later into that season, the predictive value of the risk factor may be limited, given that the impact of the risk factor may have changed significantly over time. To highlight this point, Sibold and Zizzi (2012) found that a number of psychological variables including negative life events, worry and concentration disruption were associated with the number of days until injury occurrence. To clarify, higher negative life event scores resulted in injury sooner than lower scores. These findings support the need for repeated measures designs that assess risk factors as close to injury occurrence as possible to increase their predictive validity.

In order to address the limitations highlighted in the original Williams and Andersen (1998) model, Appaneal and Perna (2014) proposed the Biopsychosocial Model of Stress, Athletic Injury and Health (BMSAIH) to serve as an independent extension (Figure 2). Based on the earlier work of Petrie and Perna (2004), the BMSAIH aimed to: (i) clarify the mediating pathways between the stress response and injury; (ii) consider other health outcomes and behavioural factors that impact on sports injury and; (iii) integrate the impact of exercise and training on athletes' health and injury occurrence (Appaneal & Perna, 2014). The central tenet of the BMSAIH is that psychosocial distress may act synergistically with high intensity training to "widen the window of susceptibility" to injury and other undesirable health outcomes such as illness, physical complaints or maladaptation to training (Appaneal & Perna, 2014, p. 4). Therefore, the BMSAIH provides a framework for future research to examine the relationship between psychosocial factors and injury occurrence proposed in Williams and Andersen's (1998) model, by including other physiological stress-related markers, that collectively may provide greater insight into the injury process.

Although offering several potential avenues for research, support for the BMSAIH remains sparse, and research has mainly focused on the hormonal response to high intensity training and injury. For example, Perna and McDowell (1995) provided promising evidence in a study that examined life event stress and cortisol response in athletes following an exhaustive graded exercise test. Participants were split into high and low life event stress (LES) groups, and the high LES group were found to have both higher cortisol in response to the graded exercise test, and increased symptomatology (e.g., muscle complaints and viral illness) over the 30 days following the graded exercise test. The study by Perna and

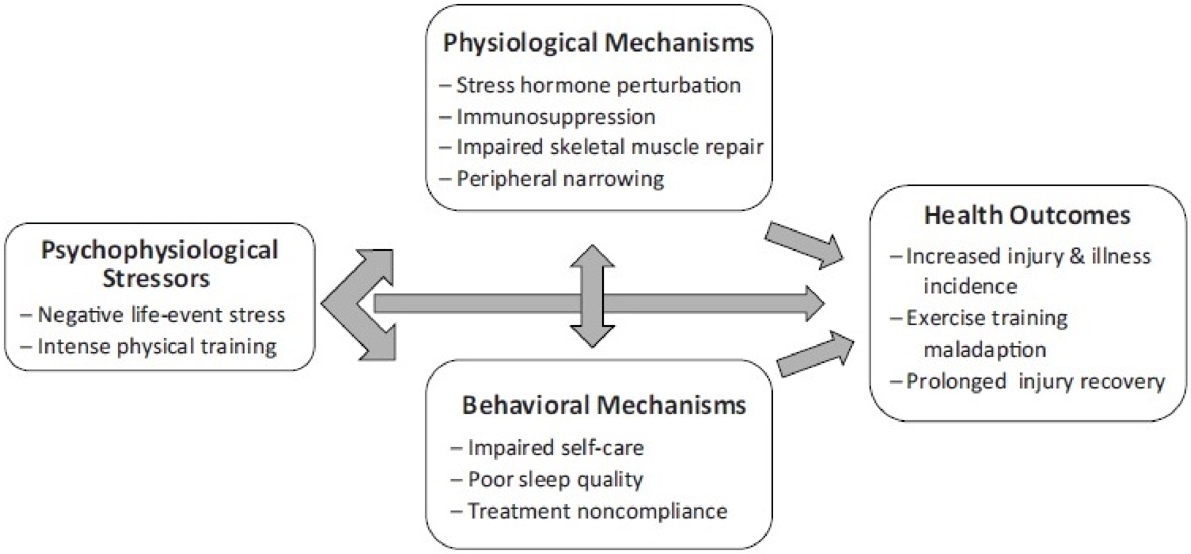

Figure 2. Biopsychosocial model of stress athletic injury and health (Appaneal & Perna, 2014, p. 4).

McDowell (1995) did not, however, explicitly examine the relationship between cortisol response to high intensity training and sports injury, and very few studies have explored the relationship further.

In addition to hormonal changes, there are several other physiological stress-related markers that could be included in research using the BMSAIH model. For example, measures of heart rate variability (Djaoui, Haddad, Chamari, & Dellal, 2017; Williams 2017), postural stability (Coco et al., 2015; Romero-Franco et al., 2014) and skeletal muscle characteristics such as stiffness (Butler, Crowell, & Davis, 2003; Pickering-Rodriguez, Watsford, Bower, & Murphy, 2017) have been of found to be related to psychological distress and are often used as markers of the physiological effects of high intensity training. Furthermore, these markers have also been linked to injury occurrence in athletes (Pickering-Rodriguez et al., 2017; Plews, Laursen, Kilding, & Buchheit, 2012; Trojian & McKeag, 2006; Williams et al., 2017), and including them alongside the psychological factors from Williams and Andersen's (1998) model may provide greater insight into the relationship between stress and injury. However, despite offering a potential framework to build upon the research stemming from William and Andersen's (1998) model, there remains a lack of research exploring the synergistic action of both psychosocial and physiological stress-related markers proposed by the BMSAIH.

One possible explanation for why sports injury research has made limited attempts to examine different sources of stress may be due to the traditional mono-disciplinary approach researchers often employ. Mono-disciplinary approaches continue to be prevalent within sports injury research despite repeated calls to move towards an interdisciplinary approach (Burwitz, Moore, & Wilkinson, 1994; Piggott, Müller, Chivers, Papaluca, & Hoyne, 2018). To gain a better understanding of the holistic nature of the stress-injury relationship, an interdisciplinary approach is necessary, and would allow different elements of both Williams and Andersen's (1998) model and Appaneal and Perna's (2014) model to be examined in more detail. For example, it would be possible to examine how psychological stress interacts with physiological markers of stress and synergistically contribute to an increased risk of injury. Additionally, to further highlight the importance of an interdisciplinary approach, Bittencourt et al. (2016) recently proposed a contemporary view of the sports injury problem, moving away from a mono-disciplinary perspective and drawing on elements from complex systems theory (Holland, 1995; Hulme & Finch, 2015). Such an approach requires an interdisciplinary perspective, combining elements from different disciplines within the sport injury literature (Buekers et al., 2017). In particular, Bittencourt et al. (2016) suggested that sports injury is an emergent phenomenon and is dependent on a multitude of factors at any one time, termed the "web of determinants" (Philippe & Mansi, 1998, p. 1). Figure 3 shows an example of different webs that might result in an ACL injury for a basketball player and a ballet dancer (Bittencourt et al., 2016). Although the injury is the same, different combinations of factors contributed to the injury, highlighting the emergent properties of sport injury. While the example mainly focuses on biomechanical properties associated with injury, additional psychological factors could easily be incorporated. As such, the complex system approach proposed by Bittencourt et al. (2016) provides a suitable backdrop for the current programme of research to explore the relationship between psychological distress, physiological stress-related markers and injury occurrence.

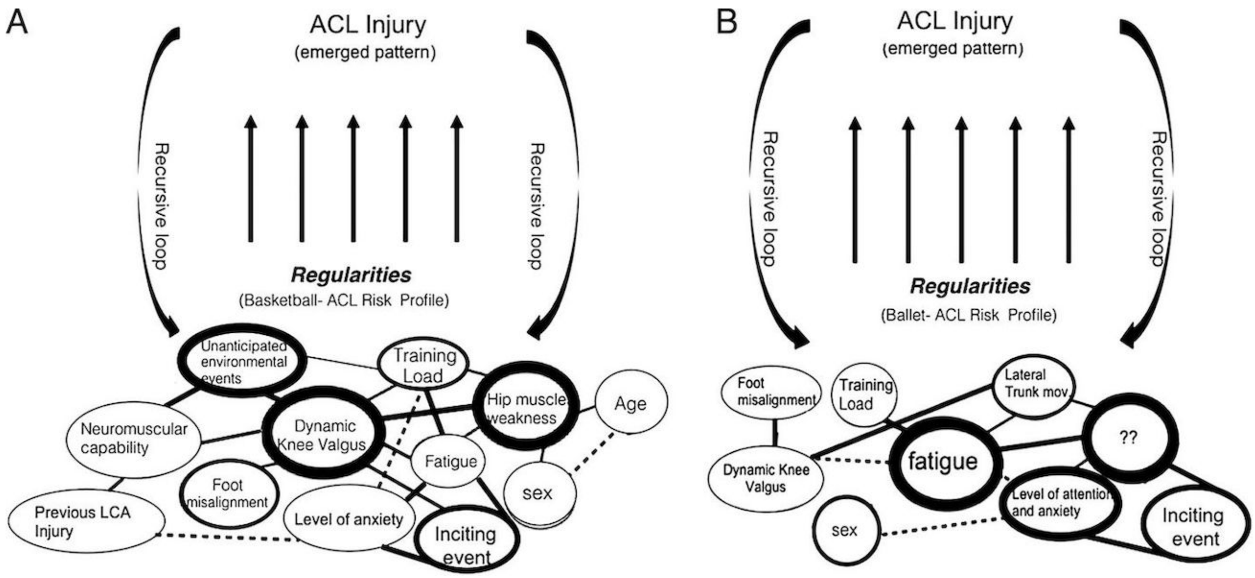

Figure 3. Two examples of complex systems approach to injury. Pattern A shows the factors that may contributed to an ACL injury for a basketball player, and pattern B shows the same injury but from a ballet dancer's perspective. Thick lines indicate a stronger relationship between variables, whereas dashed lines indicate weaker relationships. Adapted from Bittencourt et al. (2016).

Summary and recommendations for future research

While sport injury rates are continuing to rise, a large body of research has identified a multitude of factors that are related to injury occurrence. In particular, stress, from both a psychological and physiological perspective, plays a major role in injury occurrence. However, stress-related factors have often been studied in isolation, and their contribution to a more holistic view of injury occurrence has not been as well established. Recently, both Appaneal and Perna (2014) and Bittencourt et al. (2016) have provided frameworks to further sport injury research. Appaneal and Perna (2014) offered an extension to the widely cited Williams and Andersen (1998) model of stress and injury that included additional physiological stress-related markers that may act synergistically with the psychological characteristics from Williams and Andersen's (1988) model. In addition, Bittencourt et al. (2016) proposed a contemporary view of injury based on complex systems theory and highlighted the need for appropriate research designs and analysis techniques that can address the dynamic nature of injury occurrence. Together, these studies have formed the foundation for the current body of research of which the purpose was to explore the relationships between psychological sources of stress, physiological stress-related markers and injury occurrence. Specifically, the aims of this thesis were threefold: (a) to identify and evaluate the relationships between psychological and physiological markers of stress in the prediction of injury occurrence; (b) to examine the relationships between the markers of stress and injury in a prospective, repeated measures study with a large cohort of athletes; and (c) to evaluate the relationships between the markers of stress and injury using an analysis method that captures the complex nature of injury occurrence.

Chapter 3:

Measures

Measures

The purpose of this chapter is to provide a rationale for the inclusion of the variables and the measures employed in the present research programme. This includes discription of the pilot testing, and the reliability and validity of the selected measures. As highlighted earlier, one of the major criticisms of Williams and Andersen's (1998) model is that it only includes psychological predictors of sports injury, despite evidence that factors associated with an athlete's physical status and response to training are also associated with athletic injury (Appaneal & Perna, 2014; Petrie & Perna, 2004). To address this limitation, Appaneal and Perna (2014) proposed the Biopsychosocial model of stress, athletic injury and health (BMSAIH) to serve as an independent extension to Williams and Andersen's (1998) model. The BMSAIH illustrates how psychological distress and physiological stress-related markers may interact and exacerbate the stress response, increasing the risk of injury. Although research supporting the BMASIH has largely focused on the hormonal response to high intensity training, there is a growing body of literature that has identified several physical markers that can indicate an athlete's physiological status (Djaoui et al., 2017; Lee et al., 2017). These markers provide a means by which athletes and coaches can detect when the balance between the training stress required for physiological adaptation, and recovery, is not appropriate (Borresen & Lambert, 2009). For example, with reduced capacity to recover, high intensity and high volume training can have negative outcomes for an athlete's health, including over-training syndrome, increased susceptibility to illness and increased risk of athletic injury (Appaneal & Perna, 2014). Indeed, when measured in conjunction with psychological factors known to predict injury occurrence, these markers of physiological stress may contribute important insights into athletes' susceptibility to injury. However, research examining the relationships between physiological stress-related markers and sports injury remains sparse.

The purpose of the current research programme was to address the limitations of the current body of injury prediction research by employing an interdisciplinary approach to examine the synergistic action of psychological sources of stress and physiological stress metrics on injury occurrence (cf. Appaneal & Perna, 2014). The current research programme planned to use a large sample and a repeated measures design to capture changes in both psychological predictors of injury and stress-related physiological markers. In order to collect data on the large sample of participants in a timely manner, safeguarding both the rigour and viability of the study, participants needed to be able to complete the series of measures in a realistic and acceptable timeframe, over repeated administrations. This was essential, not least for participant retention in the study. In the remainder of the chapter, the specific measures used in the current research are discussed based on these considerations, and where appropriate, additional pilot studies specific to the current research are outlined.

Major life events (Appendix A)

Of the main psychological factors proposed in Williams and Andersen's (1998) model, major life events, and specifically events with a *negative* valence, have been found to consistently predict injury occurrence in athletes (Gunnoe et al., 2001; Ivarsson & Johnson, 2010; Ivarsson et al., 2017; Maddison & Prapavessis, 2005; Passer & Seese, 1983; Williams & Andersen, 2007). The most widely used measure of life event stress in the sports injury literature is the Life Event Survey for Collegiate Athletes (LESCA; Petrie, 1992). The LESCA comprises 69 items reflecting possible life events that participants may have experienced. Participants are asked to rate the perceived impact of each life event they have experienced within the last 12 months on an 8-point Likert scale anchored at -4 (extremely negative) and +4 (extremely positive). Negative and positive life event scores are calculated by summing the negative and positives score respectively. A score for total life events is calculated by summing the absolute values for both negative and positive life events. Prior to the development of the LESCA, sports injury research had been limited to general scales of life event stress such as the Social Readjustment Rating Scale (SRRS; Holmes & Rahe, 1967) and Life Experience Survey (LES; Sarason et al., 1978). However, a review by Andersen and Williams (1988) indicated the SRRS and LES scales were inappropriate for sport specific research and needed to be refined to better establish the link between major life events and sports injury. Consequently, Petrie (1992) developed the LESCA to address the need for a measure of life event stress suitable for an athletic collegiate population. Indeed, the LESCA has frequently been used in research investigating psychological characteristics associated with athletic injury (e.g., Maddison & Prapavessis, 2005; Gunnoe et al., 2001; Ivarsson & Johnson, 2010; Rogers & Landers, 2005), and remains the most comprehensive scale available to assess major life events in a population of athletes to date (Ivarsson et al., 2017).

Modifications. Although the LESCA was initially developed with collegiate athletes, several authors have used modified versions of the LESCA for younger participants (Gunnoe et al., 2001; Steffen et al., 2009) and non-collegiate athletes (Maddison & Prapavessis, 2005). In the current research, both university aged athletes and adult members of local sports clubs who were not enrolled at university, were recruited to participate in the study. Consequently, several modifications were made to the original LESCA to ensure the suitability of the items for the study population. For university students, the wording of items was adjusted to reflect cultural norms for British university students (e.g., *Beginning a new school experience [beginning college, transferring college etc]* was changed to *Beginning a new university experience [beginning university, transferring university etc]*). For non-students, items associated with college activities/behaviours were either modified to include comparable non-student events (e.g., *Being dismissed from dorm or other residence* was changed to *Being asked to vacate house/home*) or if no suitable alternative was available, the item was removed. Table 1 provides a summary of the modifications made to items in the original measure. Two Professors at the university where the research was conducted who were experienced in sports injury research scrutinized the modifications for face and content validity. In addition, the repeated measures design of the current research required a modification to the participant instructions for the LESCA. To elaborate, during the first data collection in the current research, participants were asked to report life events in the preceding 12 months, as per standard LESCA instructions. For each subsequent data point, the instructions were modified to ask participants to report all life events that had occurred *since the previous data collection.* This modification ensured that only "new" life events would be recorded at each time point, and that participants would not report the same life events more than once, unless there was some significant change.

Table 1
Summary of modifications to LESCA items.

Q	Original	Students	Non-students
19	Beginning a new school experience (beginning college, transferring college etc)	Beginning a new school experience (beginning university, transferring university etc)	Beginning a new work experience
21	Academic probation / ineligibility		Removed
22	Being dismissed from dorm or other residence	Being dismissed from halls or other residence	Being asked to vacate house/home
27	Financial problems concerning school		Financial problems
29	Conflict with room-mate		Conflict within household
36	Suspended from team for non-academic reasons		Removed
49	Being absent from school (classes) because of participation in sport	Being absent from university (classes) because of participation in sport	Being absent from work because of participation in sport
61	Major change in level of academic performance (doing better or worse)		Major change in level of performance at work (doing better or worse)
62	Making career decisions (applying to graduate school,interviewing for jobs, etc)	Making career decisions (applying for Masters degree, interviewing for jobs, etc)	Making career decisions (applying for Masters degree, interviewing for jobs, etc)

Scoring. Traditionally, the LESCA has been scored as the sum of negative and positive life event responses over a 12-month period. However, due to the repeated measures design used in the current research, several considerations were necessary regarding the most appropriate way to score participants' responses. Specifically, deriving LESCA scores at the first time point that reflect life events over a 12-month period, but for subsequent time points, reflect life events over a reduced four-month period was clearly going to be problematic comparatively; with participants likely to have experienced fewer events during the reduced time interval. This situation was further exacerbated by the potential for the effects of the life events experienced during preceding time points to still be present during subsequent data collections. Therefore, to account for the potential continuing and confounding effect of life events, a cumulative sum of life events over the study period was used as the main outcome measure. For example, if a participant reported a score of 10 life events at the first time point and 4 at the second time point, their score at the second time point would be 14. Scoring the LESCA in this way meant that the effects of previous events from preceding time points were still accounted for, while still including the new events that were reported.

Personality (Appdendix B)

Williams and Anderson (1998) proposed several personality characteristics that may exacerbate the response to a stressful athletic situation and contributed to the increased the risk of athletic injury. For example, both high trait and state anxiety have been reported to increase the risk of injury in athletes (Ivarsson & Johnson, 2010; Lavallée & Flint, 1996; Petrie, 1993). However, the evidence for a relationship between other personality traits (e.g., emotional state and locus of control) and injury has been less conclusive, with some research finding no support or contradictory results (Junge, 2000). One aspect of personality theory that has not yet been examined in relation to athletic injury is Reinforcement Sensitivity Theory (RST; Gray & McNaughton, 2000). RST is a neuropsychological theory of personality conceptualised in terms of motivation, learning and emotion that posits that all major personality traits are basic systems of approach and avoidance (Corr, 2013).

First proposed by Gray (1982), RST outlined two systems of behavioural activation and behavioural inhibition (BAS and BIS, respectively). The BAS and BIS were proposed to govern approach behaviour towards appetitive stimuli and avoidance from aversive stimuli (Montag, Smillie, Markett, Reuter, & Cooper, 2016). However, the original RST theory was updated by Gray and McNaughton (2000) who made a distinction between BIS and the Fight Flight Freeze System (FFFS; McNaughton & Corr, 2004). In the revised RST (rRST), the FFFS is proposed to be responsive to all punishing and threatening stimuli (e.g., physical pain from training or competition) and the BAS is proposed to be responsive to all rewarding and appetitive stimuli (e.g., prize money from winning a major competition). In contrast to the original theory, the BIS is thought to be activated during instances of goal conflict, for example when a threatening stimulus must be approached (Corr, 2008). Specifically, BIS is involved in the processes that will generate anxiety, which will result in more cautious approach behaviour (Corr, 2008). Indeed, rRST holds particular relevance for sports injury research due to challenges faced by an athlete in a competitive environment. For example, an athlete may be under physical pain nearing the end of a race (FFFS activated), but motivated by the thought of winning (BAS activated). In such a scenario, the BIS would be activated in an attempt to resolve the conflict between the FFFS and BAS (Hardy, Bell, & Beattie, 2014). The types of behaviour exhibited by an athlete under these conditions may expose an athlete to increased risk of injury, however, to date no research has examined the relationship between rRST and sports injury. The rRST may therefore provide a novel approach to understanding the personality characteristics associated with sports injury.

Several questionnaires have been developed to reflect the rRST model (Jackson 5, Jackson, 2009; Reinforcement Sensitivity Questionnaire (RSQ) Smederevac, Mitrović, Čolović, & Nikolašević, 2014; Reuter and Montag's rRST-Q, Montag et al., 2016); however, when reviewed by Corr (2016), the revised questionnaires were found to have significant theoretical and operational limitations. For example, the Jackson 5 scale (Jackson, 2009) only had one BAS factor, which is inconsistent with theoretical models of the BAS (Corr, 2008). To address the lack of a comprehensive psychometric measures of the rRST, Corr and Cooper (2016) proposed the Reinforcement Sensitivity Theory of Personality Questionnaire (RST-PQ). The RST-PQ comprises 65 statements that measure three major systems: FFFS (e.g., *"I am the sort of person who easily freezes-up when scared"*), BIS (e.g., *"When trying to make a decision, I find myself constantly chewing it over"*) and four BAS factors; Reward Interest (e.g., *"I regularly try new activities just to see if I enjoy* *them"*), Goal Drive Persistence (e.g., *"I am very persistent in achieving my goals"*), Reward Reactivity (e.g., *"I get a special thrill when I am praised for something I've done well"*) and Impulsivity (e.g., *"I find myself doing things on the spur of the moment"*). Corr and Cooper (2016) used both exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) to assess the validity of the RST-PQ and reported a robust six factor structure with a clear a differentiation between FFFS, BIS and separate BAS factors. The model fit indicies for the single order CFA model were: $X^2(2{,}000,\ N = 831) = 6{,}563.46$, $p < .0001$; CFI = 0.87; RMSEA = 0.052, which indicated an acceptable model fit (Corr and Cooper, 2016).

Validation of RST. The validation of the RST-PQ was examined in a separate programme of PhD research (Young, 2019). The present author was involved in the validation process as the participants from this study were used to validate the RST-PQ. Details of validation process are included here for detail but are not strictly part of this programme of research.

To establish the factorial validity of the 65-item model of the RST-PQ, Bayesian Structural Equation Modelling (BSEM; Muthén & Asparouhov, 2012) was used with responses from a sample of 419 university-level athletes (Young, 2019). BSEM was used for the validation as it had several advantages over the maximal likelihood procedures used in traditional confirmatory factor analysis (CFA). In particular, BSEM allows informative variance priors to be specified on cross-loadings, which in traditional CFA, are held at zero and can lead to a blocked or miss-specified model (Marsh et al., 2009). By recognising that some small cross-loadings and correlated residuals could be present within the items, BSEM can reduce the possibility of model miss-specification (Gucciardi & Zyphur, 2016). To perform the validation, three models were specified following the recommendations of Muthén and Asparouhov (2012). Model one specified non-informative priors on the factor loadings and exact zeros on the cross-loadings and correlated residuals. Model two specified non-informative priors on the factor loadings, informative approximate zeros on the cross-loadings and exact zeros on the correlated residuals. Lastly, model three specified non-informative priors on the factor loadings, and informative approximate zeros on the cross-loadings and correlated residuals. The variance of the informative priors was set at ± 0.10, which equates to loadings with a 95% limit of ± .20 and implies weak cross loading and correlated residuals. Loadings that were outside of the range were identified as being highly correlated or having a large source of residual variance (Muthén & Asparouhov, 2012). Estimation of the BSEM models was performed in MPLUS where a Markov chain Monte Carlo (MCMC) algorithm was used with a Gibbs sampler, in which 100,000 iterations were drawn to examine the parameter estimates and model convergence.

Model convergence was assessed using potential scale reduction factor (PSRF), where values between 1.0 and 1.1 indicated good model convergence (Gelman & Brooks, 1998; A. Gelman et al., 2013). Model fit was assessed by Posterior Predictive P (PPP) values and 95 % credibility intervals, where PPP values $\geqslant$ 0.50 and 95 % confidence interval balanced around approximately zero indicated good model fit (Muthén & Asparouhov, 2012) To compare the different BSEM models, the deviance information criterion (DIC) was used, where lower values indicated a better model-fit.

Results. The analysis revealed the 65-item RST-PQ to have both good model convergence and model fit. In addition, all the main factor loadings were statistically significant. However, several problematic items were identified based on weak factor loadings and substantial cross-loadings and correlated residuals. Consequently, the model was re-specified following an item deletion process (Young, 2019) and a 51-item RST-PQ emerged with acceptable model-fit (PPP = 0.739, 95% posterior predictive confidence intervals = -199.220, 101.356). The findings from the BSEM were replicated with a sample of 350 participants from the current research programme (PPP = 0.787, 95% posterior predictive confidence intervals = -208.405, 90.785). Consequently, the 51-item questionnaire was adopted as the chosen measure of RST in the current research.

Heart rate variability (HRV)

Heart rate variability (HRV) is a popular approach for monitoring training adaptation in athletes (Bellenger et al., 2016). Traditionally, resting heart rate was used as a marker to reflect the recovery status of an athlete, however the beat to beat variation within the cardiac cycle has provided greater insight (Plews et al., 2012). The variability in the time period between consecutive heart beats is the result of cardiac modulation through sympathetic and vagal components of the autonomic nervous system (ANS). The degree of variability in the cardiac cycle can provide insight into the ANS, which is altered during training due to homoeostatic perturbation caused by the response to stress (Dong, 2016). The ANS plays a dynamic role in both the response to, and recovery from, intense exercise, and is involved in the regulation of pain, inflammation and tissue repair (Ackermann, 2016). Consequently, some authors have suggested that HRV can be used as an indirect measure of ANS homeostasis to indicate early signs of fatigue and somatic tissue overload (Bellenger et al., 2016; Gisselman, Baxter, Wright, Hegedus, & Tumilty, 2016; Kim, Cheon, Bai, Lee, & Koo, 2018). There are, however, several important considerations to be made when designing a study using HRV as a marker of ANS homeostasis specifically regarding the measurement, calculation and interpretation of HRV data.

Over 70 variables quantifying HRV have been published in the literature that fall under three broad categories; time domain, frequency domain and non-linear methods (Quintana, Alvares, & Heathers, 2016). Time domain methods provide numerical indices summarising the variability of a heart rate signal and are calculated from the time between successive RR intervals (Malik et al., 1996). Two of the most commonly used time domain measures in the sports injury and psycho-physiological literature are the Standard Deviation of the Normal-to-Normal intervals (SDNN) and the Root Mean Square of Successive Differences (RMSSD). The SDNN reflects all the cyclic components responsible for the variability in the recoding period (Malik et al., 1996). The RMSSD reflects vagal tone and is highly correlated with high frequency HRV. Both SDNN and RMSSD are easily calculated from inter-beat interval (IBI) data, and provide reliable estimates of HRV (Al Haddad, Laursen, Chollet, Ahmaidi, & Buchheit, 2011; Laborde, Mosley, & Thayer, 2017). In contrast to time-based measures, frequency domain indices estimate the distribution of absolute or relative power across four frequency bands; ultra-low-frequency (ULF, < 0.0033 Hz), very low-frequency (VLF, 0.0033 – 0.04 Hz), low-frequency (LF, 0.04 – 0.15 Hz) and high-frequency bands (HF, 0.15 – 0.40 Hz; Shaffer & Ginsberg, 2017). Clinical interpretation of the ULF and VLF bands remain contested (Heathers, 2014), whereas the

LF and HF bands are more commonly used to quantify different spectral components of HRV in the psycho-physiological literature (Laborde et al., 2017). The LF band is thought to represent a mix of sympathetic, vagal and baroreflex influences and is affected by breathing rates from 3-9 breaths per minute (BPM). In contrast, The HF band reflects vagal tone and is influenced by breathing rates from 9-24 BPM. The LF/HF ratio has been characterised as representing sympatho-vagal balance between the parasympathetic and sympathetic nervous system (Shaffer & Ginsberg, 2017). However, use of LF/HF ratio has been heavily criticised in the literature (Heathers, 2014), due to the uncertain relationship between LF power and sympathetic nerve activation and the non-reciprocal relationship between sympathetic and parasympathetic activity (Billman, 2013; Laborde et al., 2017). Consequently, Laborde et al. (2017) recommended that researchers use HRV indices that are underpinned by clearly defined and theoretically sound physiological systems. In addition to time and frequency domain analysis, non-linear analysis has been proposed as a method to capture the complex and erratic fluctuations of the heart rate signal more adequately than traditional linear approaches (Laborde et al., 2017). However, these methods are computationally complex, and their utility has yet to be established in the literature (Sassi et al., 2015).

Both time and frequency domain indices of HRV have been used in the sports injury literature. In particular, reductions in markers such as RMSSD and HF HRV have associated with illness, burnout and increased injury incidence. For example, Williams et al. (2017) measured HRV and acute-to-chronic workload ratio (ACWR) over a 16-week period in competitive cross-fit athletes. For the analysis, the rolling 7-day average of the natural logarithm of the square root of the mean sum of the squared differences between R–R intervals (Ln RMSSD) and ACWR measures were parsed into tertiles ("low", "moderate/normal", and "high") based on within-individual z-scores. When athletes demonstrated a "low" HRV and "high" ACRW, the risk of overuse injuries substantially increased compared to when "moderate" and "high" HRV was observed. Plews et al. (2012) used a case study approach to assess HRV in two elite triathletes. HRV data were collected over a 77-day period where each athlete was training approximately 24 hours per week. During the observation period, one of the athletes performed poorly in a key triathlon event and was diagnosed as non-functionally over-reached and subsequently reactivated the dormant virus herpes zoster (shingles). The athlete's data revealed large linear reductions in the 7-day rolling average Ln RMSSD leading up to the period when the athlete was diagnosed as non-functionally over-reached. In contrast, the control athlete's data remained stable throughout the observation period, showing the potential value for repeatedly monitoring HRV through periods of high intensity training. Lima-Borges, Martinez, Vanderlei, Barbosa, and Oliveira-Junior (2018) assessed stress, recovery and several indices of HRV including LF, HF and RMSSD in sprint and endurance-based competitive swimmers during general training, specific training, and competitive stages of a 20-week season. Reductions in both HF HRV and RMSSD were associated with increased injury incidence in the sprint group, which Lima-Borges et al. (2018) attributed to the progressive activation of the sympathetic nervous system as a result of the higher intensity training in the sprint group compared to the endurance group. These studies highlight the potential predictive value of using HRV as a marker of training-related stress.

In addition to training related stress, reduced HRV indices have also been found to be associated with increased life event stress. For example, Pieper, Brosschot, Van Der Leeden, and Thayer (2007) found that both worry episodes and stressful events were independently associated with elevated heart rate and decreased levels of RMSSD. Dishman et al. (2000) assessed perceived stress, and LF and HF power in 92 healthy participants with above average cardiovascular fitness and found a negative relationship between stress and normalized HF HRV ($p = 0.038$). Dishman et al.'s (2000) findings were supported by Sin, Sloan, McKinley, and Almeida (2016) who observed that greater perceived stressor reactivity was associated with reduced HRV indices including RMSSD, SDNN and HF in a large sample of 909 participants. The combined evidence that reduced HRV is a marker of training and life event stress make HRV a suitable measure to investigate the synergistic action of both psychological and physiological sources of stress that can contribute to injury occurrence. Specifically, RMSSD has commonly been used as an index of HRV and has consistently shown a negative relationship with fatigue, overtraining, worry and stressful events (Pieper et al., 2007; Plews et al., 2012). Reductions in RMSSD are thought to represent reduced parasympathetic activation, implying impared recovery of the ANS resulting in a greater risk of negative health consequences including illness and injury (Kim et al., 2018). However, few studies have assessed psychological predictors of injury in conjunction with a marker such as HRV and examining the link between these variables will provide greater insight into the injury process. There are, however, several methodological factors that need to be considered when designing a study measuring HRV.

Measurement issues. As outlined above, a range of different techniques have been developed for both the recording and analysis of HRV data (Quintana et al., 2016). In an attempt to standardise measurement techniques across different studies, Malik et al. (1996) published a set of recommendations to guide HRV research however inconsistencies in the standards of HRV measurements and reporting remain (Kim et al., 2018). One criticism of Malik et al.'s (1996) guidelines is that they are now over two decades old, and do not reflect the advances in technology that make the measurement of HRV available to a wide variety of researchers with different backgrounds (Quintana et al., 2016). Consequently, authors from psychiatry (Quintana et al., 2016) and psychophysiology (Laborde et al., 2017) have recently provided guidelines for their specific fields of research to advance both the interpretation and reproducibility of HRV research. The recommendations from Quintana et al. (2016) and Laborde et al. (2017) were relevant to the current study given the measurement of psychological characteristics in combination with markers of training related stress. Therefore, the recommendations from Malik et al. (1996), Quintana et al. (2016) and Laborde et al. (2017) were used to guide the collection and analysis of HRV in the current study.

HRV indices. Although the HF band has been established as a suitable marker of vagal tone, recording and calculating HF power presents several additional problems compared to using time-domain methods (Esco, Williford, Flatt, Freeborn, & Nakamura, 2018). For example, respiration rate has been shown to greatly influence the HF component of the inter-beat interval signal, and the HF band only reflects vagal tone when respiration rate is above nine and below 24 cycles per minute (Berntson et al., 1997). Athletes who have respiration rates near 9 BPM are at the lower end of the HF spectrum (0.15Hz) and inconsistencies in HF results have been reported in athletes with breathing rates near 9

BPM (Saboul, Pialoux, & Hautier, 2014). Furthermore, in a repeated measures study design, breathing rate would need to be similar across all measurement occasions to allow comparison across multiple time points. In the current study, assessment of breathing rate would have added additional time to the data collection process potentially reducing participant retention. Additionally, the calculation of HF requires complex algorithms that can be greatly influenced both by the number of artefacts in, and the cleaning process used for, the IBI data (Quintana et al., 2016).

In contrast to HF, time domain measures such as RMSSD are less affected by breathing rate and provide a more stable measure of HRV under spontaneous breathing conditions across repeated measurements (Hill, Siebenbrock, Sollers III, & Thayer, 2009). Furthermore, time domain methods require less sophisticated calculations, and artefacts in the IBI recording can be removed proportionally (Quintana et al., 2016). Given that time-domain measures such as RMSSD are highly correlated with HF measurements (Laborde et al., 2017), and that calculation of time-domain indices minimise the potential issues with frequency-domain calculations (Quintana et al., 2016), the current research used RMSSD to quantify HRV (Munoz et al., 2015).

Equipment. Electrocardiogram (ECG) traces are the recommended gold standard for recording the IBI needed to calculate HRV (Malik et al., 1996). However, access to ECG recording equipment may be limited due to the high cost and high level of expertise needed to operate the equipment. Consequently, several alternative methods for recording IBI data have been developed. The recent popularity of HRV in sports science has been largely driven by advances in technology that have provided athletes, coaches and researchers with affordable, robust and reliable means of recording inter-beat interval data in the form of heart rate monitors (HRMs) with wireless chest strap electrodes (Giles, Draper, & Neil, 2016). HRMs have an advantage over traditional methods of assessing HRV due to the portability and relatively lower cost in comparison with ECG equipment meaning HRV can be collected in a variety of different settings. One such device that has been validated in the literature is the PolarV800 (Polar OY, Finland). IBI data collected using PolarV800 has been found to be highly comparable to data obtained from ECG recordings (Giles et al., 2016). In addition, HRV parameters calculated from inter-beat interval and ECG data have shown a strong correlation ($r = 0.99$) under spontaneous breathing conditions (Plews et al., 2017). Based on this information, a PolarV800 was used in the current study to collect IBI data.

Recording duration. The length of recording necessary to establish reliable indices of HRV has been widely addressed in the literature (Berntson et al., 1997; Malik et al., 1996). A minimum of 5 min is recommended for short-term recordings to ensure comparability of results across studies (Malik et al., 1996). However, research has shown durations as short as 60 s have good reliability compared to 5 min recordings (Esco & Flatt, 2014). Munoz et al. (2015) found it was unnecessary to use recordings longer than 120 s to obtain accurate time-domain measurements of both SDNN and RMSSD. Short duration recordings are particularly desirable in an applied sport setting as they place minimal burden on the participant and can be collected under standardised conditions (Munoz et al., 2015). Despite the evidence of reliable short term HRV recordings, Laborde et al. (2017) recommended that researchers use the standard 5 min recording period where possible to allow comparison with clinical studies. Therefore, in-line with the recommendations of both Malik et al. (1996) and Laborde et al. (2017), 5 min recordings were used in the current study.

Confounding variables. Several factors have been reported to influence the results of IBI recordings including; age, gender, habitual levels of alcohol consumption and cardioactive medication (Laborde et al., 2017). In addition, transient variables prior to the data collection need to be considered (Laborde et al., 2017; Quintana et al., 2016). For example, the amount of intense physical training 24-hours before the recording, caffeine and food consumption in the 2-hours prior to the collection and alcohol consumption 24-hours prior to collection can all influence HRV recordings. To control for potential confounding variables in the current study, the lead researcher communicated with participants to establish the most suitable time to collect data. For example, data collections were scheduled on days following a day of rest and before any physical activity on the day of testing. The day of the week and time of day were recorded for each participant and subsequent data collections were scheduled at similar times to maintain consistency across time points.

Methods to calculate HRV indices. There are a several software packages available that can be used to calculate HRV indices. For example, Kubios HRV (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2014) is a widely used software package capable of calculating commonly cited HRV indices in both the time and frequency domain, such as RMSSD and HF band. Kubios HRV software is not open source, meaning it is impossible to ascertain exactly how each parameter is calculated. Replication of results in HRV studies by other researchers can therefore be difficult when the Kubios HRV software is used to calculate HRV indices.

In contrast to Kubios HRV, R (R Core Team, 2019a) is a freely available, open source programming language where users can create packages that can be used for a wide range of data analysis tasks. The RHRV package (García Martínez et al., 2017) provides a comprehensive set of functions to calculate many of the widely used HRV indices in both the time and frequency domain. In addition, the R language allows the user to define functions that can loop over many data files with ease. Given the flexibility provided by the R language and RHRV package, a custom script (Appendix C) was written by the author to enable fast computation of IBI files, minimising the potential for user error.

Muscle stiffness

Musculoskeletal characteristics such as tone, and mechanical properties including stiffness and elasticity have been identified as important factors for sports performance (Lee et al., 2017). Objective measurement of these characteristics can greatly enhance the ability to detect abnormal changes in the muscle that commonly precede muscular injury (Mullix, Warner, & Stokes, 2012). Indeed, the link between muscle stiffness and injury was proposed as a possible mechanism for injury in Williams and Andersen's (1998) model, with a heightened stress response predicted to result in increased physiological activation causing increased muscle stiffness. The increased stiffness was proposed to cause decreased flexibility and reduced motor coordination, potentially increasing the risk of athletic injury

(Williams & Andersen, 1998). However, few studies have provided evidence for the relationship between acute changes in muscle stiffness as a result of the stress response in relation to athletic injury.

In their extension to Williams and Andersen's (1998) model, Appaneal and Perna (2014) suggested that athletic injury was the result of the synergistic action between psychological stressors and training related stress from high intensity exercise. Muscle stiffness is often increased in response to high intensity exercise as a result of eccentric muscle contractions that occur in a variety of movements (Hedayatpour & Falla, 2015). When an athlete is exposed to prolonged periods of high intensity training cellular changes within the muscle structure can cause increased muscle stiffness in the resting muscle (Hedayatpour & Falla, 2015). Therefore, measurement of muscle stiffness provides a marker of training related stress that may precede injury occurrence.

Traditionally, clinical assessment of muscle stiffness has been quantified with subjective measurement scales. For example, the Modified Ashworth Scale (MAS) is a six-category ordinal scale used to assess the resistance encountered during passive muscle stretching performed by a trained physical therapist; however, the reliability of the MAS has been found to be poor, and more objective methods of quantifying muscle tension are preferred (Craven & Morris, 2010). In contrast to the MAS, shear wave elastography (SWE) is an objective technique to measure muscle properties such as stiffness and is considered the current gold standard in objective assessment of skeletal muscle (Kelly et al., 2018). SWE uses focused ultrasound radiation forces to generate a wave that travels horizontally though the tissue to the point of application to estimate properties of the tissue (Gennisson et al., 2010), and has been found to be highly reliable across several muscle groups (Lacourpaille, Hug, Bouillard, Hogrel, & Nordez, 2012). However, researchers have limited access to SWE due to the high cost per unit and specialist expertise needed to use the equipment.

Recently Kelly et al. (2018) reported a significant correlation between SWE measures of stiffness and a novel handheld device (MyotonPRO, Myoton AS, Tallinn, Estonia) capable of measuring skeletal muscle properties. The relative cost of the MyotonPRO is significantly lower than SWE and may offer a suitable alternative for assessing skeletal muscle characteristics. Unlike SWE, which uses ultrasound to estimate properties in the muscle tissue, the MyotonPRO generates an oscillation in soft tissues by exerting a brief mechanical tap on the surface of the skin. The oscillation is recorded by a three-axis digital accelerometer in the device, and several skeletal muscle characteristics are calculated simultaneously by the device using the oscillation signal including; natural oscillation frequency (Hz) characterising muscle tone, logarithmic decrement of natural oscillation frequency (D) characterising muscle elasticity, dynamic stiffness (N/m) creep and mechanical stress relaxation time (ms). The reliability of the MyotonPRO has been well established within the literature, with studies reporting good to excellent inter-rater (Agyapong-Badu et al., 2013), intra-rater (Aird, Samuel, & Stokes, 2012) and between day reliability (Agyapong-Badu, Warner, Samuel, & Stokes, 2016). Consequently, the MyotonPRO has been used as a tool to examine skeletal muscle properties in both clinical and applied settings.

In a clinical setting, the MyotonPRO has been used to distinguish between participants with symptomatic and asymptomatic Achilles tendons (Morgan, Martin, Williams, Pearce, & Morris, 2018), to identify changes in stiffness and elasticity levels in shoulder muscles following treatment for chronic shoulder pain (Gordon, Andrasik, Schleip, Birbaumer, & Rea, 2016) and quantify musculoskeletal characteristics in stroke patients (Chuang et al., 2013; Chuang, Wu, & Lin, 2012). In the sports science literature, the MyotonPRO has often been used alongside markers of dynamic stiffness (e.g., vertical hopping, Pruyn, Watsford, & Murphy, 2014) to investigate the relationship between stiffness and athletic performance (Kalkhoven & Watsford, 2017; Pruyn, Watsford, & Murphy, 2015). Considerably fewer studies have used the MyotonPRO to examine the relationship between muscle properties and athletic injury. One study that did examine this relationship was conducted by Pickering-Rodriguez et al. (2017), who measured stiffness at four sites on the lower body (lateral gastrocnemius [LG], medial gastrocnemius [MG], soleus [SOL] and Achilles aponeurosis [ACH]) in a group of 29 netballers. Injury occurrence was monitored prospectively over one season, and a total of 12 injuries sustained by 10 players were recorded. Pickering-Rodriguez et al. (2017) reported that injured players had increased SOL ($p = 0.037$) and ACH ($p = 0.004$) stiffness compared to healthy players, thus providing evidence of a relationship between increased muscle stiffness as measured by the MyotonPRO and athletic injury. However, the study was limited by a small sample size and is not generalisable to a wider sporting populating given that all the participants were female netball players. The present research aimed to address this limitation and examine the relationship between muscle characteristics such as stiffness in a large group of participants from a variety of different sports and ability levels. Given the findings from Pickering-Rodriguez et al. (2017) and the established reliability and validity of the device, the MyotonPRO was used in the current research to quantify musculoskeletal properties as markers of stress including tone, stiffness and elasticity.

Pilot study. Due to the repeated measures design of the current research, a reliable method of determining the testing sites was necessary to allow for measurements to be compared across different time points in the study. One commonly used method for identifying testing sites involves finding the midpoint of the muscle by measuring the distance between two anatomical landmarks (Bailey, Dinesh, Warner, Stokes, & Samuel, 2013). For example, to identify the belly of the biceps brachii Bailey et al. (2013) measured the halfway point between the anterior aspect of the lateral tip of the acromion and the medial border of the cubital fossa. However, a limitation of this technique is that anatomical landmark locations can vary in different people depending on body type and size. The resulting measurement may be invalid due to incorrect identification of the muscle belly. In comparison, Chuang et al. (2013) used a visual-placatory technique, which included visually identifying and palpating the target muscle belly. The visual-palpatory technique allows for a degree of clinical interpretation of the testing location and can be used for a range of different body types. Therefore, the visual-palpatory technique was chosen to accommodate a range of body types in participants from different sports and genders in the current research, however, the consistency of the technique on repeated trials needed to be established.

A pilot study was conducted to determine the consistency with which the visual-palpatory technique could be applied to identify different testing sites over repeated trials. The trial had the following aims: (a) quantify the difference between testing locations identified using repeated application of the visual-palpatory technique; and (b) test for statistical differences in the measures obtained using the MyotonPRO at the sites identified using the repeated application of the visual-palpatory technique.

Participants. Five sports students (male, n = 3 female, n = 2, age = 25.6 ± 1.8, mass = 74.4 ± 11.1, height = 177.8 ± 12.7) from a British University were recruited to take part in the study. All participants were members of the university athletics team and were injury free at the time of data collection.

Site identification. Four testing sites including the muscle belly of the rectus femoris (RF), bicep femoris (BF), medial gastrocnemius (MG) lateral gastrocnemius (LG) on both the left and right legs were chosen as testing locations. These sites had been used in previous literature and represent the major muscle groups in the lower extremities (Pruyn et al., 2015). A visual-palpatory technique similar to that described by Chuang et al. (2013) and based on the SENIAM guidelines (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000) was used to identify the testing locations. The technique involved a combination of the participant performing a contraction of the target muscle, visual inspection of the target muscle and palpation of the muscle belly. The contraction helped to visually identify the muscle, and palpation helped identify the bulk of the muscle.

The testing sites were identified in the following order; left and right RF, left and right BF, left MG and LG, right MG and LG. All sites were initially marked with an ultraviolet pen (UVP; Invisible ink pen, MainStreet Unlimited, Michigan), that was invisible to the tester until a UV light was used to illuminate the area. After a period of 10 min, the process of identifying the sites was repeated with a surgical marker pen (SMP). Once all sites had been marked twice, once with the UVP and once with the SMP, images were taken of each site. A tape measure was included in each image to provide a reference point for the measurement between each point (Figure 4).

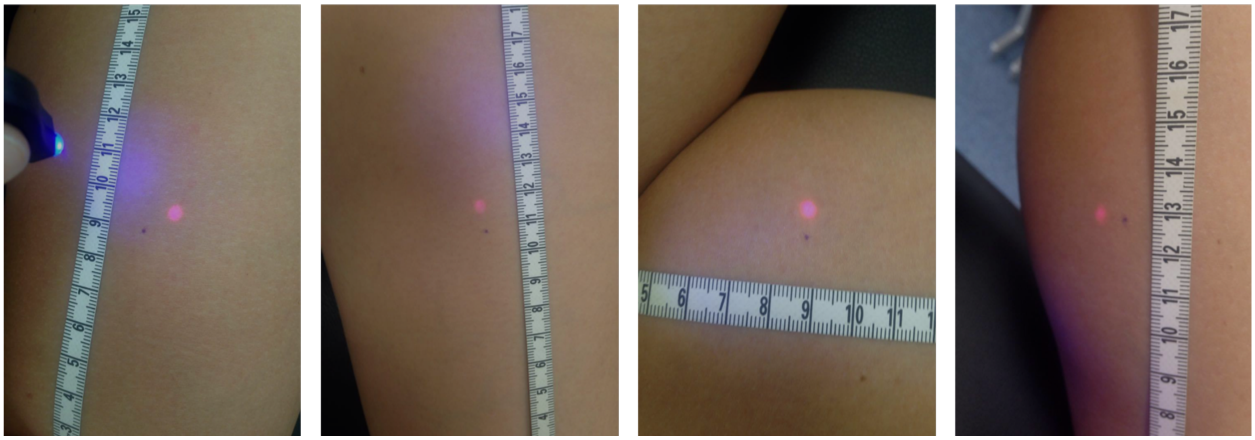

Figure 4. Testing sites identified with repeated application of the visual-palpatory technique. SMP = black mark, UVP = pink mark. Left to Right; RF, BF, MG, LG.

Images were uploaded to a computer and the distance between the UVP and SMP locations was measured digitally using a Java based image processing app (ImageJ, Laboratory for Optical and Computational Instrumentation, University of Wisconsin). First, the scale was set by drawing a line that was equivalent to 10mm against the measuring tape in each image. A line was then drawn linearly between the centre of the two marked sites. The software calculated the length of the calibrated line between the two marks and was saved for further analysis (Figure 5).

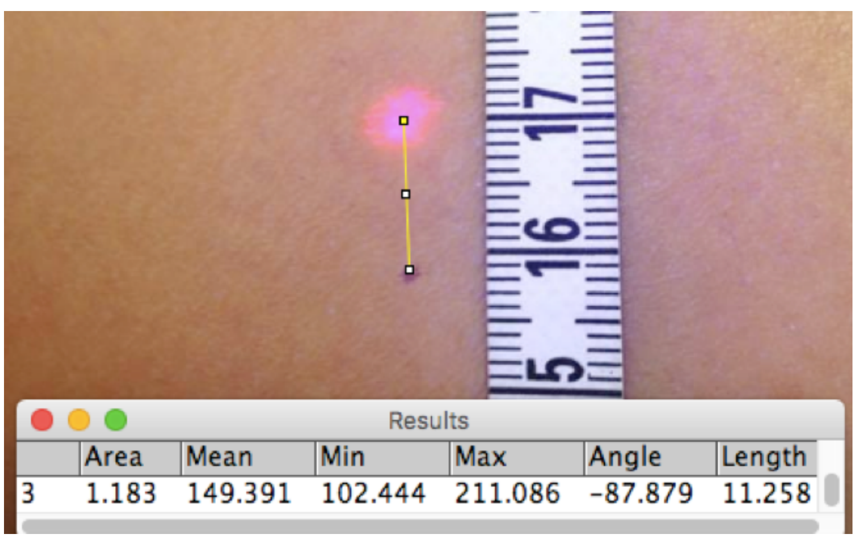

Figure 5. Screenshot of the measurement process using the ImageJ software.

Myoton measurement. Two sets of measurements were recorded using the MyotonPRO. The first set included all the sites marked with the UVP, and the second set included all sites marked with the SMP. At each site, the MyotonPRO delivered a mechanical impulse of 0.4N for a period of 15 ms under a constant pre-load (0.18N) of the subcutaneous tissue layer above the muscle that was being measured. The device end (d = 3mm) was positioned on the marked location and held perpendicular to the surface of the skin. After each mechanical impulse, the oscillations of the tissue deformation were recorded by the accelerometer in the device. The device was set to multi-scan mode and five consecutive measurements separated by a one second interval were taken at each site with the median of the five measurements saved for further analysis (Morgan et al., 2018). The device automatically calculated the coefficient of variation (CV) for each set of five measurements, and a measurement set was repeated if the CV was greater 3% as recommended by the MyotonPRO user guidelines (myoton.com).

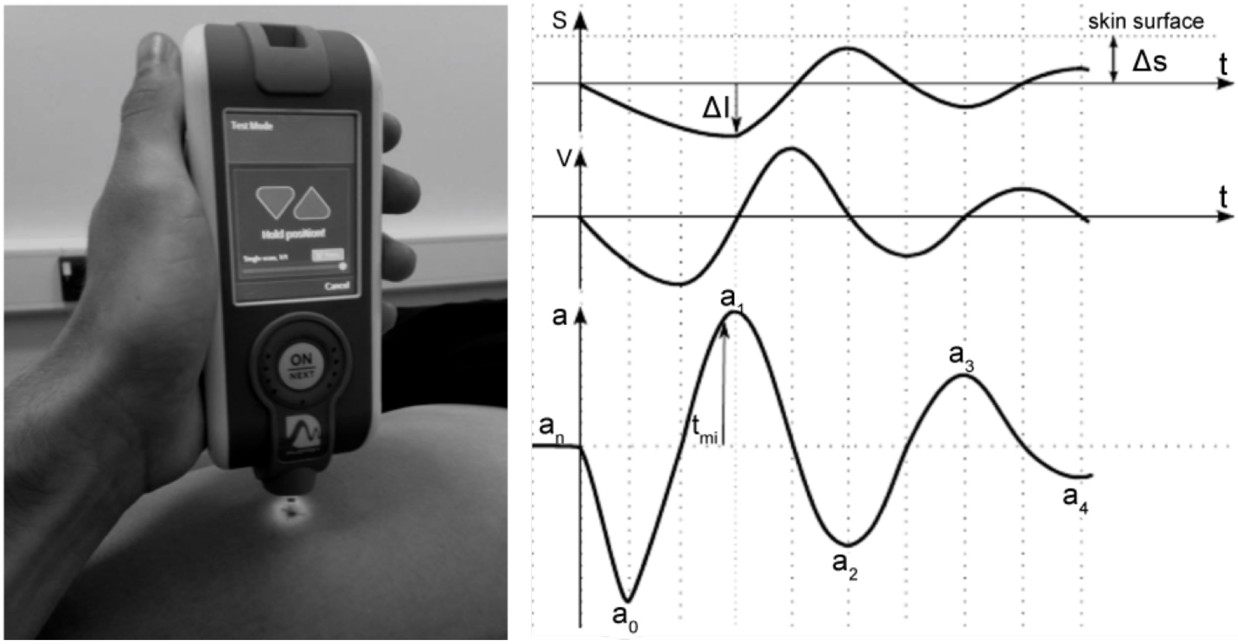

Figure 6. Left: MyotonPRO being applied to soft tissue. Right: Waveforms recorded by the device following mechanical impulse illustrating the relative displacement (S; mm), velocity (V; m/s) and acceleration (a; mG) of the soft tissue oscillation. a0 : Maximum acceleration; tmi: End of mechanical impulse; a1: Maximum acceleration representing the maximum displacement of the tissue i.e. maximum tissue resistance (mG); a2: Maximum acceleration at the point of opposite displacement due to residual inertia of the tissue oscillation; a3: Maximum acceleration of the second period of oscillation–occurs due to recuperation of stored residual mechanical energy in the tissue. Adapted from Bailey et al. (2013)

The following musculoskeletal characteristics were calculated from the acceleration signal.

F: Oscillation frequency [Hz]

Oscillation frequency indicates the tone of a muscle and is defined as the maximum frequency (F = f_{max}) computed from the signal spectrum by Fast Fourier Transform (FFT).

S: Dynamic stiffness ($\mathrm{N} \cdot \mathrm{m}^{-1}$)

Dynamic stiffness ($\mathrm{N} \cdot \mathrm{m}^{-1}$) is defined as the ability of the tissue to resist a force that modifies its shape.

$$S = m_{probe} \left(\frac{\alpha_{max}}{\Delta l} \right) \tag{1}$$

Where m_{probe} is the mass of the testing end of the MyotonPRO (kg), α_{max} is the maximum acceleration of the oscillation ($m \cdot s^{-2}$), and Δl is deformation depth of the muscle tissue.

D: Logarithmic decrement

The logarithmic decrement of the oscillation indicates the elasticity of the muscle. Elasticity is defined as the tissues ability to restore its shape after being deformed. Elasticity (logarithmic decrement) is expressed in arbitrary units as:

$$D = \ln \left\{ \frac{a_1}{a_3} \right\} \tag{2}$$

Where ln is the natural logarithm, a_{max} is the maximal amplitude of oscillation and a_1 is the oscillation amplitude. Differences between the identified sites were examined with descriptive statistics (range, mean and standard deviation). The BEST package (Kruschke, 2013) in R (R Core Team, 2019a) was used to perform Bayesian paired-sample t-tests to compare the difference in means between set one and set two for each characteristic and location.

Results. Differences between the measurement locations identified using the visual-palpatory technique are presented in Table 2. The BF site had the largest measured differences at 17 mm and 19 mm for the left and right legs respectively. The right MG had the smallest error of 5.8 mm. Figure 7 provides a visual representation of the results from the Bayesian paired sample test. The difference between the SMP and UVP measurements for all parameters and locations were close to 0 (0.05 $\pm$ 0.58), and the probability that differences where less than or greater than zero were 0.40 $\pm$ 0.22 and 0.60 $\pm$ 0.22 respectively.

Table 2

Difference (mm) between identified measurement locations using the visual-palpatory technique.

	Left side				Right side			
Location	Min	Mix	*M*	*SD*	Min	Max	*M*	*SD*
RF	6.0	11.0	9.20	1.90	1.0	13.0	6.20	4.60
BF	5.0	17.0	8.80	4.46	3.0	19.0	11.20	5.80
MG	6.0	9.0	7.40	1.40	2.0	9.0	5.80	2.40
LG	5.0	12.0	8.20	2.65	2.0	16.0	8.40	5.14

Note. RF = rectus femoris, BF = biceps femoris, MG = medial gastrocnemius, LG = lateral gastrocnemius

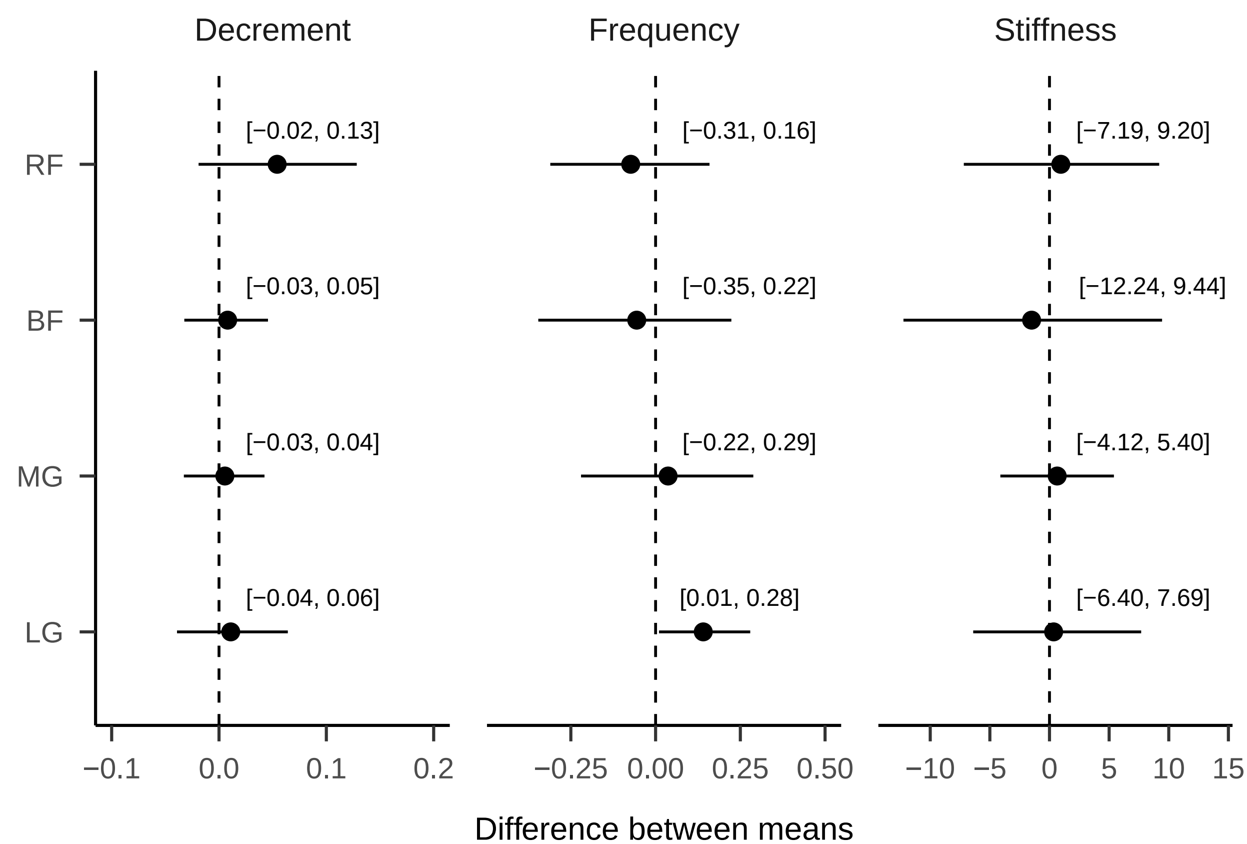

Figure 7. Mean difference (95% CI) between measurements taken at sites identified with the UV and Surgical marker pen. Vertical dashed line indicates no difference between the means.

Summary. The pilot study confirmed the use of the visual-palpatory technique as a reliable method to identify testing locations. The results showed the error between testing sites identified using the visual-palpatory technique did not result in differences between the measurement sets (probability of the difference between means $\neq 0 < 1$). Therefore, the visual-palpatory technique was used to identify testing locations across all time points in the current study.

Balance

The ability to maintain an upright posture depends on the complex interaction between vestibular, somatosensory and visual systems in the body (Paillard & Noé, 2015). Maintaining postural stability is necessary to accomplish movement and motor tasks that require the displacement of body segments or the entire body (Brachman et al., 2017) and is therefore an important factor for all sports, which often require rapid body displacement to achieve sport specific tasks (Hahn, Foldspang, Vestergaard, & Ingemann-Hansen, 1999). Given the importance of postural stability for movement, improved postural stability is often the goal of athletic training programmes (Hrysomallis, 2011). Elite athletes often exhibit superior postural stability compared to their less proficient counterparts (Paillard et al., 2006; Sell, Tsai, Smoliga, Myers, & Lephart, 2007), and balance training has been found to improve vertical jump, agility and shuttle run performance in athletes (Kean, Behm, & Young, 2006; Yaggie & Campbell, 2006). In contrast, impaired postural stability has been identified as a predictor of lower extremity injury (McGuine, Greene, Best, & Leverson, 2000; Romero-Franco et al., 2014; Trojian & McKeag, 2006; Tropp, Ekstrand, & Gillquist, 1984; Wang, Chen, Shiang, Jan, & Lin, 2006). In addition, both fatigue (Salavati, Moghadam, Ebrahimi, & Arab, 2007), and perceived psychological stress (Coco et al., 2015; Doumas, Morsanyi, & Young, 2018), have been found to have a negative impact upon postural stability, potentially exacerbating the risk of injury. Assessment of postural stability may therefore provide an important marker of the synergistic action of both training related, and psychological sources of stress experienced by athletes that contributed to increased risk of injury (Appaneal & Perna, 2014).

Several methods have been described in the literature for assessing postural stability. In a clinical setting, the use of force platforms to measure changes in centre of pressure (COP) displacement is considered the gold standard for postural stability assessment (Paillard & Noé, 2015). In the sports injury literature, Dingenen et al. (2016) reported increased COP displacement during a double to single leg transition task predicted subsequent non-contact lower extremity injury in a group of 50 female athletes. For the transition task (TT), participants transitioned from a double leg stance to a single leg stance, with each stance being held for 13 seconds and eyes closed throughout. Increased COP displacement in the first three seconds following the transition from double to single leg stance was found to predict non-contact lower extremity injury (Dingenen et al., 2016). The TT outlined by Dingenen et al. (2016) therefore provides a suitable measure of postural stability for the proposed study; however, the protocol may be too time consuming to complete with a large sample of participants.

In contrast to quantitative force plate measures, several qualitative techniques have been developed to provide a simple means of assessing postural stability. The Balance Error Scoring System (BESS) is one such measure that has been widely used in the sports injury literature (Bell, Guskiewicz, Clark, & Padua, 2011). The BESS protocol contains three stance positions; double-leg stance (hands on the hips and feet together), single-leg stance (standing on the non-dominant leg with hands on hips), and a tandem stance (non-dominant foot behind the dominant foot) in a heel-to-toe fashion completed on both a firm and foam surface. Each stance is held for 30s with no visual input (eyes closed) and errors observed in maintaining the stance position are recorded by the tester. Errors are defined as: (a) lifting hands off iliac crests; (b) opening eyes; (c) stepping, stumbling, or falling; (d) moving the thigh into more than 30 degrees of flexion or abduction; (e) lifting the forefoot or heel; and (f) remaining out of the testing position for more than five seconds (Riemann, Guskiewicz, & Shields, 1999). At the end of the test, the errors are summed together to give a total score. The BESS has demonstrated the ability to distinguish between injured and non-injured athletes (Riemann et al., 1999), and participants with and without functional ankle instability (Ross, Linens, Wright, & Arnold, 2011). Therefore, the BESS provides an alternative to the single leg transition task outlined by Dingenen et al.

(2016) for assessing postural stability in a large sample of participants.

Pilot study. To determine which method was most appropriate for the proposed study, both the single leg TT and BESS were trialed. A group of 10 sports students (males = 8, females = 2, age = 22.9 ± 4.2, height = 172.9 ± 6.0, mass = 71.3 ± 9.5) who were injury free at the time of the test were recruited to take part in the trial. All participants first completed the single leg transition task outlined by Dingenen et al. (2016), followed by the BESS protocol (Riemann et al., 1999). The time taken to complete each trial for both methods, and the total time to complete each method with all 10 participants was recorded. Table 3 shows the mean (±SD) and total time (minutes) taken to complete each test.

Table 3
Mean, SD and total time taken for the BESS and TT.

Method	*M*	*SD*	Time (Min)
BESS	3.85	0.49	38
TT	8.45	1.04	85

Note. BESS = Balance error scoring system; TT = Transition task.

The trial revealed several difficulties with using the TT compared to the BESS. Several participants found the TT protocol challenging, and trials had to be repeated when participants failed to complete the transition task successfully. The repetition of failed trials added a considerable amount of time to the protocol. Approximately 90 min were needed to record data for all 10 participants, which was longer than the desired data collection protocol of 60 min for all measures in the current study. In contrast, participants found the BESS protocol simple and the testing was completed within the target 60 min. However, participants found the double leg stance on both firm and foam surfaces simple, ultimately contributing no errors to the total error score. In a previous study, Hunt, Ferrara, Bornstein, and Baumgartner (2009) examined the contribution of each of the stances used in the BESS and found the double leg stance contributed only 0.17% of the variance within the test. Furthermore, the test-retest reliability of the BESS increased from

0.67 to 0.71 when the double leg stance was removed (Hunt et al., 2009). Based on the findings from the pilot study and those of Hunt et al., (2009) the double leg stance was removed for the proposed research. In addition, a limitation of the original protocol was that only one single leg stance on the non-dominant leg was examined. Injuries may occur unilaterally on either limb, and athletes may have impaired postural stability on either the dominant or non-dominant side. Indeed, postural stability assessment of both limbs would enable a comparison between dominant and non-dominant legs and asymmetries between the limbs. Particularly pertinent to the current research, asymmetry between limbs has previously been found to predict non-contact injury in athletes (Smith, Chimera, & Warren, 2015). Therefore, an additional single leg stance on the dominant leg was included in the BESS protocol for the current research.

In summary, the BESS protocol used in the current research modified the original protocol in two ways. 1) The double leg stance was removed based on the findings from the pilot study and the recommendations from Hunt et al. (2009). 2) A second single leg stance on the dominant leg was included to enable comparison between limbs as asymmetry in balance performance has previously been associated with lower leg injuries (Smith et al., 2015). All scoring of the modified BESS protocol (mBESS) used in the current research was in accordance with the original protocol.

Hormonal response to training

The measurement of salivary free cortisol has been widely used as a biomarker of the stress response in a variety of psychological investigations (e.g., Anderson & Wideman, 2017; Pulopulos, Vanderhasselt, & De Raedt, 2018), and is commonly used to monitor athletes' response to training due to its non-invasive nature. Cortisol is released from the adrenal gland when the hypothalamic-pituitary adrenal-axis (HPA) is activated in response to an environmental or psycho-social challenge (Tsigos & Chrousos, 2002). The main role of cortisol in response to exercise is to increase the availability of substrates for metabolism, both during exercise and into recovery (Anderson & Wideman, 2017). However, in addition to high intensity exercise, cortisol is also released in response to psychological stress such as to major life events and the attendant emotional distress that follows (Pulopulos et al., 2018; Roos, Levens, & Bennett, 2018). In both high intensity training and exposure to psychological stress, the degree to which the cortisol response is activated is dependent on an individual's appraisal of the severity, and their perceived capacity to cope with the situation (McEwen, 2007). Therefore, the combined reaction of exposure to psychological stress and high intensity training, as proposed by the BMSAIH model, may lead to increased levels of cortisol. Specifically, the BMSAIH predicts that an athlete who performs high intensity training and perceives high intensity training and life events as *severe* stressors, will have prolonged emotional reactivity and a concomitant exacerbated cortisol response (Appaneal & Perna, 2014). A heightened cortisol response has been proposed to have several negative effects, for example, elevated evening cortisol has been associated with a suppressed immune system, poor sleep and reduced growth hormone release, all of which can inhibit recovery following intense exercise and increase the risk of athletic injury (Brownlee, Moore, & Hackney, 2005; McEwen, 2008; O'Donnell, Bird, Jacobson, & Driller, 2018).

Despite the promising theoretical foundations for a relationship between cortisol and sports injury, literature supporting the relationship remains inconclusive. Perna and McDowell (1995) provided promising evidence in a study that examined life event stress and cortisol response in athletes following an exhaustive graded exercise test. Participants were split into high and low life event stress (LES) groups, and the high LES group were found to have both higher cortisol in response to the graded exercise test, and increased symptomatology (e.g., muscle complaints and viral illness) over the subsequent 30 days following the graded exercise test. The study by Perna and McDowell (1995) did not, however, explicitly examine the relationship between cortisol response to high intensity training and sports injury. Other literature that has examined the role of cortisol in high intensity training has focused on the relationship between cortisol and sports performance (Anderson, Lane, & Hackney, 2016; Cormack, Newton, McGuigan, & Cormie, 2008; Rowell et al., 2018; Siart, Nimmerichter, Vidotto, & Wallner, 2017; Strahorn, Serpell, McKune, & Pumpa, 2017). For example, Rowell et al. (2018) used testosterone and cortisol concentrations as markers of training load and match performance in a group of 23 football players. High intensity training was found to increase the concentration of both testosterone and cortisol, however no relationship between testosterone and cortisol concentration and match performance was found. Furthermore, the degree of increase in cortisol was dependent on the playing position, indicating an individualised cortisol response to training. In a similar study, Siart et al. (2017) examined concentrations of cortisol and testosterone in relation to performance in 19 track and field athletes competing at the 2016 European Games. Performance was negatively correlated with testosterone and cortisol (p = 0.08, r = -0.49), however the relationship between cortisol and performance was only evident after the three least competitive athletes were removed from the analysis (Siart et al., 2017). While these studies have highlighted the link between training and cortisol, there is a need to establish whether there is also an relationship between cortisol and athletic injury. Such an relationship will provide support for the BMSAIH model and strengthen the argument for cortisol acting as a potential mediating pathway in the relationship between stress and injury.

Collection of saliva. Several approaches to assessing the concentration of cortisol exist within the literature. Salivary cortisol is often preferred over other forms such as serum cortisol obtained from blood samples and urinary cortisol due to the relative ease with which it can be collected. Furthermore, Gozansky, Lynn, Laudenslager, and Kohrt (2005) reported that more physiologically relevant data were obtained from salivary cortisol compared to total serum cortisol when measuring dynamic hypothalamic-pituitary-adrenal activity. Salivary cortisol is also preferred over hair cortisol which is more suited to measuring chronic levels of cortisol, as opposed to an acute cortisol response following intense exercise (Gerber et al., 2012). Two commonly used salivary techniques are passive drool and oral swab (Gröschl, Read, Hughes, & Riad-Fahmy, 2008). The passive drool technique requires participants to provide approximately 2 ml of saliva through a straw into a collection tube. In comparison, the oral swab technique requires participants to place a small piece of absorbent material (e.g., polyethylene or cotton) under their tongue for a standardised time (typically 1-2 min). Both techniques provide a reliable means of collection and each have their merits (Gröschl et al., 2008). For example, the oral swab technique requires only minimal instruction and can increase participant compliance.

However, swabs can interact with other analytes in the same sample and/or negatively influence assay performance. In comparison, the passive drool technique requires greater participant compliance and relies upon the participant to provide a sample of the necessary quality and volume. However, samples obtained by passive drool are often of better quality to those obtained from swab methods (Hashiguchi, Kaji, Kozaki, Tochihara, & Yasukouchi, 2009).

Pilot study. To determine the best approach for the current research, both the passive drool and oral swab methods were trialed with a group of five participants. Saliva collection aids and 4 ml cryovials (Salimetrics, USA) were used to obtain the passive drool samples and SalivaBio oral swab (Salimetrics, USA) were used to collect oral swab samples (Figure 8). Participants provided the passive drool sample followed by the oral swab sample. Participants were read instructions provided by Salimetrics for each technique prior to providing the sample. After both techniques had been completed, participants were asked which technique they preferred. All participants reported that the passive drool technique was easier and more comfortable compared to the oral swab technique. Given the advantages the passive drool technique has been proposed to have over oral swab collection, and based on the feedback from trial participants, passive drool was chosen as the preferred technique to collect samples of saliva in the current study.

Figure 8. Left: Oral swab being place in the mouth. Right: passive drool tube with collection aid.

Summary

The purpose of this chapter was to provide a rationale for the inclusion of the specific variables and the measures employed in the present research programme. Several suitable measures were identified, and where appropriate, based on findings from the pilot studies, minor modifications were made to make the measures appropriate for the intended sample of study, and also allow for data to be collected in a reasonable amount of time. Where possible, the reliability of the modified measures and techniques used was established. The main modifications were; 1) Minor changes to the wording of items on the LESCA questionnaire were made to improve the suitability for the intended sample (Table 1). 2) A custom R script was developed to enable HRV parameters to be calculated in a reproducible and time efficient way (Appendix C). 3) A visual-palpatory technique was developed to enable testing locations to be identified reliably over repeated measurement occasions. 4) An additional single leg stance on both firm and foam surfaces was included in the BESS test to enable balance asymmetry to be calculated.

Chapter 4:

Study 1

Study 1: An interdisciplinary examination of stress-related markers and injury occurrence in athletes.

Over the last four decades sport related injuries have received increased research attention (Ivarsson et al., 2017). This attention is unsurprising given the high incidence (Rosa et al., 2014; Sheu et al., 2016), and numerous undesirable physical and psychological effects of sports injuries (Brewer, 2012; Leddy et al., 1994). In an attempt to address this and mitigate against both the increasing incidence and undesirable consequences of injury, research that has focused on identifying factors related to injury occurrence has identified several psychological (Slimani et al., 2018), anatomical (Murphy, Connolly, & Beynnon, 2003), biomechanical (Hughes, 2014; Neely, 1998) and environmental (Meeuwisse et al., 2007) factors associated with sports injury. Indeed, several models of injury causation have been proposed that highlight the multifactorial nature of injury occurrence (Kumar, 2001; Meeuwisse et al., 2007; Wiese-Bjornstal, 2009), of which one of the most widely cited and influential models was developed by Williams and Anderson (Andersen & Williams, 1988; Williams & Andersen, 1998).

Williams and Andersen's (1998) model proposed that when faced with a potentially stressful athletic situation, an athlete's personality traits (e.g., hardiness, locus of control, sense of coherence, competitive trait anxiety, achievement motivation and sensation seeking), history of stressors (e.g., daily hassles, major life events, previous injuries) and coping resources (e.g., general coping behaviours, social support, psychological skills) will contribute to their response, either interactively or in isolation. Central to the model is the stress response, which reflects the bi-directional relationship between athletes' appraisal of, and response to, a stressful athletic situation. The model predicts that athletes who have a history of many stressors, personality traits that intensify the stress response and few coping resources to deal with the situation, will exhibit greater attentional (e.g., peripheral narrowing) and/or physiological (e.g., increased muscle tension) responses that put these individuals at greater risk of injury.

Within Williams and Andersen's (1998) model, although the stress response is the mechanism through which injuries are thought to occur, personality traits, history of stressors and coping resources have received the most research attention (Johnson et al., 2014). Of these variables the major life events component of an athlete's history of stressors appears to most consistently predict injury occurrence (Williams & Andersen, 2007). Specifically, major life events with a negative, as opposed to positive, valence have been found to be most related to injury occurrence (Maddison & Prapavessis, 2005; Passer & Seese, 1983). However, personality traits and coping resources have also been found to predict injury, with athletes more likely to sustain an injury if they have low social support, low psychological coping skills, high trait anxiety and elevated competitive state anxiety; compared to athletes with the opposite profile (Ivarsson & Johnson, 2010; Lavallée & Flint, 1996; Smith, Smoll, & Ptacek, 1990). The evidence for a relationship between other personality traits and injury has been less conclusive, with some research finding no support or even contradictory results (Junge, 2000). Furthermore, the amount of variance explained by the psychosocial factors proposed by the model has been modest, typically between 5 - 30% (Galambos et al., 2005; Ivarsson & Johnson, 2010); suggesting other factors are also likely to contribute to injury occurrence.

Although the majority of injury prediction research has examined the effect of the psychosocial factors in Williams and Andersen's (1998) model, other research has attempted to examine the mechanisms through which these factors are proposed to exert their effect. To elaborate, the model suggests that injuries are likely to occur through either increased physiological arousal resulting in increased muscle tension and reduced flexibility or attentional deficits caused by increased distractibility and peripheral narrowing. However, to date, the research exploring the mechanisms has largely focused on attentional deficits (Andersen & Williams, 1999; Rogers & Landers, 2005; Swanik et al., 2007; Wilkerson, 2012). For example, Andersen and Williams (1999) measured peripheral and central vision during high and low stress conditions and found athletes with high life event stress coupled with low social support had greater peripheral narrowing under stressful conditions compared to athletes with the opposite profile; these athletes went on to sustain an increased number of injuries during the following season. Rogers and Landers (2005) supported Andersen and Williams's (1999) earlier findings reporting that peripheral narrowing under stress mediated 8.1% of the relationship between negative life events and injury. However, few attempts have been made to explain the remaining variance between negative life events and athletic injury through the other proposed mechanisms in Williams and Andersen's (1998) model, such as increased muscle tension and reduced motor control.

One possible reason for this is the multifactorial nature of injury and the possible contribution of other non-psychological factors to the stress response (Meeuwisse et al., 2007; Wiese-Bjornstal, 2009). For example, a large body of research indicates that training-related stress is also likely to be related to the stress response and injury occurrence (Djaoui et al., 2017; Lee et al., 2017), and may account for the unexplained variance from the psychological predictors of injury. Considering these limitations, Appaneal and Perna (2014) proposed the bio-psychosocial model of stress athletic injury and health (BMSAIH) to serve as an extension to Williams and Andersen's (1998) model. To elaborate, the BMSAIH aimed to clarify the mediating pathways between the stress response and injury, consider other health outcomes and behavioural factors that impact sports participation, and integrate the impact of exercise training on athletes' health (Appaneal & Perna, 2014). The central tenet of the BMSAIH is that psychosocial distress (e.g., negative life events) may act synergistically with training-related stress as a result of high-intensity and high-volume sports training, and "widen the window of susceptibility" (Appaneal & Perna, 2014, p. 74) to a range of undesirable health outcomes including illness and injury. Consequently, the BMSAIH provides a framework for future research to build upon research that supports the relationship between psychosocial factors and injury occurrence proposed in Williams and Andersen's (1998) model, by including other physiological markers of training-related stress, which together may provide greater insight into the injury process.

Although research supporting the BMSAIH has mainly focused on the relationship between hormonal responses to training and injury occurrence (Perna, Antoni, Baum, Gordon, & Schneiderman, 2003; Perna & McDowell, 1995; Perna, Schneiderman, & LaPerriere, 1997), other research has identified other markers of training-related stress that are associated with an increased risk of injury; for example, heart rate variability (Bellenger et al., 2016; Williams et al., 2017), postural stability (Romero-Franco et al., 2014) and muscle stiffness

(Pruyn et al., 2015). Unfortunately, these markers are often studied in isolation and without an assessment of the psychosocial factors that are known to contribute to injury, limiting our understanding of how psychosocially and physiologically derived stress may contribute synergistically to injury occurrence. Recently, Bittencourt et al. (2016) proposed that to better understand the multifactorial nature of sports injuries, research needs to move away from studying risk factors in isolation and instead adopt a complex systems approach to injury. Such an approach posits that injury may arise from a complex "web of determinants" (Bittencourt et al., 2016, p. 3), where different factors interact in unpredictable and unplanned ways, but result in a global outcome pattern of either adaptation or injury. Complex systems approaches have been used in health care to model the large number of risk factors associated with different types of diseases (Plsek & Greenhalgh, 2001); however, very few studies have attempted to address sport injury occurrence using such an approach (Hulme, Thompson, Nielsen, Read, & Salmon, 2018).

Despite offering a possible framework to build on the research stemming from Williams and Andersen's (1998) model, researchers have largely overlooked the potential to explore other physiological stress-related markers proposed by the BMSAIH, in addition to the already well-established psychological characteristics known to be related to injury (Appaneal & Perna, 2014). Furthermore, research has typically only recorded one wave of measurements, assuming that the time interval between measurement and actual injury occurrence does not influence subsequent injuries (Johnson et al., 2014). Such an approach fails to capture changes in both psychosocial factors and stress-related physiological markers that may occur preceding an injury. Assessing how these variables change with respect to time is essential if we are to understand what effect repeated exposure to major life events and other stress-related factors, such as high intensity training, has on injury occurrence. Viewed through the lens of a complex systems approach the interaction between psychosocial sources of stress, stress-related physiological markers and injury occurrence may provide new insight into the injury process. Therefore, the purpose of the current study was to examine the relationship between psychosocial factors, physiological stress-related markers and occurrence of injury in athletes. Furthermore, as these variables are likely to change over time a repeated measures design was employed.

Method

Participants. The participants were 351 athletes (male: $n = 231$, female: $n = 120$), with an average age of (21.6 ± 6.3) years who represented a range of team (football, rugby, netball, cricket, lacrosse, basketball and field hockey) and individual sports (athletics, tennis, weightlifting, gymnastics, judo, swimming and golf) from a British university and local sports clubs (see table 4 for full participant information). Participants self-rated competitive level ranged from recreational to international standard. A total of 162 (46.15%) participants had sustained an injury in the 12 months prior to the start of the study (male: $n = 114$ [49%], female; $n = 48$ [40%]). At the start of the study, all participants were injury free (no modifications to their usual training routine due to a sport related medical problem for a minimum of four weeks). Participants were engaged in training for their respective sports for at least five hours per week. Ethical approval was obtained from Cardiff Metropolitan University ethics committee prior to the start of the study and all participants provided informed consent.

Table 4
Participant demographics.

	Male ($n = 231$)	Female ($n = 120$)
Demographics M (SD)		
Age (yrs)	24.3 (10.0)	20.2 (1.7)
Height (cm)	167.0 (7.9)	177.9 (8.7)
Body mass (kg)	66.9 (10.5)	81.7 (14.8)
Hours per week training	7.7 (4.1)	10.6 (8.4)
Current competitive level n (%)		
Recreational	6 (5)	10 (4)
University	78 (65)	188 (81)
National/International	36 (30)	33 (14)

Measures.

Major life events (Appendix A). A modified version of the Life Events Survey for Collegiate Athletes (LESCA) was used to measure participants history of life event stress (Petrie, 1992). The LESCA comprises 69 items that reflect possible life events that participants may have experienced. Example items include, “Major change in the frequency (increased or decreased) of social activities due to participation in sport”, “Major change in the amount (more or less) of academic activity (homework, class time, etc)” and “Major change in level of athletic performance in actual competition (better or worse)”. Participants were asked to rate the perceived impact of each life event they had experienced within the last 12 months on an 8-point Likert scale anchored at -4 (*extremely negative*) and +4 (*extremely positive*). Negative and positive life event scores were calculated by summing the negative and positive scores respectively. A score for total life events was calculated by summing the absolute values for both negative and positive events. Petrie (1992) reported test-retest reliabilities at 1-week and 8-weeks with values ranging from 0.76 to 0.84 ($p < .001$) and 0.48 to 0.72 ($p < .001$) respectively. Petrie (1992) also provided evidence of discriminant, convergent and predictive validity. The LESCA is the most widely used measure of major life events for athletes in the sports injury literature. For this study, reliability was assessed using composite reliability (Fornell & Larcker, 1981), rather than the more widely used Cronbach’s alpha. Composite reliability is preferred because it does not assume parallelity (i.e., all factor loadings are constrained to be equal, and all error variances are constrained to be equal) and instead takes into consideration the varying factor loadings of the items in the questionnaire. Composite reliability for the LESCA was 0.84.

The Reinforcement Sensitivity Theory Personality Questionnaire (RST-PQ; Appendix B). The RST-PQ was used to measure motivation, emotion, personality and their relevance to psychopathology (Corr & Cooper, 2016). The revised version of the RST-PQ presented in Chapter 2 comprises 51 statements that measure three major systems: 1) Fight-Flight-Freeze System (FFFS; e.g., “I am the sort of person who easily freezes-up when scared”), 2) Behavioural Inhibition System (BIS; e.g., “When trying to make a decision, I find myself constantly chewing it over"), and, 3) Behavioural Approach System (BAS) factors; 1) Reward Interest (RI; e.g., "I regularly try new activities just to see if I enjoy them"), 2) Goal Drive Persistence (GDP; e.g., "I am very persistent in achieving my goals"), 3) Reward Reactivity (RR; e.g., "I get a special thrill when I am praised for something I've done well") and 4) Impulsivity (I; e.g., "I find myself doing things on the spur of the moment"). Participants rated each item on a scale from 1 (*not at all*) to 4 (*highly*) to reflect how well each statement described their personality in general. The responses to items associated with each subscale (FFFS, BIS, RI, GDP, RR and I) were summed to give a total score that was subsequently used for further analysis. The composite reliabilities for each subscale were; BIS = 0.92, FFFS = 0.77, GDP = 0.87, I = 0.71, RI = 0.77, RR = 0.81.

Heart rate variability (HRV). A Polar V800 heart rate monitor (HRM) and Polar H7 Bluetooth chest strap (Polar OY, Finland) was used to collect inter-beat interval (IBI) data. IBI recordings using the Polar V800 are highly comparable (ICC = >0.99) with ECG recordings (Giles et al., 2016), which are considered the gold standard for assessing HRV. In addition, HRV indices calculated from IBI and ECG data have shown a strong correlation ($r = 0.99$) in athletes (Caminal et al., 2018) and under spontaneous breathing conditions (Plews et al., 2017).

Musculoskeletal properties. A handheld myometer (MyotonPRO, Myoton AS, Tallinn, Estonia) was used to measure muscle stiffness. The MyotonPRO is a non-invasive, handheld device that applies a mechanical impulse of 0.40N for 0.15ms perpendicular to the surface of the skin. The impulse causes natural damped oscillations in the tissue, which are recorded by a three-axis digital accelerometer sensor in the device. The raw oscillation signal is then processed, and the stiffness parameter is calculated (Agyapong-Badu et al., 2016). The MyotonPRO has previously been reported to be a reliable and valid tool for the measurement of in-vivo tissue stiffness properties (Chuang et al., 2013; Nair, Dougherty, Schaefer, Kelly, & Masi, 2014; Pruyn, Watsford, & Murphy, 2016), and has demonstrated good internal consistency (coefficient of variation < 1.4%) over sets of 10 repetitions (Aird et al., 2012).

Postural stability. Postural stability was assessed with a modified version of the balance error scoring system (mBESS) based on the protocol recommended by Hunt et al. (2009). In total, each trial of the mBESS was performed without shoes (McCrory et al., 2013) and included six stances in the following order; dominant leg (DL; standing on the dominant foot with the non-dominant foot at approximately 30-degrees of hip flexion and 45-degrees of knee flexion), non-dominant leg (NDL; standing on the non-dominant foot with the dominant foot at approximately 30-degrees of hip flexion and 45-degrees of knee flexion) and tandem leg stance (TS; standing heel-to-toe with the non-dominant foot behind the dominant) on firm and foam (Alcan airex AG, Sins, Switzerland) surfaces respectively (Figure 9). To determine leg dominance, participants were asked their preferred leg to kick a ball to a target, and the chosen limb was labelled as dominant (cf. Cingel, Hoogeboom, Melick, Meddeler, & Nijhuis-van der Sanden, 2017). Participants were asked to maintain each stance for a total of 20 seconds. Participants hands were placed on hips at the level of the iliac crests. A Sony DSC-RX10 video camera (Sony Europe Limited, Surrey, United Kingdom) was used to record each participants performance during the mBESS.

The error identification criteria from the original BESS protocol was used by the lead researcher who scored all the BESS trials. One error was recorded if any of the following movements were observed during each trial: a) lifting hands off iliac crests; b) opening eyes; c) stepping, stumbling, or falling; d) moving the thigh into more than 30 degrees of flexion or abduction; e) lifting the forefoot or heel; and f) remaining out of the testing position for more than five seconds (Riemann et al., 1999). A maximum score of 10 errors was possible for each stance. Multiple errors occurring simultaneously were recorded as one error. A participant was given the maximum score of 10 if they remained out of the stance position for more than five seconds. To calculate limb asymmetry, the DL and NDL leg score was calculated by summing the DL and NDL errors respectively. A total score was calculated by summing the total number of errors recorded on all stances (DL, NLD and TS, on foam and firm surfaces). To assess the intra-rater reliability, a single measurement, absolute agreement, two-way mixed effects model for the intraclass correlation (ICC; Koo & Li, 2016) was used on a sample of 40 participants from the first time point. The

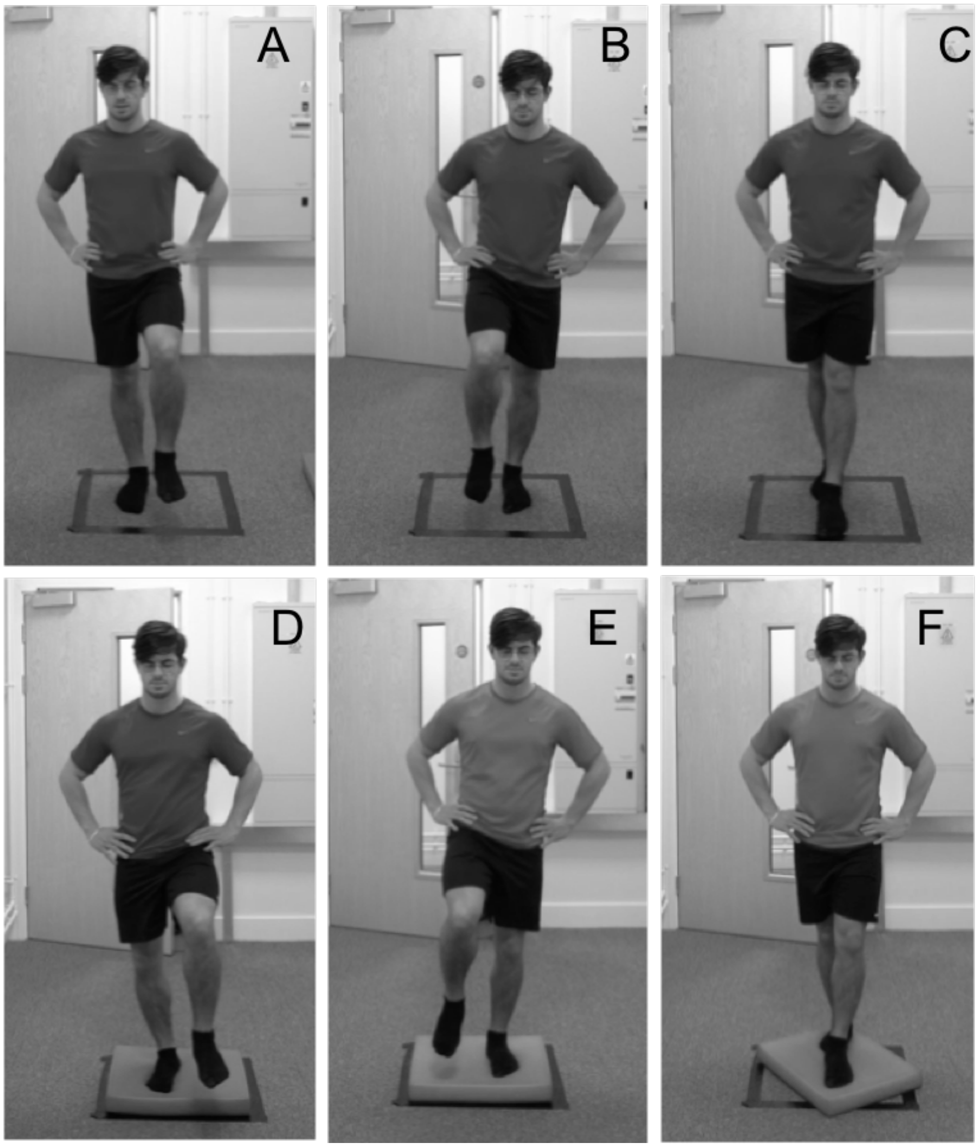

Figure 9. mBESS positions (A-F). Top row, firm surface. Bottom row, foam surface. Left column, dominant leg stance. Middle column, non-dominant leg stance. Right column, Tandem leg stance.

test-retest scoring of BESS resulted in a "good" to "excellent" ICC score (ICC = 0.93, 95% confidence interval = 0.88 - 0.96), indicating the scoring was reliable (Koo & Li, 2016).

Injury. Participants self-reported any injuries they sustained at each data collection during the study period. An injury was defined as any sports related medical problem causing the athlete to miss or modify their usual training routine. Minor scrapes and bruises that may require certain modifications (e.g., strapping or taping) but did not limit continued participation were not considered injuries (cf. Appaneal, Levine, Perna, & Roh, 2009). Injury status (did / did not sustain an injury) served as the main outcome measure.

Procedure. At the start of the academic year (September 2016 and 2017), coaches of sports teams at a British university and local sports clubs were contacted and informed about the study. With the coaches' permission, the lead researcher attended training sessions to inform the athletes about the overall aim of the study and the requirements of participation. To be eligible for the study athletes had to be injury free (no modifications to their usual training routine due to a sport related medical problem for a minimum of four weeks) and training a minimum of five hours per week. Athletes who met the criteria and volunteered to take part in the study were invited to attend scheduled testing sessions.

A repeated measures prospective cohort design was used to assess athletes' major life events, stress-related physiological markers and injury status over a twelve-month period between September 2016 and September 2018. Each participant was asked to attend a total of four data collections during the twelve-month period, with each data collection separated by a four-month interval (figure 10). Participants provided informed consent before data collection commenced. A maximum of 12 participants were scheduled to attend each data collection session, which lasted approximately one hour. Participants were asked to avoid wearing any tight-fitting shorts or leggings to the data collection session.

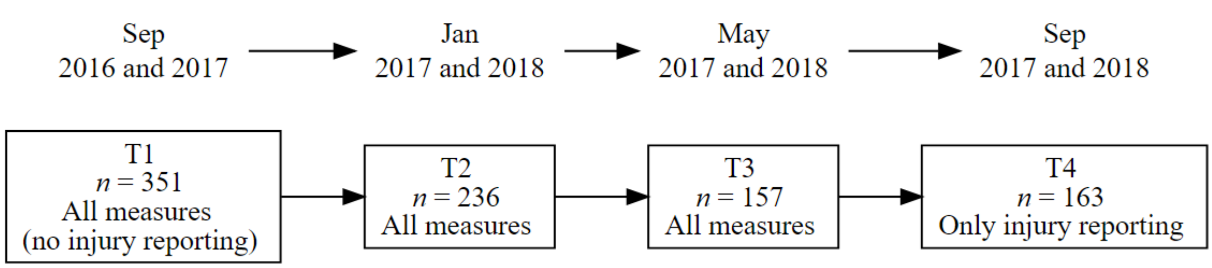

Figure 10. Study design. For each time point (T), each box contains the number of participants who completed the data collection (n), the measures used for data collection and the approximate date of the data collection.

All data were collected in a dedicated laboratory space at the university. For the first three data collections (T1, T2 and T3), participants followed the same protocol in a specific order (Figure 11). To ensure all measures could be collected within an hour, participants were separated into two groups. The first group completed all computer-based measures followed by all physical measurements, whereas the second group completed all physical measurements followed by computer-based measures. Participants were randomly assigned to one of the two groups and remained in those groups across all time points.

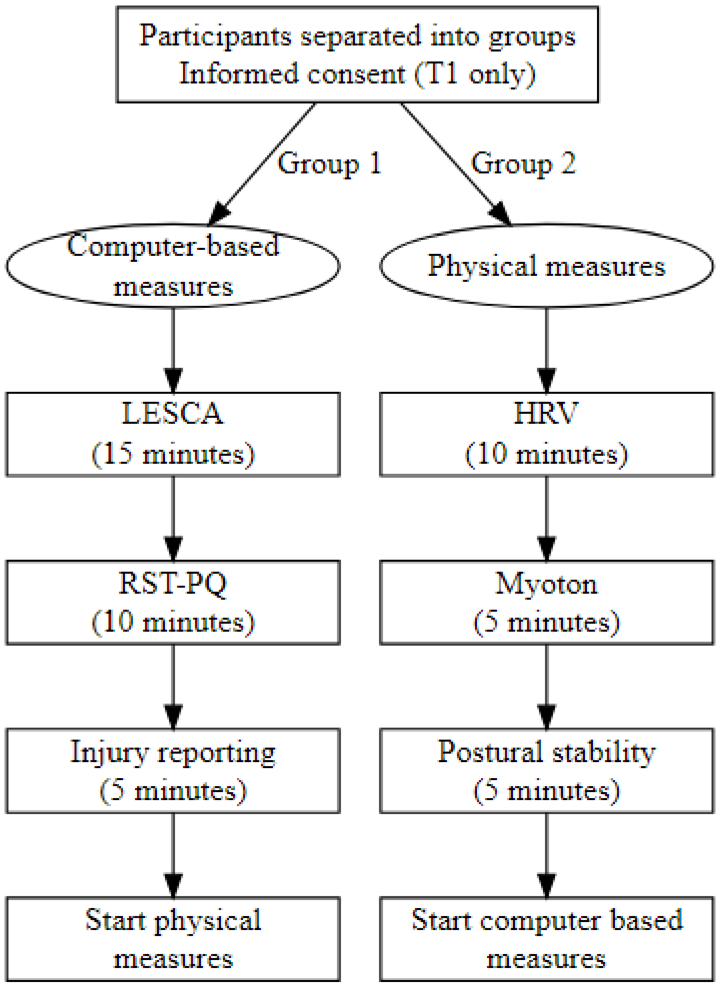

Figure 11. Outline of the protocol for each data collection.

Questionnaires. The questionnaires, which included demographic information, the LESCA, RST-PQ (T1, T2, T3) and injury status (T2, T3, T4) were completed via an on-line survey (SurveyMonkey Inc., USA, www.surveymonkey.com). The instructions for the LESCA were modified at T2 and T3 and asked participants to report major life events that had occurred since the previous testing session. For injury reporting, participants were asked to record any injuries they had sustained since the last data collection. The data were downloaded from surverymonkey.com and imported into R (R Core Team, 2019a) for analysis purposes.

HRV. To minimise potential distractions, participants were directed to a designated quiet area in the laboratory where IBI data were recorded. A total of seven heart rate monitors were used which allowed IBI recordings for up to seven participants simultaneously. Participants were instructed to turn off their mobile devices to avoid any interference with the Bluetooth sensor. Each chest strap was dampened with water and adjusted so it fitted tightly but comfortably, as outlined by Polar's guidelines. Participants were seated and asked to remain as still as possible for the duration of the recording. No attempt was made to control participants respiratory frequency or tidal volume (Denver, Reed, & Porges, 2007). Inter-beat interval (IBI) data was collected for 10 min at a sampling frequency of 1000 Hz.

Raw, unfiltered IBI recordings were exported from the Polar Flow web service as a space delimited .txt file and imported into R (R Core Team, 2019a) where a custom script using the *RHRV* package (Rodriguez-Linares et al., 2019) was used to calculate HRV indices. Raw IBI data was filtered using an adaptive threshold filter, and the first 3 min and last 2 min of each recording were discarded, leaving a 5 min window that was used to calculate the root mean square of successive differences (RMSSD) in RR intervals following the recommendations for short term IBI recordings (Laborde et al., 2017; Malik et al., 1996). RMSSD was calculated as:

$$\overline{RR} = \frac{1}{N}\sum_{i=1}^{n} RR_i \tag{3}$$

Where N is the length of the time series, and RR_i the RR interval between beats i and $i-1$, where each beat position corresponds to the beat detection instant. The R script used in the current study for the HRV analysis is available in Appendix C.

Muscle stiffness. To assess muscle stiffness, participants lay horizontally on a massage plinth and four testing sites were identified on each lower limb. The muscle belly of the rectus femoris (RF), biceps femoris (BF), medial gastrocnemius (MG) and lateral gastrocnemius (LG) sites were identified using a visual-palpatory technique to determine the exact location of each site (Chuang et al., 2012). The visual-palpatory technique required the participant to contract the target muscle to aid the lead researcher to visually identify the muscle. The participant was then asked to relax the muscle and the muscle was palpated to locate the muscle belly. A skin safe pen (Viscot all skin marker pen, Viscot Medical LLC, NJ) was then used to mark the testing site in the centre of the muscle belly.

After the eight testing sites had been identified, the testing end of the MyotonPRO (diameter = 3 mm) was positioned perpendicular to the skin on the testing site. A constant pre-load of 0.18 N was applied for initial compression of subcutaneous tissues. The device was programmed to deliver five consecutive impulses, separated by a one second interval (Morgan et al., 2018). For each impulse, the device computed stiffness values, with the median of the five values being saved by the device for further analysis. In accordance with the recommendation of Myoton.com, a set of five measurements with a coefficient of variation (CV) of less than 3% was accepted. Sets of measurements above 3% were measured again to ensure the reliability of the data. The CV was calculated in real time by the device after each set of measurements. Measurements saved on the device were uploaded to a computer using MyotonPRO software and imported in R for further analysis. For each participant, the sum of all eight testing sites was calculated to provide a total lower extremity stiffness score and was used for further analysis.

Postural stability. Participants entered a private office located in the dedicated laboratory where a research assistant who had received training in delivering the mBESS protocol conducted the postural stability assessment. Instructions for the mBESS were read to each participant and a demonstration of the positions was provided by the research assistant. For each position, participants were instructed to close their eyes, rest their hands on their iliac crests and remain as still as possible for 20 s. Participants were instructed to get back into the testing position as quickly as possible if they lost their balance. The research assistant started the video recording prior to the first stance position and stopped the recording after all stances had been completed. Each completed mBESS protocol took approximately 4 min. Only one trial was performed to avoid familiarisation effects across the repeated measurement occasions (cf. Valovich, Perrin, & Gansneder, 2003). The video recordings for each participant were imported from the recording equipment (Sony DSC-RX10) and the lead researcher scored each trial using the error identification criteria.

Data analysis. A Bayesian Network (BN) was used to explore the relationships between the psychological measures, physical markers of stress and sports injury. A BN is a graphical representation of a joint probability distribution among a set of random variables, and provides a statistical model describing the dependencies and conditional independences from empirical data in a visually appealing way (Scutari & Denis, 2014). A BN consists of arcs and nodes that together are formally known as a directed acyclic graph (DAG), where a node is termed a parent of a child if there is an arc directed from the former to the latter (Figure 12; Pearl, 1988). However, the direction of the arc does not necessarily imply causation, and the relationship between variables are often described as probabilistic instead of casual (Scutari & Denis, 2014). The information within a node can be either continuous or discrete, and a complete network can contain both continuous and discrete nodes; however, discrete networks are the most commonly used form of BN (Chen & Pollino, 2012). In discrete networks, conditional probabilities for each child node are allocated for each combination of the possible states in their parent nodes and can be used to assess the strength of a dependency in the network.

Learning the structure of the network is an important step in BN modelling. The structure of a network can be constructed using expert knowledge and/or data-driven algorithm techniques (e.g., search and score, such as hill climbing and gradient descent algorithms; Scutari & Denis, 2014). The learned structure can then be used for inference by querying the network[1] and obtaining the posterior probabilities of a particular node for a given query. The posterior distribution can be obtained by $Pr(X|E,B) = Pr(X|E,G,\Theta)$, where the learned network B with structure G and parameters Θ, are investigated with new

[1] The term "query" in relation to Bayesian Networks stems from Pearl's (1988) expert systems theory. A query can be submitted to an expert (in this case, the network is the expert) to get an opinion, the expert then updates the querier's beliefs accordingly. Widely used texts on Bayesian Network analysis (Koller & Friedman, 2009) have adopted the terminology in favour of that used in traditional statistics.

evidence E using the information in B (Scutari & Denis, 2014). In the example network presented in Figure 12, new values assigned to each of the parent nodes (e.g., both set to "Low") could be used to investigate what effect the new information has on the state of the child node (conditional probability of a particular state of the child node). In a more complex network containing many nodes, the outcome of a particular node can be assessed conditional on the states of any subset of nodes in the network. BNs therefore provide a unique and versatile approach to modelling a set of variables to uncover dependency structures within the data.

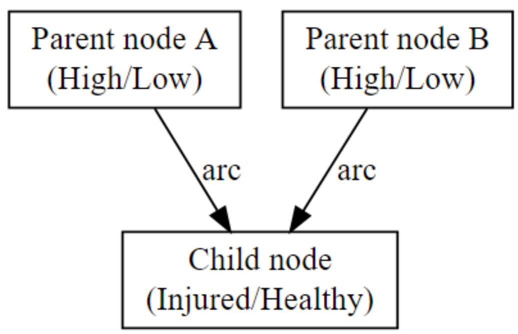

Figure 12. A simple discrete Bayesian network contain nodes, possible states of the nodes and the arcs connecting nodes.

BNs have recently been used in the sport psychology literature (Fuster-Parra, Vidal-Conti, Borràs, & Palou, 2017; Olmedilla, Rubio, Fuster-Parra, Pujals, & García-Mas, 2018) and offer several benefits over traditional statistical analysis. For example, predictions can be made about any variable in the network, rather than there being a distinction between dependent and independent variables in the data, such as in linear regression models that are often used within the sport psychology literature (Bittencourt et al., 2016; Olmedilla et al., 2018). Furthermore, the structure of a network can be obtained from both empirical data *and* prior knowledge about the area of study; the latter being particularly useful when there are a large number of variables in the network, or only a small number of observations are available in the data (Xiao-xuan, Hui, & Shuo, 2008). In such instances, a purely data driven approach to learning the network would be time-consuming due to the large parameter space, and inefficient at identifying an approximation of the true network structure. Prior knowledge about dependencies between variables can therefore be included in the network structure, while still allowing a data driven approach for unknown dependencies, to improve the overall computation of the network structure (Heckerman, Geiger, & Chickering, 1995; Xu, Zhao, Chen, & Han, 2015). The following sections detail the steps taken in the current study to firstly prepare the data for the network, and then obtain the structure of the network that was used for inference.

Data preparation. To prepare the data for the BN, missing values in the dataset were imputed. Out of the 668 total measurements across all time points in the current study, there were 31 (4.64%) missing Myoton files and 70 (10.48%) missing heart rate recordings. The missing data were due to technical faults in the data collection equipment and were considered to be missing completely at random. A missing rate of 15-20% has been reported to be common in psychological studies, and several techniques are available to handle missing values (Enders, 2003; Lang, Jorgensen, Moore, & Little, 2013). In the current study, the *caret* package (Kuhn, 2008) was used to impute the missing values. A bagged tree model using all of the non-missing data was first generated and then was used to predict each missing value in the dataset. The bagged tree method is a reliable and accurate method for imputing missing values in data and is superior to other commonly used methods such a median imputation (Kuhn, 2008).

Preliminary correlation analysis of the data revealed no strong linear dependencies in the data, and therefore the data were binarised to create “Low” and “High” levels within each variable. Binarising variables is a common procedure for BN modelling and can help improve the fit of the model by reducing the number of possible states for each node (Beuzen, Marshall, & Splinter, 2018). All variables were approximately normally distributed; therefore, the median of each variable was used as the cut-off point to create the “Low” and “High” levels.

For the LESCA, a cumulative total of the current, and previous time points was calculated at each time point to account for the potential continuing effect of the life events experienced by athletes over time. Given the weak support for a relationship between positive life events and injury, only negative and total life events were included in the network (Williams & Andersen, 2007). Cumulative negative and cumulative total life event scores were first log scaled and then binarised based on the median at each time point (nlelg and tlelg respectively). In addition to the log scaled cumulative values, an untransformed NLE score from the first time point was included as an additional variable based on previous literature that indicates this variable should have a strong relationship with injury outcome (Ivarsson et al., 2017). The complete table of variables that were included in the initial network structure is provided in Appendix D, table D1.

Network structure. To obtain the network structure, several steps were taken to ensure that both a theoretically realistic network, and a network that was an appropriate fit to the collected data, was used for inference. Prior knowledge about the network structure was included by providing a list of arcs that are always *restricted* from being in the network (blacklist), and a list of arcs that are always *included* in the network (whitelist). Additionally, there are several scoring functions such as Bayesian Information Criteria (BIC) and Bayesian Dirichlet equivalent uniform (BDeu) that can be used to compare network structures with certain nodes and arcs included or excluded (Scutari & Denis, 2014).

In order to account for the repeated measures design in the current study, and to maximise the use of the data, pairs of complete cases (e.g., participants who completed T1 + T2, and T2 + T3) were used in a two-time Bayesian network (2TBN) structure (Murphy, 2002). In the 2TBN, variables measured T2 could depend on variables measured at T1 (e.g., T1 → T2) and variables measured at T3 could depend on variables measured at T2 (e.g., T2 → T3). However, arcs were blacklisted between T2 → T1 and T3 → T2 to preserve the order in which data was collected. Variables were separated into two groups; "explanatory", for variables that did not change during the study (e.g., gender), or "independent", for variables that were measured at each time point and could vary during the study. Independent variable names were suffixed with _1 for time point T, and _2 for time point T+1 (e.g., T1_1 → T2_2 and T2_1 → T3_2). Formatting the data in this way meant participants who completed T1 and T2, but did not complete T3, could still be included in the analysis. Table 5 provides an example of the formatted data frame. Participants 1 and 3 have complete data, and therefore have two rows of data each representing variables from

Table 5
Example of the data arrangement used for the network.

Participant	X_1		X_2
1	T1	->	T2
1	T2	->	T3
2	T1	->	T2
3	T1	->	T2
3	T2	->	T3

T1 → T2 and T2 → T3, respectively. Participant 2 did not complete the final data collection at T3 and therefore only has one row of data representing the variables collected at T1 and T2. In addition to the blacklisted arcs between T2 → T1 and T3 → T2, the direction of arcs was restricted between independent variables and explanatory variables (e.g., independent → explanatory), however arcs were not restricted between explanatory → independent variables. Finally, arc direction was restricted between specific nodes within the explanatory variables. Arcs from clevel → gender, nlebase → gender and nlebase → ind_team were included in the blacklist, as arcs in these directions did not make logical sense. All subsequent models used the same blacklist.

Preliminary network structures. Prior to the final network structure presented in the results section, several structures were investigated. Networks were learned using a Tabu search algorithm (Russell & Norvig, 2009) and BIC was used to score and compare different models. A higher BIC value indicates the structure of a network is a better fit to the observed data. BIC values for each combination of variables of interest are reported as the combination of variables with the highest BIC value, followed by the relative scores of the other variables in the model.

Initially, both negative life events and total life events were included in the network structure, however the network score was improved when only nlelg or tlelg was included (highest BIC value = nleleg, BIC values relative to nlelg; tlelg only = -79.64, tlelg and nlelg = -217.54). Additionally, despite strong evidence in the literature that both negative and total life event stress are related to injury occurrence (Williams & Andersen, 2007), network structures learned using the Tabu search algorithm failed to identify a relationship between NLE and injury or TLE and injury in the data. Given that nlelg provided the highest network score, and there is a stronger relationship between negative life events and injury in the literature, an arc was whitelisted between nlelg_1 and injured_1 and nlelg_2 and injured_2 in the final network structure. Total life event score was not included in the final structure.

The subscales representing the BAS system (RR, RI, GDP and I) showed limited connection to other variables in the network. Therefore, several models were run with each scale individually to find the scale that resulted in the highest BIC value (values are shown relative to the highest value). RI provided the highest BIC value, compared to RR (-13.36), GDP (-21.10) and I (-28.44). Including all the variables (RR, RI, GDP and I) resulted in a significantly lower score -907.97) indicating that including all the variables was not beneficial to the model structure and did not offset the cost of the additional parameters. Therefore, only RI was included in the final structure.

Finally, both total score and asymmetry were included in the initial network. However, visual inspection of the network revealed no arcs between bal_asym_1 or bal_asym_2 and any other node in the network. Therefore, balance asymmetry was dropped from the final network structure. To summarise, Table 6 includes the variables that were included in the final network structure.

Table 6

Variables included in the final Bayesian network structure.

Variable	Definition	State 1	State 2
clevel	Current competitive level	Club_university_county	National_international
gender	Gender of the participant	Female	Male
hours	Number of hours spent training per week	0-9 (Low)	>9-35 (High)
ind_team	Participate in an individual or team based sport	Individual	Team
pi	Previous injury - Whether an injury had been sustained in the previous 12 months prior to the study	No Injury	Injury
nlebase	Untransformed NLE at TP 1	0-13 (Low)	>13-93 (High)
FFFS	Fight-Flight-Freeze System	8-16 (Low)	>16-30 (High)
BIS	Behavioural Inhibition System	17-38 (Low)	>38-68 (High)
RI	Reward Interest	4-10 (Low)	>10-16 (High)
stiffness	Sum of all stiffness locations	1543-2330 (Low)	>2330-4518 (High)
rmssd	Root mean squared difference of successive RR intervals	2.03-4.02 (Low)	>4.02-5.94 (High)
balance	Total balance score	5-15 (Low)	>15-46 (High)
nlelg_1	Log NLE at TP 1	0-2.64 (Low)	>2.64-4.54 (High)
nlelg_2	Log NLE at TP 2	0-3.04 (Low)	>3.04-5.19 (High)
nlelg_3	Log NLE at TP 3	0-3.18 (Low)	>3.18-4.79 (High)

Preliminary network structures also revealed strong dependencies between the same variables at subsequent time points. For example, the probability that stiffness_1 and stiffness_2 were both “High”, or both “Low” was approximately 80%. Including the arcs between the same variables from X_1 → X_2 did not provide any theoretically meaningful information to the network structure as the majority of participants would either be in a “Low” or “High” state for each pair of variables in the network. To more appropriately assess changes *within* variables over time, a second BN was investigated by modelling the differences between variables at different time points. The use of differential equations to model changes in variables over time is a common procedure in Bayesian network analysis when there are repeated measurements in the data (Scutari et al., 2017). To obtain the structure, variables suffixed with _1 were subtracted from variables suffixed with _2 to calculate the difference between variables measured at time points T1 → T2 and T2 → T3. Independent variables were then standardized to allow relative changes between variables to be compared. The "injured" variable was also modified to represent whether a participant had sustained an injury at any point over the duration of the study or were healthy for the duration of the study. The result was a network that explicitly modelled the *amount* of change within variables between time points, as opposed to the first network that would only have captured changes when the median threshold was crossed from "Low" to "High". Identical blacklists to the first network were used for arcs between independent and explanatory variables. The nlebase variable was also dropped from the list of explanatory variables to allow the *changes* in negative life events to be the only life event variable in the network. Table 7 shows the pre-standardised variables that were included in the network, all explanatory variables were identical to Table 6.

Table 7
Mean and SD of the change between time points for independent variables.

Variable	*M*	*SD*
balance	0.02	4.45
BIS	0.00	6.20
FFFS	0.07	3.26
nlec	6.55	8.30
RI	0.08	1.91
rmssd	0.09	0.74
stiffness	26.20	230.89

To obtain the final networks, the appropriate blacklist and whitelists were provided and a

Tabu search algorithm identified the remaining structure of the network. The final network structured was obtained by averaging 1000 bootstrapped models (Efron & Tibshirani, 1994) to reduce the impact of locally optimal, but globally suboptimal network learning, and to obtain a more robust model (Olmedilla et al., 2018). Arcs that were present in at least 30% of the models were included in the averaged model. The strength of each arc was determined by the percentage of models that the arc was included in, independent of the arc's direction. An arc strength of 1 indicates that the arc is always present in the network, with the value decreasing as arcs are found in fewer networks. In the current study arcs above 0.5 were considered "significant" with arcs below 0.5 and above 0.3 "non-significant" (Scutari & Nagarajan, 2013). Arcs below 0.3 were not included in the model. Appendix D (Table D2) provides a table of arc strengths for each network.

Network inference. Conditional probability queries (CPQ) were used to perform inference on both network structures. To conduct a CPQ, the joint probability distribution of the nodes was modified to include a new piece of evidence. The query allows the odds of a particular node state (e.g., injured_1 = "injured") to be calculated based on the new evidence. CPQs were performed using a likelihood weighting approach; a form of importance sampling where random observations are generated from the probability distribution in such a way that all observations match the evidence given in the query. The algorithm then re-weights each observation based on the evidence when computing the conditional probability for the query (Scutari & Denis, 2014). Inference was first performed on arcs that had a strength greater than 0.50 between the explanatory variables and independent variables and between different independent variables in the network. Of particular interest in the current study were the variables that were connected to "injured" nodes. To examine the variables that were associated with injured nodes in the network, the Markov blanket of "injured_1" and "injured_2" were examined. A Markov blanket contains all the nodes that make the node of interest conditionally independent from the rest of the network (Fuster-Parra et al., 2017). CPQs were used to determine what effect the variables in the Markov blanket of injured nodes had on the probability of the injured node being in the "injured" state.

The second network contained both continuous and discrete data. To examine dependencies between continuous variables with arc strengths above 0.5 in the second network random samples were generated based on the conditional distribution of the nodes included as evidence in the query. The samples were then extracted and examined with Bayesian linear regression models using the *brms* package (Bürkner, 2017) to determine the relationship between nodes in the network. Similar to the first network, the Markov blanket of the "injured" node was also investigated by determining the highest probability of injury with combinations of variables in the Markov blanket below the mean change, at the mean change and above the mean change.

Results

During the study, 26% of participants reported at least one injury with an average severity of 10.6 ± 31.0, days (range = 2 - 365). Both male and female participants reported a greater number of acute compared to chronic injuries (male, acute = 85 [69%], chronic = 39 [31%]; female, acute = 38 [72%] chronic = 15 [28%]), and non-contact injuries were more common than contact injuries (male, non-contact = 83 [67%], contact = 39 [31%]; female, non-contact = 35 [66%] contact = 18 [34%]). Table 8 shows the number and percentage of injury types sustained by both male and female participants.

Table 8
The number and percentage (%) of types of injuries sustained by male and female participants.

	Female		Male	
	Lower body	Upper body	Lower body	Upper body
Joint / ligament	14 (36)	5 (36)	37 (43)	14 (38)
Muscle / tendon	17 (44)	6 (43)	45 (52)	12 (32)
Other	8 (21)	3 (21)	5 (6)	11 (30)

Note. Other included bone, skin and brain injuries.

First network structure. The final network structure obtained from the data is shown in Figure 13. Several of the explanatory variables showed strong connections with independent variables in the network. The arc from nlebase → RI_1 had a strength of 0.84, and the probability of RI_1 being in the “High” state increased from 0.22 to 0.46 when nlebase increased from “Low” to “High”. The ind_team node had strong arcs to hours (0.90) and nlebase (0.84). Individual athletes were more likely to have “High” hours per week (0.84) compared to team-based athletes (0.61). Individual athletes were also more likely to have “High” negative life events in the 12 months preceding the start of the study compared to team based athletes (individual athletes = 0.65, team-based athletes = 0.41). The arcs from gender → stiffness_1 and gender → stiffness_2 were 0.76, and 0.65 respectively, with males more likely to have “High” stiffness compared to females (males =

0.63, females = 0.40). The arc from pi → stiffness_1 was 0.55 with athletes who reported an injury in the preceding 12 months more likely to have "High" (0.65) compared to "Low" (0.34) stiffness. The arc from clevel → balance_1 had a strength of 0.51, with lower level performers more likely to have decreased balance ability (0.48), compared to national level athletes (0.29).

Arcs were also present between independent variables in the network. Strong arcs were present between BIS_1 → FFFS_1 (0.98) and BIS_2 → FFFS_2 (0.68). In both instances, "High" FFFS was more likely when BIS was "High" (0.65 for _1, 0.61 for _2) compared to "Low" (0.32 for _1, 0.37 for _2). The arc between nlelg → BIS had a strength of 0.62 for nlelg_1 → BIS_1, however no arc was present between nlelg_2 and BIS_2. For nlelg_1 → BIS_1, "Low" negative life events increased the probability of BIS being in the "High" state from 0.33 to 0.55.

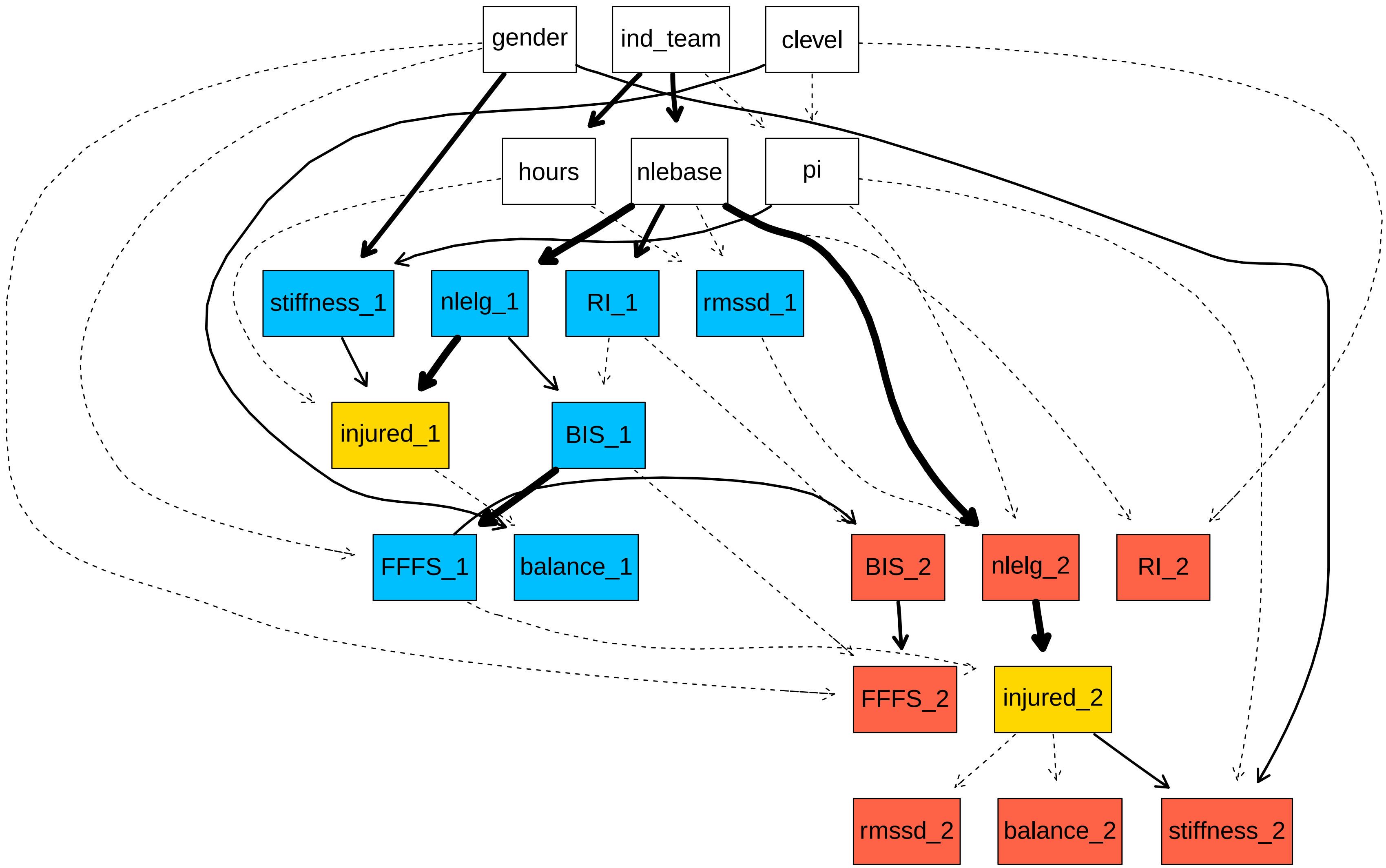

Figure 13. The full Bayesian network structure was plotted using the *strength.plot* function in bnlearn. The strength of each arc is shown graphically by the style of the arc. Thin, dashed arcs indicate the weakest arcs (arc strength below 0.50), whereas thick solid arcs indicate the strongest arcs (arc strength of 1). White nodes in the network indicate the explanatory variables, blue nodes indicate T1_1 and T2_1 variables, and red nodes indicated T2_2 and T3_2 variables. The injured_X nodes have been coloured gold as they are the main nodes of interest within the network.

Markov blanket for injured_1. The Markov blanket for injured_1, which contained hours spent training per week (hours), negative life events (nlelg_1), muscle stiffness (stiffness_1), current competitive level (clevel) and balance (balance_1), is shown in Figure 14. The arc between nlelg_1 and injured_1 was fixed in the network, so has the maximum strength of 1.

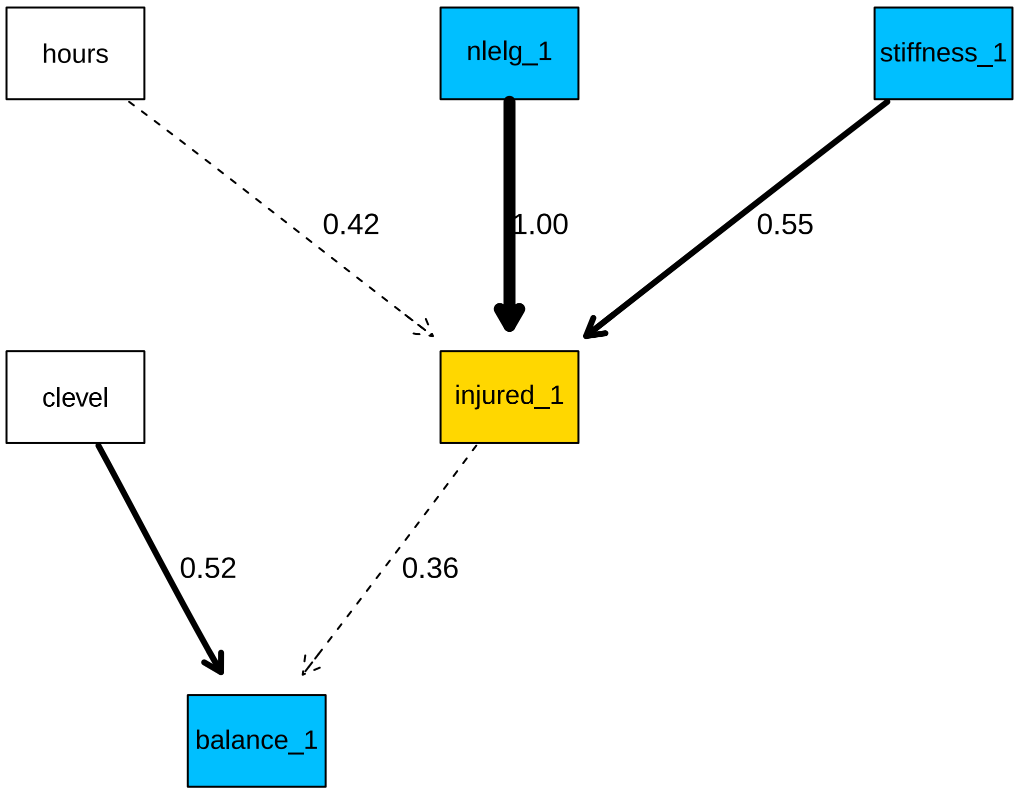

Figure 14. Markov blanket of injured_1. Arc strengths are included as arc labels.

The CPQ for injured_1 in the "injured" state for all variables that were directly linked to injured_1 (parents and children) is shown in Table 9. The probability of injured_1 = "injured" rose from 0.16 to 0.31 when stiffness was "High" compared to "Low". Negative life events had a negligible effect on the probability of injury when moving from the "Low" (Pr = 0.24) to "High" (Pr = 0.26) state.

Table 9
Probability of injured_1 being in the "injured" state, conditional on each variable.

Variable	Low	High
balance_1	0.21	0.30
hours	0.18	0.29
nlelg_1	0.24	0.26
stiffness_1	0.16	0.31

The second CPQ investigated the outcome of injured_1 being "injured" conditional on all variables in the Markov blanket. The Markov blanket contained five nodes, each with two possible states resulting in 2^5 combinations of variables, therefore only the three lowest and highest probabilities are shown in Table 10 (full Table in Appendix D, table D3). The combination of lower competitive level, "High" hours per week, "Low" negative life events, "High" balance and "High" stiffness resulted in a probability of 0.53 for injured_1 being in the "injured" state. When all variables were in the "Low" state the probability of "injured" was approximately 0.04. Negative life events only had a substantial effect on injured_1 when all other variable were fixed to "Low". In this instance the probability of injured_1 being "injured" rose from 0.04 to 0.19, when negative life events was in the "Low" and "High" states respectively.

Table 10

Highest and lowest probability of injured_1 being in the "injured" state, conditional on all variables in the Markov blanket for injured_1.

Probability	clevel	hours	nlelg_1	stiffness_1	balance_1
Highest					
0.53	club_university_county	High	Low	High	High
0.46	national_international	High	Low	High	Low
0.44	national_international	High	Low	High	High
Lowest					
0.06	national_international	Low	Low	Low	Low
0.05	national_international	Low	Low	Low	High
0.04	club_university_county	Low	Low	Low	Low

Table 11 shows the states of all explanatory and independent variables suffixed with _1 that resulted in the highest and lowest probability of injured_1 being in the "injured" state. The probability of injured_1 being "injured" rose from 0.03 to 0.56 with the combination of variable states in Table 11. Notably, hours per week, stiffness_1 and balance_1 had the greatest effect on raising the probability of injured_1 being in the "injured" state.

Table 11

Probability of injured_1 being in the "injured" state conditional on explanatory variables and independent variables suffixed with _1.

	High risk	Low risk
Probability	0.56	0.03
Variable		
pi	no injury	no injury
clevel	club_university_county	club_university_county
gender	female	male
hours	High	Low
ind_team	individual	individual
nlebase	Low	High
stiffness_1	High	Low
balance_1	High	Low
RI_1	High	High
BIS_1	Low	Low
FFFS_1	Low	Low
nlelg_1	Low	Low
rmssd_1	High	Low

Markov blanket of injured_2. The Markov blanket for injured_2 is shown in Figure 15 and contained gender, previous injury, FFFS_1, stiffness_2, balance_2 and rmssd_2. The arc between stiffness_2 and injured_2 was comparable to the arc between stiffness_1 → injured_2. Very weak arcs (0.30) between injured_2 → balance_2 and injured_2 → rmssd_2 were also present in the Markov blanket for injured_2. Results of the first query for injured_2 in the "injured" state are presented in Table 12. Similar to injured_1, stiffness_2 doubled the probability of injured_2 being "injured" from 0.13 in the "Low" state to 0.27 in the "High" state. FFFS_1 in the "Low" state increased probability of injured_2 being "injured" by 0.19 compared to the "High" state. "High" negative life events decreased the probability of injury from 0.24 to 0.19.

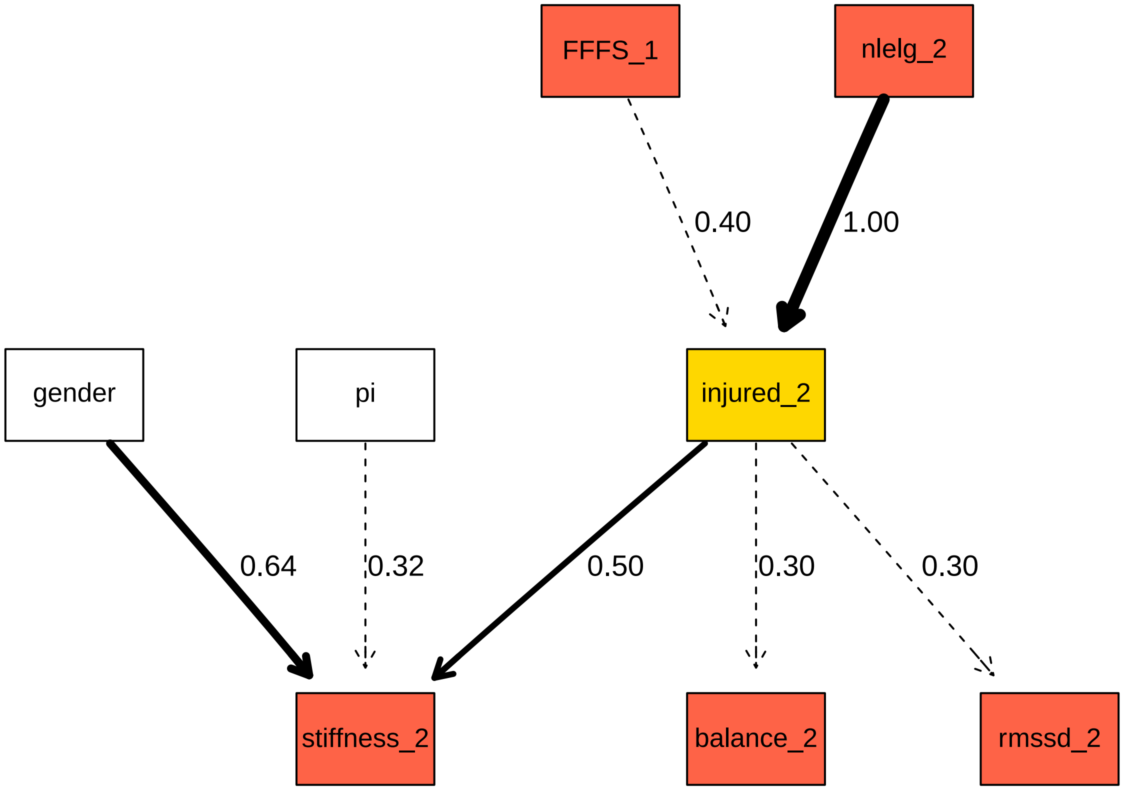

Figure 15. Markov blanket for injured_2.

Table 12
Probability of injured_2 being in the "injured' state, conditional on each variable in the Markov blanket for injured_2.

Variable	Low	High
balance_2	0.17	0.27
FFFS_1	0.30	0.11
nlelg_2	0.24	0.19
rmssd_2	0.25	0.18
stiffness_2	0.13	0.27

The conditional probabilities based on all the variables in injured_2 Markov blanket are presented in Table 13. Again, only the three lowest and highest probabilities are shown. The combination “Low” FFFS_1, “High” stiffness_2, “High” balance resulted in the greatest probability of injured_2 being “injured”, with the highest probability of injury being 0.53. With all other variables held in the “High” state, the probability of injured_2 being “injured” rose from 0.14 to 0.34 when FFFS_1 was in the “Low” compared to “High” state. The combination of “Low” stiffness, “Low” balance and “High” FFFS resulted in the lowest probability of injured_2 being “injured”.

Table 13
Highest and lowest probability of injured_2 being in the ’injured’ state, conditional on all variables in the Markov blanket for injured_2.

Probability	FFFS_1	nlelg_2	stiffness_2	rmssd_2	balance_2
Highest					
0.53	Low	Low	High	Low	High
0.45	Low	High	High	Low	High
0.41	Low	Low	High	High	High
Lowest					
0.07	High	High	Low	Low	Low
0.05	High	Low	Low	High	Low
0.04	High	High	Low	High	Low

Second network structure - changes within variables. The network for changes within variables is presented in Figure 16. An arc between BIS → FFFS with strength 1 was present in the network. Arcs between clevel → BIS and gender → stiffness had a strength of 0.60. The arc between RMSSD → FFFS was 0.56. The arcs between BIS → FFFS and RMSSD → FFFS were examined further by drawing random observations from the conditional probability distribution and examining the relationship in a Bayesian linear regression model. The use of a separate Bayesian linear regression enabled the both the main effects and interaction between FFFS, BIS and RMSSD to be examined.

Results from the Bayesian linear regression model are presented in table 14 and include 95% credible intervals (CrI). Increases in BIS were associated with increases in FFFS (b =

0.38, 95% CrI = [0.33, 0.44]), whereas positive changes in RMSSD where associated with decreased changes in FFFS (b = -0.18, 95% CrI = [-0.24, -0.13]). There was no clear interaction between RMSSD and BIS (b = -0.01, 95% CrI = [-0.07, 0.04]).

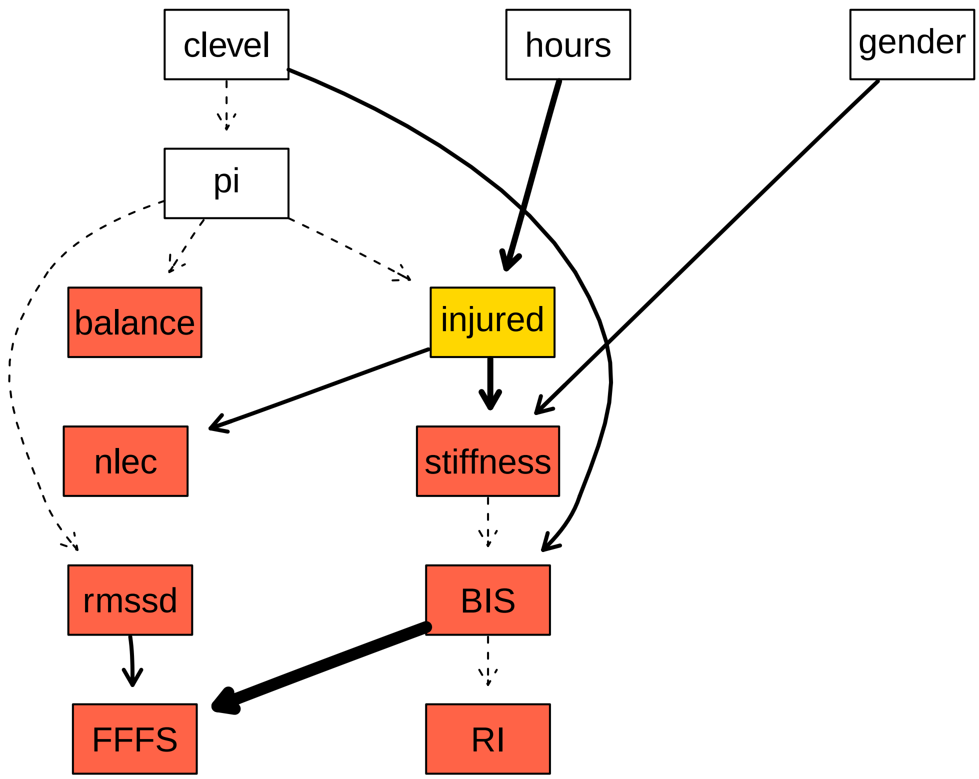

Figure 16. Network structure of the changes within variables between time points.

Table 14
Estimate, error and 95% credible intervals for the fixed effects in the linear model containing FFFS, BIS and RMSSD.

Term	Estimate	Error	95% CrI
Intercept	0.03	0.03	[-0.03, 0.08]
RMSSD	-0.18	0.03	[-0.24, -0.13]
BIS	0.38	0.03	[0.33, 0.44]
RMSSD:BIS	-0.01	0.03	[-0.07, 0.04]

The Markov blanket for the “injured” node contained previous injury, gender, hours, stiffness and nlec (Figure 17). For stiffness and nlec the values in the nodes represent the standardised change between time point. Combinations of nlec and stiffness at one SD below the mean change, at the mean change, and 1SD above the mean change are presented in Table 15. Changes in both nlec and stiffness 1SD above the mean change resulted in a probability of being injured of 0.7 over the duration of the study. With stiffness held at the mean change, the probability of “injured” rose from 0.35 to 0.64 with nlec at 1SD below an 1SD above respectively.

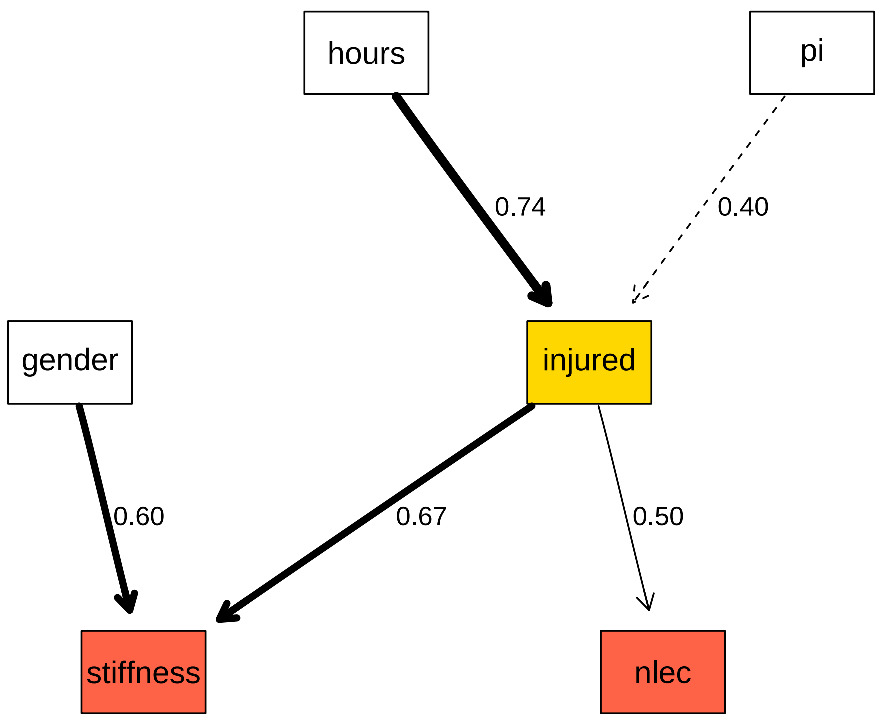

Figure 17. Markov blanket for the injured node in the network reflecting changes within variables between time points.

Table 15

The probability of injury with values of stiffness and nlec held at 1SD below the mean change, at the mean change and 1SD above the mean change.

Probability of injury	nlec	Stiffness
0.70	+1SD	+1SD
0.64	+1SD	mean
0.63	+1SD	-1SD
0.53	mean	+1SD
0.45	mean	mean
0.43	mean	-1SD
0.43	-1SD	+1SD
0.35	-1SD	mean
0.34	-1SD	-1SD

Table 16 shows the three highest and lowest probabilities for injury for all variables in the Markov blanket. The combination of 1SD above the mean change for nlec and stiffness and "High" hours per week and previous injury resulted in the highest probability that an injury would be sustained during the study (0.77). In contrast, below average changes in nlec and stiffness combined with "Low" hours per week and no previous injury resulted in the lowest probability of an injury (0.12).

Table 16
Highest and lowest probability of injury, conditional on all variables in the Markov blanket for "injured".

Probability	hours	pi	nle	stiffness
Highest				
0.77	High	injury	+1SD	+1SD
0.75	High	no injury	+1SD	+1SD
0.72	Low	injury	+1SD	+1SD
Lowest				
0.16	Low	no injury	-1SD	+1SD
0.12	Low	no injury	-1SD	mean
0.11	Low	no injury	-1SD	-1SD

Discussion

This study investigated the relationships between psychosocial factors, stress-related physiological markers and injury occurrence. The relationships were investigated using two BN structures; the first was a two-time Bayesian Network that investigated the relationships between variables *across* time points in the study (Figure 13), and the second network used differential equations to model the changes in variables *between* time points (Figure 16). Informed by Appaneal and Perna's (2014) extension to the widely cited Williams and Anderson's (1998) stress-injury model, the study aimed to address several of the criticisms in the sport injury literature (Johnson et al., 2014), and apply the concepts from Appaneal and Perna's (2014) extension to Williams and Anderson's (1998) model. Specifically, it examined how measures of psychological stress and physiological stress-related markers may interact and act synergistically to increase the risk of injury.

The first network revealed several links between the injured nodes and other variables in the network. For example, Figure 14 and Figure 15 show the Markov blankets for the injured_1 and injured_2 nodes in the first network and include all the variables that had a direct effect on the probability of injury. The combination of high stiffness and poor balance resulted in the highest probability of injury in the Markov blankets for injured_1 and injured_2. The presence of these variables at both injured nodes indicates that the combined action of these variables is important for determining an athlete's risk of injury. In the second network, the highest probability of injury was observed when changes in stiffness *and* negative life events were both greater than average (table 15), indicating that the combination of changes in psychological and physiological measures of stress may combine additively to increase the risk of injury (Appaneal & Perna, 2014). Table 13 shows the combinations of variables that resulted in the highest and lowest probabilities of injury conditional on all explanatory variables and variables suffixed with _1. Although only the highest and lowest values are shown, there were over 16000 possible combinations of variable states that could influence the probability of injury. The adopted approach attempts to take the complex systems view of injury recommended by Bittencourt et al. (2016), where injury is an emergent property from a web of determinants that interact in different ways.

Of all the variables measured in the study, muscle stiffness appeared to be most strongly related to injury. Both "High" levels of stiffness in the first network, and greater than average increases in stiffness in the second network were found to increase the risk of injury. In the current research, a novel hand-held device (MyotonPRO) was used to measure muscle stiffness. To date, one of only a small number of studies that have used the MyotonPRO to explore the relationship between muscle stiffness and sports injury, has found that increased muscle stiffness in the soleus and Achilles tendon was related to increased injury incidence in elite level netball players (Pickering-Rodriguez et al., 2017). The findings from the current study build upon these findings, with a larger sample of athletes from a range of different sports, strengthening the evidence for a relationship between higher levels of muscle stiffness and injury. However, high levels of muscle stiffness, as measured by the MyotonPRO, have also been found to be related to improved performance, with elite level athletes having increased lower extremity stiffness (Kalkhoven & Watsford, 2017; Pruyn et al., 2015). Collectively, these findings suggest that while muscle stiffness plays a vital role in performance, increased levels of stiffness also increase the probability of injury, and each athlete is likely to have an optimum level of stiffness that maximises performance while minimising the risk of injury (Butler et al., 2003).

Additionally, high levels of stiffness may only increase the risk of injury if other factors are also present. To elaborate, the combination of high stiffness *and* poor balance was found to result in the greatest probability of injury. In contrast, athletes with high stiffness and good balance were less likely to be injured, suggesting that improved postural stability may counteract the potential harmful effects of high levels of muscle stiffness. Several studies have identified how balance (Romero-Franco et al., 2014; Trojian & McKeag, 2006) and muscle stiffness (Butler et al., 2003; Pickering-Rodriguez et al., 2017) are related to injury individually, however the current study has demonstrated how these two factors may interact in relation to injury occurrence.

In addition to stiffness, balance is also linked to injury at both injured nodes in the first network, however the strength of the arc was only 0.35 and 0.30 from balance → injured_1 and balance → injured_2 respectively. Despite the weak arc strength, a “High” balance score, indicating impaired postural stability, was found to increase the probability of injury. This finding is consistent with previous research that has reported a relationship between decrements in postural stability and increased injury risk (Riemann et al., 1999; Romero-Franco et al., 2014; Trojian & McKeag, 2006). Postural stability is often used as an indicator of athlete performance level, with higher level athletes demonstrating better postural stability over their lower level counterparts (Paillard et al., 2006). In the current study, athletes who competed at a higher level were also more likely to have good balance (“Low” balance), compared to their lower level counterparts. These findings suggest that better postural stability is associated with both a higher level of performance and a lower probability of sustaining an injury, reinforcing the importance of postural stability as a feature of athletic training programmes designed to prepare athletes for the demands of high intensity training and competition (Hrysomallis, 2011).

Of the psychological variables in the study, negative life events have previously been reported to be most strongly associated with injury (Ivarsson et al., 2017; Williams & Andersen, 2007). In the current study, the second network revealed that greater than average increases in negative life event stress increased the probability of being injured during the study period. However, negative life event stress had almost no effect on the probability of injury in the first network. This finding suggests that the *relative change* in life events may be more important than the *absolute score* for life events, despite the latter being commonly used in sports injury research to date. For example, an athlete who reports a negative life event score of one during the first time point, but then a score of five at the second time point will have a 400% increase in their life event score. Although the absolute score would be “Low”, the relative increase could have been caused by a significant event in the athlete’s life, that could have considerable psychological and physiological effect (Appaneal & Perna, 2014). Future research should therefore consider study designs and appropriate analysis methods that enable relative changes in an individual athlete’s life events to be assessed (cf. Ivarsson, Johnson, Lindwall, Gustafsson, & Altemyr, 2014).

The finding that negative life events had almost no impact on the probability of injury in the first network was surprising given that the majority of research has consistently identified major life events, particularly those events with a *negative* valence, as the strongest predictor of injury in Williams and Andersen’s (1998) model (Ivarsson et al., 2017). During the initial network structure development, no arcs between the negative life event nodes and injured nodes were found by the Tabu search algorithm. However, given the strength of the literature indicating that negative life events are related to injury, an arc was fixed between nlelg_1 → injured_1 and nlelg_2 → injured_2 to allow this relationship to be examined more closely. When negative life events were “High” the probability of injury showed a negligible change at the injured_1 node and decreased by -0.05 at the injured_2 node. One possible explanation for these findings may be due to the use of the LESCA questionnaire in a repeated measures design. In the original LESCA instructions, participants are asked to report major life events that have occurred over the previous 12 months (Petrie, 1992). However, in the current study, participants completed the LESCA at approximately 4-month intervals after baseline and were therefore asked to report any events which had occurred *since the previous data collection session*, to avoid inflated scores caused by reporting the same event on multiple occasions. The reduced four-month time interval between data collections may have reduced the likelihood for life events listed in the LESCA to have taken place. For example, at the second and third time points, 26% of participants reported zero negative life events for the preceding four-month period. Simply, it may be that the items on the LESCA are less suitable for repeated measurements with durations shorter than the original 12 months than a measure that captures minor life events (cf. Fawkner, McMurrary, & Summers, 1999).

Another possible explanation for the findings for major/negative life events is that participants in the study may have had access to the necessary coping resources to mitigate against the effects of any negative life event stress they experienced. Williams and Andersen's (1998) model proposed a number of coping resources that were either directly related to injury or moderated the relationship between life stress and injury occurrence; for example, general coping strategies (e.g., good sleeping habits and self-care), social support systems and stress management skills. Although coping was not measured in the current study, several studies have found high levels of social support can reduce the risk of injury (Johnson et al., 2014; Petrie, 1993; Petrie, Deiters, & Harmison, 2014). Therefore, future research should consider including a measure of coping alongside that of life event stress to help explain the possible moderating effect.

Of the remaining variables, both FFFS and RMSSD were also linked to injury. A weak arc was observed between RMSSD_2 → injured_2 (arc strength = 0.30), however no arc was present between RMSSD_1 and injured_1, suggesting the link between RMSSD and injury was weaker than muscle stiffness and balance, where stronger arcs were observed at both of the injured nodes. Despite the uncertainty regarding the relationship between injury and RMSSD in the first network, "Low" RMSSD increased the probability of injury from 0.17 (RMSSD = "High") to 0.27 (RMSSD ="Low"). This finding is consistent with previous research that has found reduced RMSSD to be indicative of illness or maladaptation to training due to decreased parasymapthic activity, which often precedes injury (Bellenger et al., 2016; Gisselman et al., 2016; Williams et al., 2017). An arc between FFFS_1 and injured_2 (arc strength = 0.40) was also observed in the first network, where the probability of injury was increased from 0.13 to 0.29 with FFFS in the "High" and "Low" states respectively. Interestingly, the "Low" FFFS score was also related to injuries at subsequent time points; for example, the "Low" FFFS recorded at the first time point was related to injuries recorded at the second time point in the study. One possible explanation for this finding could be that those athletes who reported “Low” FFFS scores were less fearful, and may therefore engage in more risk taking behaviours, increasing the probability of injury. The RST theory proposes that higher levels of FFFS increase avoidance motivation (Corr et al., 2016), and therefore “High” FFFS may have acted as a deterrent from taking risks while training and competing, reducing exposure to situations that could have resulted in injury.

Although not directly related to injury, the first network revealed an interesting relationship between BIS and FFFS, and the second network revealed arcs between BIS, FFFS and RMSSD. In the first network, “High” BIS was associated with “High” FFFS, while in the second network, increases in BIS were associated with increases in FFFS. RST proposes that the combination of high BIS and high FFFS is likely to result in a more anxious disposition due to high levels of avoidance and high goal conflict characterised by high levels of FFFS and BIS (Corr, 2013). High levels of anxiety and anticipation of stressful situations have been associated with reductions in HRV indices including RMSSD (Chalmers, Quintana, Abbott, & Kemp, 2014; Pulopulos et al., 2018), which is supported by the negative relationship between FFFS and RMSSD in the second network (Table 14). These findings agree with the proposed actions of the RST theory (Corr et al., 2016). For example, high levels of BIS are proposed to be the result of goal conflict between the FFFS (avoidance) and BAS (approach) systems. The goal conflict is likely to elicit a physiological response (e.g., decreased HRV) in preparation to engage in the required behaviour to resolve the goal conflict (Corr et al., 2016). To extend these findings, the BAS should also be considered. Specifically, to establish how the BAS and FFFS interact, and how these two systems affect the BIS. However, in the current study, initial network structures revealed the BAS sub-scales to have limited connectivity with other measures in the network, therefore only one of the BAS sub-scales (RI) was included in the final network structure. In the first network, RI_1 was connected to both BIS_1 and BIS_2, and in both instances, the probability of “High” BIS was increased when RI was also “High”. However, the arcs between RI_1 and BIS were weak (< 0.50), and RI represents only one component of the BAS system. Other BAS factors such as impulsivity may be more closely related to risk-taking behaviours and may reveal additional links to sports injury.

Therefore, a more detailed examination of the different elements of RST, and specifically the BAS in relation to injury occurrence is warranted.

The current research had several strengths, including the repeated measures design and modelling approach. A major critique of the sport injury literature has been the use of only one wave of measurement that may not be reflective of and capture the dynamic nature of the variables that are associated with injury (Johnson et al., 2014). The repeated measures design of the current study allowed *changes* between time points to be explored. Another significant strength of the current research was the interdisciplinary approach that enabled an examination of the complex interplay between psychological and physiological markers of stress. Although there are unique and significant challenges with research employing a repeated measures design, the level of detail obtained is needed to further understand the dynamic relationships between stress-related factors and injury occurrence in athletes. Sport injury research has been criticised for adopting analytic approaches that are reductionist in nature (Bittencourt et al., 2016) and fail to account for the complex, emergent behaviour that is characteristic of injury occurrence. To address this issue, Bayesian networks (BN) were used in the current study to utilise an analytical approach more closely aligned with the complex, multifactorial nature of injury. The networks allowed several markers of stress that were free to interact with each other, as well as injury, to be explored. Consequently, BNs provided a contemporary approach that improved upon traditional methods such as logistic regression (Olmedilla et al., 2018).

As with all research, there were limitations with the present study. Firstly, the choice was made to binarise variables in the first network so only “Low” and “High” states were observed. Although binarising variables is a common procedure in Bayesian network analysis and has several advantages, Qian and Miltner (2015) highlighted that both a loss of statistical accuracy and potential difficulty in subsequent interpretation of the model may arise when following a binarising procedure. For example, the meaning of a “Low” and “High” value in the current study is only meaningful for the population that was studied, and there could be additional levels within each category that were not investigated. A second limitation was the nature of the physiological measures used in the current study.

In order to collect data on a large sample of participants, relatively simple measures were required to ensure the viability of the data collection; however, some of these measures may not have been sensitive enough to detect more subtle variation in athletes. For example, postural stability could have been assessed with the use of a force plate, which is considered the gold standard, to provide detailed data and enable a more fine-grained analysis (Ross et al., 2011).

In addition to the future directions already outlined, the findings from the current study offer several avenues for future research. Although the current study used a range of measures to capture “stress” from both a psychological and physiological perspective, there may be additional measures available that could provide further insight into the relationship between stress-related factors and sports injury. For example, stress hormones such as cortisol have been found to be a marker of both psychological and training-related stress (Appaneal & Perna, 2014; Perna & McDowell, 1995), and could help elucidate the relationship between stress and injury. Additionally, although the LESCA is the most widely used measures of major life events in sports injury research, the current study found several limitations with using the LESCA in a repeated measures design, including how the items were scored. For example, there is no way to differentiate between an athlete who has answered four items as moderately negative, and one item as extremely negative. Both responses would be scored a “-4”; however, there could be vastly different psychological and physiological effects between moderately negative and extremely negative events. Therefore, future research could develop a modified version of the LESCA that could distinguish between these types of responses and their effects.

Chapter 5:

Study 2

Study 2: The relationship between negative life events, muscle stiffness, cortisol and injury occurrence in team sport athletes.

The findings from the first study raised some important questions about the role of different physiological and psychological markers of stress in the prediction of injury. Firstly, given the widespread support in the research literature for negative life events being a predictor of athletic injury, the at best weak relationship between negative and major life events and injury was very surprising (cf. Passer and Seese (1983); Maddison and Prapavessis (2005); Gunnoe et al. (2001); Ivarsson and Johnson (2010); Ivarsson et al. (2017); Williams and Andersen (2007)). Although the Life Event Survey for Collegiate Athletes (LESCA) is a widely used measure of life event stress in the sports injury literature, the way the questionnaire is scored may disguise some important information about the nature of the life events experienced. Specifically, the *number* of events that make up the score are not considered. To elaborate, an athlete who answers four events with a -1 (moderately negative) would get the same score as someone who answers one event as a -4 (extremely negative). The psychological and physiological responses to four moderately negative events compared to one extremely negative event are unlikely to be equivalent (Ganzel, Morris, & Wethington, 2010), so a distinction between the nature of the life events that athletes' report may provide a more nuanced understanding of the role of life events and the predictive ability of the LESCA.

Secondly, in addition to the markers of "stress" used in the first study, there is evidence to suggest that stress hormones including testosterone, cortisol, adrenaline and norepinephrine may also be linked to athletic injury (Appaneal & Perna, 2014; Cormack et al., 2008; Mangine et al., 2018; Perna & McDowell, 1995). Of these hormones, cortisol has been mostly widely studied as a marker of stress and athletic activity (Paridon, Timmis, Nevison, & Bristow, 2017). Cortisol is released in response to both psychological stress, such as to major life events and the attendant emotional distress that follows (Roos et al., 2018), and high intensity and high volume training, in which athletes regularly engage (Brownlee et al., 2005). Although cortisol plays a vital role in response to stress by increasing substrate mobilisation (Anderson et al., 2016), prolonged exposure to increased levels of cortisol can have several negative effects. For example, elevated evening cortisol has been associated with a suppressed immune system, poor sleep and lessened growth hormone release, all of which can inhibit recovery following intense exercise, and increase the risk of athletic injury (Brownlee et al., 2005; McEwen, 2008; O'Donnell et al., 2018). However, the link between cortisol and injury has not been well established within the literature. Perna and McDowell (1995) provided promising evidence in a study that examined life event stress and cortisol response in athletes following an exhaustive graded exercise test. Participants were split into high and low life event stress (LES) groups, and the high LES group were found to have both higher cortisol in response to the graded exercise test, and increased symptomatology (e.g., muscle complaints and viral illness) over the subsequent 30 days following the graded exercise test. Perna and McDowell (1995) did not, however, explicitly examine the relationship between cortisol response to high intensity training and sports injury.

Finally, in terms of how stress-related factors relate to injury *severity*, in the first study, injury was treated as a binary variable, as is often common in sports injury research (Bittencourt et al., 2016). However, treating injury as a binary variable may result in important information being lost regarding the stress-related markers of an athlete who sustains more severe injuries. For example, there may be differences in the psychological and physiological profile of an athlete who sustains a minor hamstring strain that results in a week of missed training, compared to an athlete that suffers a full ACL tear needing surgery and six months rehabilitation. Examining not only the difference between healthy and injured athletes, but also the severity of the subsequent injury, may therefore provide greater insight into the stress-injury relationship.

The purpose of this study was twofold; first, to establish whether an alternative approach to scoring the LESCA questionnaire might elucidate additional insight into the relationships between life event stress and injury, and secondly, to examine the possible relationships between life event stress, the cortisol response to high intensity training and injury severity. In addition, given the strength of the relationship between muscle stiffness and injury in the first study, muscle stiffness was included as an additional factor that may also be related to injury severity. In line with the relevant literature and findings from the first study, the following hypotheses were established with regard to injury occurrence and severity: a) higher average negative life event scores would have a positive relationship with both injury occurrence and severity; b) greater increases in cortisol following high intensity exercise would be associated with injury occurrence; and c) high levels of muscle stiffness would increase the risk of injury occurrence and result in greater days lost to injury. With regard to how the predictor variables may interact, the following hypotheses were established: a) higher average negative life event score would be associated with greater cortisol response following high intensity exercise; b) there would be a positive relationship between average negative life event score and muscle stiffness; and c) muscle stiffness would have a positive relationship with cortisol response following high intensity exercise.

Method

Participants. Participants, who were a sub-sample from Study 1 (chapter 4), comprised 51 male members of the football and rugby teams based at a British University (Table 17). Both teams played in a national level league and regularly performed high intensity training throughout the season. All participants were injury free at the start of the study.

Table 17
Participant demographics.

	Football ($n = 22$)		Rugby ($n = 29$)	
	M	*SD*	*M*	*SD*
Age (yrs)	22.0	2.2	19.4	1.3
Height (cm)	180.6	6.5	181.0	9.1
Body mass (kg)	80.0	5.5	99.7	15.5
Hours per week spent training	8.5	2.4	26.8	8.2

Measures.

Cortisol. In addition to the LESCA and the muscle stiffness measure outlined in Study 1, salivary cortisol concentrations pre-and post-high intensity training sessions were collected. Salivary cortisol is reported to be a better measure of dynamic hypothalamic–pituitary–adrenal (HPA) activity than serum cortisol and provides a non-invasive method that can be conducted in a field-based setting (Gozansky et al., 2005). Saliva collection aids and polypropylene cryovials (Salimetrics LLC, California, USA) were used to collect samples of whole saliva via the passive drool method (Figure 18).

Figure 18. Example of the passive drool method using saliva collection aid and polypropylene cryovial.

Injury. The number of days lost due to injury was the main outcome measure. Injury was defined as any sports related medical problem causing the athlete to miss or modify their usual training routine. Participants were asked estimate how long they missed or modified their usual training routine when they reported injuries.

Procedure. The study followed the same design as Study 1 with the addition of a saliva sample pre-and post-a high intensity training session (Figure 19). In the month preceding each data collection the lead researcher communicated with the head coach for each team to establish when high intensity training sessions were planned. Data collection was then scheduled to coincide with each team's identified sessions. The content of each training session was decided by each team's head coach and no attempt was made by the researcher to influence the content of the session. Each session lasted approximately 120 min and included a warm-up, technical drills (e.g., passing, shooting and tackling), high intensity interval training (e.g., shuttle runs and circuit training) and match specific practice (e.g., small sided games lasting between 20 - 30 min of high intensity). Table 18 shows the mean for each team for both the maximum and average heart rates achieved, in addition to the percentage of time spent above 85 % of each players maximum heart rate, during the first high intensity training session. Work above 85% of maximum heart rate is often used as a marker of high intensity in athletes (Birkett et al., 2019). Training sessions at time points two and three were similar to the first session and the details of each training session are provided in Appendix E.

Table 18
Maximum and average heart rate, and percentage of time spent above 85 % of maximum heart rate during the first high intensity session for each team.

	Football		Rugby	
Variable	*M*	*SD*	*M*	*SD*
max hr (bpm)	199	3	201	1
avg hr (bpm)	140	14	159	8
% above 85	22.98	14.25	29.48	22.24

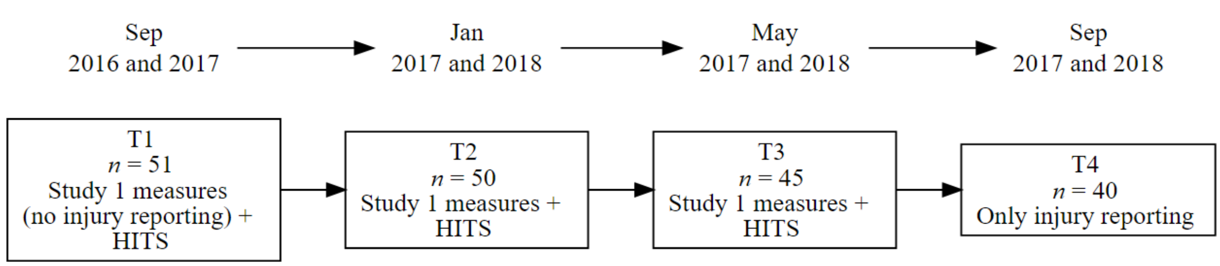

Figure 19. For each time point (T), the number of participants who took part in the data collection (n) and the measures recorded. HITS = High intensity training session.

On the day identified by the coach, participants first completed the standard data collection procedure outlined in the main study. Participants then provided 2 ml of saliva via the unstimulated passive drool technique pre-and post-training. Pre-training samples were collected between 1650 - 1700 hours prior to the warm-up beginning. Post-training samples were collected between 1900 - 1910 hours after the session had finished (Figure 20).

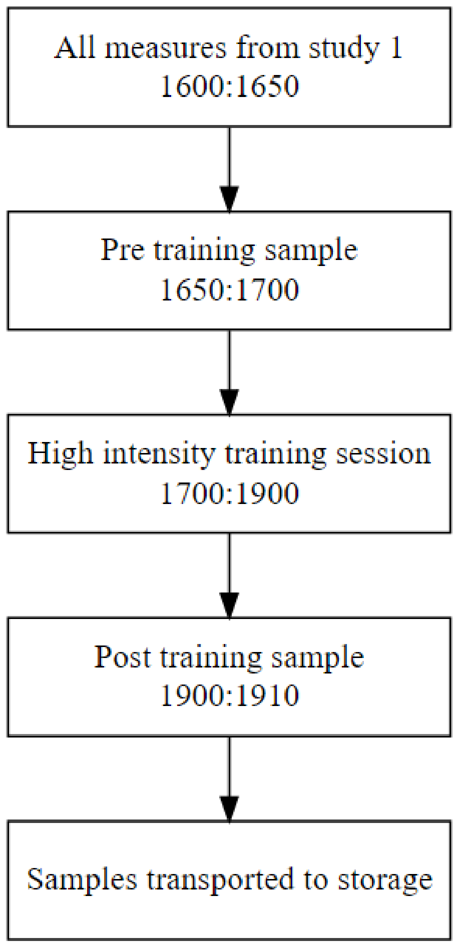

Figure 20. Order and timing of the data collection protocol.

Prior to the data collection, participants were asked to avoid eating a large meal (60 mins prior) and drinking alcohol (12 hours prior) to reduce contamination of the samples. Additionally, following the recommendations provided by Salimetrics for salivary cortisol collection, participants were instructed to rinse their mouths with water for 10 minutes prior to providing each sample. Once both pre-and post-training samples had been obtained, the samples were immediately frozen at -80°C. After the completion of all data collection, cortisol concentrations (µg/dL) were determined in duplicate by enzyme-linked immunoassay (Appendx G; Salimetrics, USA) using a micro plate reader (SpectraMax 190, Molecular Devices, CA, USA). The micro plate reader was calibrating by a laboratory technition using an absorbance test plate prior to the analysis. In addition, a new standard curve was calculated from the control samples provided in the Salimetrics assay kits for each of the seven plates used in the analysis, as recommended in the Salimetrics instruction manual (Salimetrics, 2018). The inter-assay coefficient of variation ($n = 7$) was 13.31% ($\pm$2.85). The intra-assay coefficient of variation ($n = 248$) was 5.17%. Salimetrics recommend that inter-assay values below 15% and intra-assay values below 10% are acceptable.

Data analysis. Several steps were taken to prepare the independent variables prior to the analysis. The LESCA scoring was modified to represent the average response for negative life events at each time point. The modified score was calculated by dividing the negative life event score (calculated with the standard LESCA scoring instruction) by the number of negative life events reported. The average score ranged from 0 (no life events) to 4 (all events answered as extremely negative). This approach was employed to ensure that the LESCA scores captured and more accurately reflected the perceived severity of life events and differentiated between athletes who may have high scores based on several minor events compared to a smaller number of major events.

Equation (4) was used to calculate the change in cortisol (C) concentration following high-intensity training.

$$\Delta C = \frac{Post}{Post + Pre} \tag{4}$$

Equation (4). Delta values for cortisol. ΔC = Change in cortisol; Pre = Pre-trainng values; $Post$ = Post-training values.

ΔC is a dimensionless ratio between 0 and 1. Values above 0.5 represent an increase in cortisol levels and numbers below 0.5 a decrease in cortisol levels between pre-and post-training (Siart et al., 2017). Finally, muscle stiffness was calculated as the sum of all eight testing locations as outlined in the first study. Summary statistics of the predictor variables, and days lost to injury are in presented in Table 19. All independent variables were mean centred and standardised to one standard deviation prior to the analysis.

Table 19
Summary statistics (mean and SD) of the variables included in the analysis.

	Football		Rugby	
Variable	*M*	*SD*	*M*	*SD*
Average NLE score	1.68	0.86	1.67	1.17
Days missed	15	35	10	23
ΔC	0.49	0.18	0.54	0.18
Stiffness ($\mathrm{N \cdot m^{-1}}$)	2586	319	2541	191

A Bayesian hurdle regression model was used to explore the relationships between the negative life events, muscle stiffness, change in cortisol and number of days lost due to injury. A hurdle model contains two parts. The first part is a logistic regression that provides an estimate for whether days missed was 0 (healthy) or greater than 0 (sustained an injury). The second part is a zero-truncated negative binomial regression that predicts the number of days lost due to injury. A score of zero is ignored in this instance as the model assumes the counting process (e.g., sustaining an injury) has not taken place (Hu, Pavlicova, & Nunes, 2011). Each part of the model can have different independent variables; however, in the current study all predictors were present in all parts of the model. To account for the potentially different hormonal responses in the two teams due to different high intensity training sessions, an interaction between ΔC and sport was included in the model. In addition, a varying intercept was included to account for the repeated measurements on the same individuals (Goldstein, Bryk, & Raudenbush, 2006).

Two further Bayesian linear regression models were used to investigate the relationship between the average NLE score, (ΔC) and muscle stiffness. In the first model, (ΔC) was the dependent variable and both muscle stiffness and average NLE score were the independent variables. In the second model, muscle stiffness was the dependent variable and average NLE score was the independent variable. Figure 21 shows a path diagram that outlines the relationships investigated.

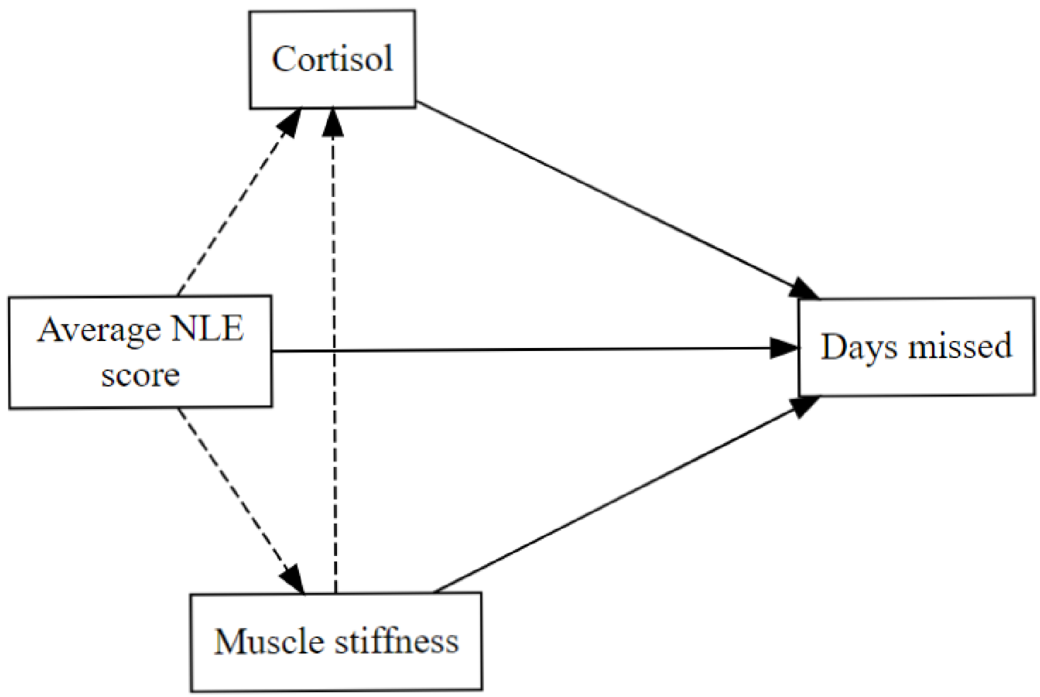

Figure 21. Path diagram of the structural relationships modelled. Solid lines represent relationships modelled within the hurdle model, and dashed lines represent relationships modelled in the multiple regression models.

All Bayesian models were created using Stan computational framework (Carpenter et al., 2017), accessed via the *brms* package (Bürkner, 2017) in R (R Core Team, 2019a). The full R code for all models is available in Appendix F, and in the thesis repository on Github (https://github.com/HarryFisher1/phd-thesis). In order to ensure the analysis was performed with rigour, the WAMBS checklist (Depaoli & van der Schoot, 2017) was used as guidance for each stage of the analysis. The checklist outlines three main areas to consider when conducting Bayesian analysis; the choice of priors and their influence on the model, model convergence, and the estimates generated by the model. Each of these points were considered with regards to the current analysis, and the steps taken are outlined in the following sections.

Priors. To improve convergence and guard against over-fitting, weakly informative priors were specified for the model parameters. All predictor variables were standardised so a one-unit change represented a change in one standard deviation. Therefore, priors with a normal distribution, centred at 0, with a standard deviation of 2.5 were used for each predictor. For the intercepts in each model, non-informative priors were used with a normal distribution centred at 0 with a standard deviation of 10. A half-Cauchy prior with mode of 0 and scale of 1 was used as a prior distribution for the variance parameter of the varying intercepts as recommended by Gelman (2006). As the priors used were only weakly regularizing, no sensitivity analysis was conducted (Depaoli & van der Schoot, 2017).

Convergence. For each model, a total of four chains containing 2000 samples were initially used to generate posterior estimates. Convergence was checked via Rhat values and visual inspection of the trace plots of the MCMC chains (Andrew Gelman & Shalizi, 2013). The number of iterations were then doubled to 4000, and convergence was checked again (Depaoli & van der Schoot, 2017). In both instances, the models showed convergence (Rhat values < 1.01; similar trace plots for each chain for all parameters). The ShinyStan interface (Gabry, 2018) was used to visualise the trace plots, and can be accessed by running the "launch_shinystan" function with each model object (Appendix F).

Output. To ensure the estimates were reasonable, and were a close fit to the observed data, posterior predictive checks were carried out to assess the model predicted values (Heino, Vuorre, & Hankonen, 2018). Several functions are available in the *brms* package to perform posterior predictive checks (Bürkner, 2017). For example, Figure 22 shows the observed (dark blue) and predicted estimates for muscle stiffness. Major discrepancies between the observed and predictor values can indicate that the model has not converged well, however in this instance, the estimates are reasonably relative to the observed data.

Credible intervals. Throughout the results section, 95% credible intervals (CrI) are obtained for the posterior distributions of coefficiants generated by the model. Credible intervals differ from frequentist confidence intervals in that they can be interpreted as having 95% probability (although not limited to 95%, any justifiable interval can be used) of containing the true population value (Morey, Hoekstra, Rouder, Lee, & Wagenmakers,

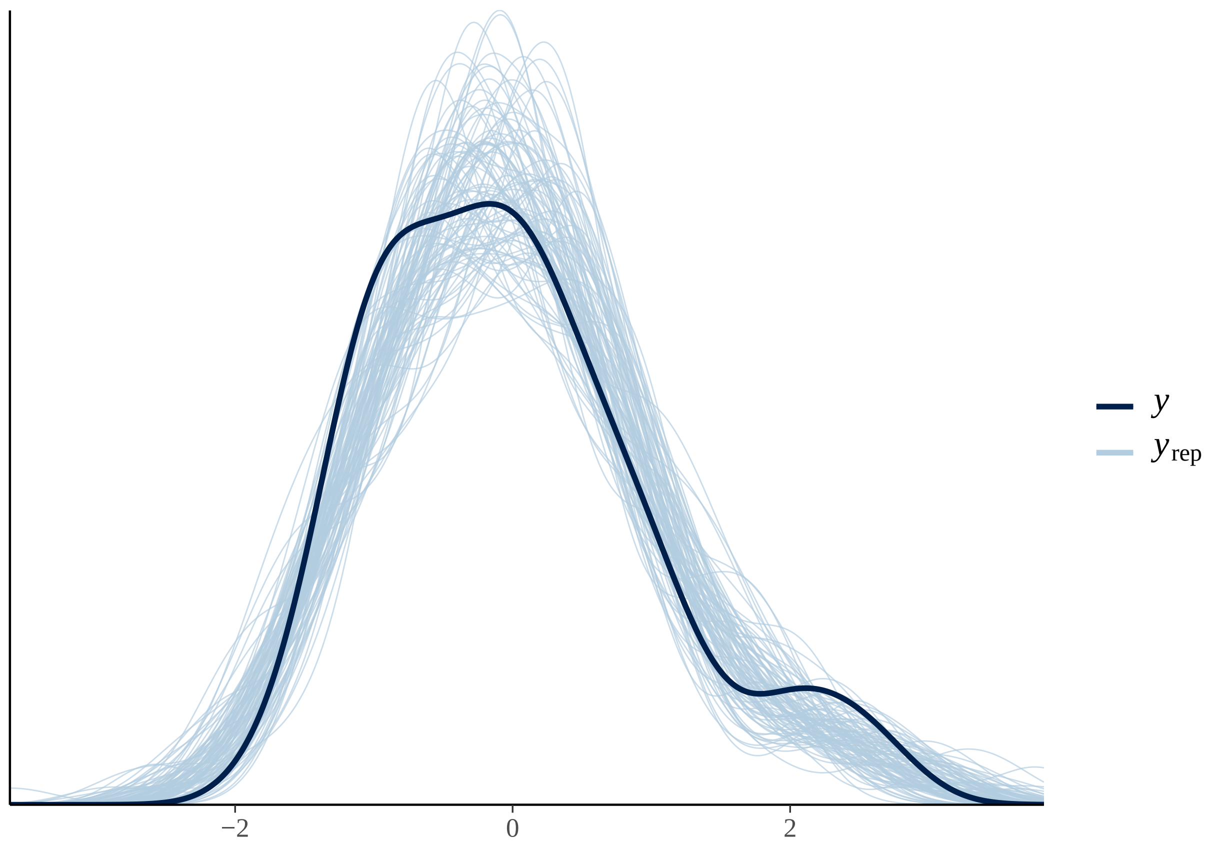

Figure 22. Observed (y) and predicted values (y_{rep}) of muscle stiffness in the second model.

2016). In contrast, a 95% confidence interval implies that over long run frequencies, 95% of the intervals obtained in the same manner (e.g., with the same sample size) would contain the true population value (Depaoli & Schoot, 2017). Morey et al. (2016) provide a detailed review of the implications of using confidence intervals, and why credible intervals should be preferred when conducting Bayesian inference.

Results

A total of 42 injuries were sustained by 28 participants (football = 12 [0.55%], rugby = 16 [0.55%]). Ten participants sustained two injuries and one participant sustained four injuries in the study period. On average, each injury resulted in 13 ± 30 days lost (range = 1 - 180) training and participation. The ΔC values for each team at each time point are presented in Table 20. There was large variation in athletes' cortisol response between the different teams and each time point in the study.

Table 20

Pre-, post- and change in cortisol (ΔC) following high intensity training (M $\pm SD$).

Time	Pre-training (µg/dL)	Post-training (µg/dL)	ΔC
Football			
1	0.17 ± 0.12	0.20 ± 0.13	0.52 ± 0.23
2	0.20 ± 0.12	0.20 ± 0.15	0.48 ± 0.15
3	0.22 ± 0.08	0.42 ± 0.24	0.62 ± 0.13
Rugby			
1	0.25 ± 0.12	0.30 ± 0.21	0.53 ± 0.14
2	0.21 ± 0.12	0.14 ± 0.10	0.40 ± 0.22
3	0.17 ± 0.07	0.24 ± 0.18	0.53 ± 0.16

Estimates for the hurdle model are presented in Table 21. For the logistic regression part of the model, higher values of both average NLE score and stiffness decreased the probability of days missed being 0, indicating a greater probability of being injured. However, the 95% credible interval for both predictors is large and crosses 0 (average NLE score = [-1.06, 0.57], muscle stiffness = [-1.46, 0.55]) indicating a high level of variability in the data. Conversely, greater ΔC concentrations were found to increase the probability of days missed being equal to 0 for both teams. However, again the 95% credible intervals were large, and the estimates should be interpreted with caution. The negative binomial part of the model revealed a similar trend, with higher values of both average NLE score and stiffness associated with a greater number of days lost due to injury (average NLE score = 0.13, 95% CrI = [-0.30, 0.55], muscle stiffness = 0.10, 95% CrI = [-0.19, 0.40]). However, similar to the logistic regression part of the model, the estimates are small and should be interpreted with caution. The relationship between ΔC concentration for the rugby team indicated a positive relationship with increases in cortisol associated with increased number of days lost, however the relationship was not clear for the football team. Additionally, the 95% CrI for estimates for both teams were large indicating uncertainty around the estimate (Table 21). Figure 23 shows the conditional effects for each predictor, with other predictors held at their mean value for both the logistic regression and negative binomial parts of the hurdle model.

Table 21
Parameter estimates for the hurdle model.

Term	Estimate	Error	95% CrI
Logistic parameters			
Intercept	0.90	0.53	[-0.07, 2.03]
Average NLE score	-0.24	0.40	[-1.06, 0.57]
Stiffness	-0.47	0.49	[-1.46, 0.55]
ΔC - Football	1.10	0.71	[-0.06, 2.69]
ΔC - Rugby	0.38	0.48	[-0.53, 1.35]
Negative binomial parameters			
Intercept	3.53	0.22	[3.18, 3.88]
Average NLE score	0.13	0.26	[-0.30, 0.55]
Stiffness	0.10	0.18	[-0.19, 0.40]
ΔC - Football	0.10	0.32	[-0.43, 0.62]
ΔC - Rugby	0.54	0.30	[0.05, 1.05]

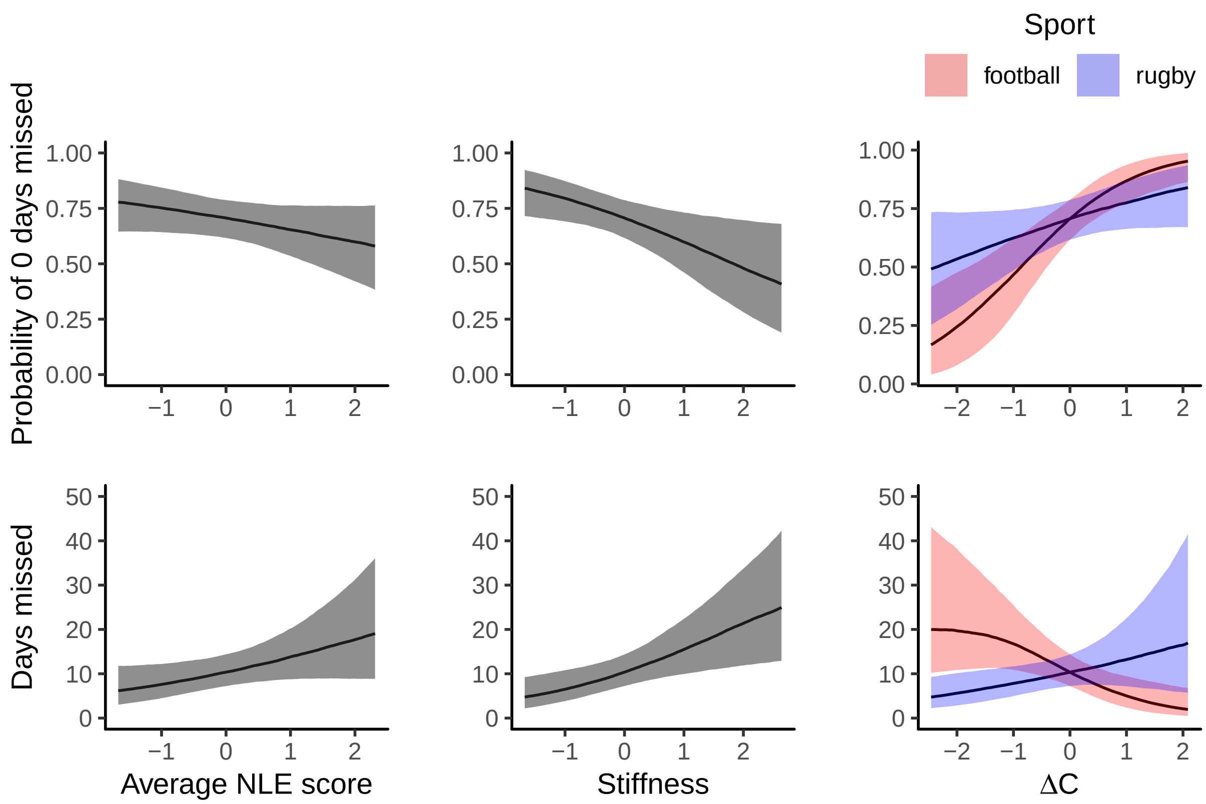

Figure 23. Conditional effects for each predictor with other predictors held at their mean value (0). Top row is the logistic regression estimates, bottom row is the negative binomial estimates.

The estimates from the multiple regression between ΔC, muscle stiffness and average NLE score are presented in Table 22. No relationship was found between average NLE score and change in cortisol (β = 0.01, 95% CrI = [-0.19, 0.20]). Weak positive relationships were found between increases in cortisol and muscle stiffness(β = 0.07, 95% CrI = [-0.32, 0.48], and between stiffness and average NLE score was unclear (β = 0.05, 95% CrI = [-0.06, 0.16]). However, these estimates were again small with credible intervals that crossed zero meaning there is uncertainity around the true estimate.

Table 22

Parameter estimates for the relationships between delta, average NLE score and muscle stiffness.

Term	Estimate	Error	95% CrI
ΔC			
Intercept	0.00	0.11	[-0.22, 0.21]
Average NLE score	0.01	0.10	[-0.19, 0.20]
Stiffness	0.07	0.20	[-0.32, 0.48]
Stiffness			
Intercept	-0.07	0.14	[-0.29, 0.16]
Average NLE score	0.05	0.06	[-0.06, 0.16]

Discussion

This study investigated the relationships between psychological and physiological markers of stress, and injury severity in a sample of national level athletes. In this regard, the purpose of the study was twofold. First, to determine whether a modified method of scoring the LESCA that accounted for perceived severity of the life event experienced was related to injury. Secondly, to investigate the relationships between negative life event stress, muscle stiffness and changes in cortisol (ΔC) following high intensity training in relation to injury occurence.

Individuals who perceived life events as more severe (had a higher average NLE score) were both more likely to sustain an injury, and also have more severe injuries (Figure 23). However, estimates had wide credible intervals that crossed zero highlighing the uncertainty within the estimate (Table 21). This finding suggests there may be a need to distinguish between those athletes that experience multiple low severity events from those that have fewer events which are perceived as more stressful. To elaborate, more major events that hold greater significance are likely to contribute more towards increased stress levels than multiple minor life events that the individual may be able to adequately deal with. The relationship between more major events and injury may be explained by the concurrent heightened physiological response that is likely to accompany the perceived major event stress. Several studies have shown how the *perception* of stressful events plays an important role in the physiological stress response (Dishman et al., 2000; Edwards, Walsh, Diment, & Roberts, 2018; McEwen, 2005; Otter, Brink, Diercks, & Lemmink, 2015). Indeed, when coupled with the physiological distress experienced during high intensity training, the heightened responsivity to major life events may act synergistically with other sources of physiological stress to increase the risk of injury (cf. Appaneal & Perna, 2014).

To add further support to the relationship between perceived psychological stress and physiological activation, the current study also found a weak positive relationship between muscle stiffness and average NLE score (Table 22). Williams and Andersen's (1998) model proposed increased muscle stiffness, as a result of the stress response, as one of the potential mechanisms through which injury may occur. While the cause of the increased muscle stiffness cannot be ascertained in the current study, the existence of both major life events and high muscle stiffness is likely to increase the risk of injury. Although the weak relationship between muscle stiffness and average NLE score was not conclusive (reflected by large 95% credible intervals), the current findings offer initial support for both Williams and Andersen's (1998) stress-injury model and Appaneal and Perna's (2014) BMSAIH.

With regard to the findings for cortisol, no conclusive evidence for a relationship between ΔC and injury occurrence or severity was found. This finding was in contrast to the predicted role of cortisol in the injury process outlined in Appaneal and Perna's (2014) BMSAIH model. There are several possible explanations for this finding, including the large variation in pre, post and delta values for cortisol for each team at different time points in the study (Table 20). Although each session was denoted as "high intensity" by the respective team coach, the content of each session varied between time points and the two sports. In addition, there was no measure of "intensity" for individual players for all sessions, and some players may have potentially performed at a lower intensity than others. Indeed, Hill et al. (2008) found that lower exercise intensities may actually reduce the concentration of cortisol post exercise. Given the varied content of each session, and potential individual differences in effort in each session, the uncertainty (wide credible intervals in Table 21) in the estimates for ΔC is to be expected. Although the field-based nature of the current study provides for high ecological validity, greater control for potential confounding variables is needed to establish the relationship between ΔC and injury. The results for the relationship between average NLE score and ΔC were unclear, with wide credible intervals ($\beta = 0.01$, 95% CrI = [-0.19, 0.20]), implying the effect was not well supported by the data. Of note was a weak positive relationship between muscle stiffness and ΔC, with higher levels of muscle stiffness associated with greater ΔC concentrations ($\beta = 0.07$, 95% CrI = [-0.32, 0.48]). One possible explanation could be that the higher-level performers within each team may have been training at a higher intensity compared to their lower level counterparts, resulting in a greater change in cortisol post training. This suggestion would be consistent with the notion that higher levels of stiffness are associated with a higher level of performance (cf. Kalkhoven & Watsford, 2017; Pruyn, Watsford, & Murphy, 2015).

Consistent with the findings from the first study, muscle stiffness was again found to be related to injury, and there was also a positive relationship between muscle stiffness and injury severity. The relationship between muscle stiffness and injury is complex, as high levels of muscle stiffness are associated with both a greater risk of injury (Pickering-Rodriguez et al., 2017), but also high levels of performance (Kalkhoven & Watsford, 2017; Pruyn et al., 2015). This complexity may have been reflected in the wide credible intervals associated with the esimate for muscle stiffness in the hurdle model (Table 21), implying variability with the data regarding the relationship between stiffness and injury. The higher levels of muscle stiffness observed in the current study could be attributable to a higher number of exposure hours likely experienced by certain players. One of the effects of high expsoure is likely to be in an increase muscle stiffness due to the repeated contraction of skeletal muscle, potentially increasing the risk of injury (Hedayatpour & Falla, 2015). Addintionally, high levels of exposure are also known to increase the risk of injury (S. Williams, Trewartha, et al., 2017), and therefore these factors together may amplify the risk of injury further. As such, increased muscle stiffness may provide an early indication of physiological maladaptation caused by high level of exposure to training. There is however, a contrasting viewpoint regarding the effect of increased exposure to training. Increased exposure to high intensity training has been found to reduce the risk of injury in certain instances (Gabbett, 2016). For example, low training volume and intensity may not provide the necessary physiological adaptations needed to tolerate the higher loads that are likely to occur over the course of a season (Gabbett, 2016). Evidently, training loads that are excessive will still likely result in injury, however there is likely to be an optimum level of load needed to elicit the required adaptations to perform optimally, while still remaining healthy (Gabbett, 2016). Taken together, both exposure levels and muscle stiffness highlight the need for methodologies that account for individual differences, and an interdisciplinary approach to understand the complex relationships between different sources of stress and injury.

The present study had a number of strengths and limitations. One strength was the use of a hurdle regression model that can account for both injury occurrence and injury severity. Traditionally, logistic regression models have been used within sport injury research (Bittencourt et al., 2016); however, hurdle regression extends the logistic model by also including a negative binomial model to predict the number of days lost due to injury. As such, greater detail can be obtained from the predictors in the model by examining both their influence on injury occurrence *and* severity. The study also used a hurdle model within a Bayesian framework, which has several advantages over a frequentist method, most notably the ability to account for small samples sizes such as the one used within the current study. Of the study limitations, the sample was restricted to only two male team sports, and therefore findings may not be generalisable across other individual based sports or for female athletes. The study also suffered from a high dropout rate, with only 19 out of 51 participants completing all three time points in the study. Given the high drop out rate, the sample size was relatively small compared to the first study. A large sample would help to combat the large credible intervals present in the model estimates that made drawing conclusive conclusions regarding the different releationships difficult. Additionally, the field-based nature of the data collection meant there was an absence of control over training intensity.

Future research should aim to clarify the relationship between negative life event stress, cortisol and injury as proposed by Appaneal and Perna (2014). While the current study was unable to find a clear relationship, there were several confounding variables that may have impacted upon the results. In addition, a stronger positive relationship was observed between cortisol and muscle stiffness, which could provide an interesting avenue for research to explore further. Future research could also further investigate the novel scoring approach outlined in the current research. The scoring is easily implemented and can be included as an additional variable in research that uses the LESCA to assess life event stress in athletes. Establishing whether events that are perceived as more severe have a stronger relationship with injury may lead to a more fine-grained understanding of the relationship between life event stress and injury.

Chapter 6:

General Discussion and Conclusions

General discussion and conclusions

The purpose of the final chapter is to draw together the findings and highlight the implications of this programme of research. The chapter is organised into six sections that provide: (a) a summary of the two studies, including key aims and findings; (b) a discussion of the conceptual and measurement issues that have emerged from this thesis; (c) the practical implications of the results of the research; (d) directions for future research; e) the strengths and limitations of the current research; and (f) conclusions that synthesizes the central aspects of the thesis.

Summary of studies

Despite acknowledging that sport injury is a complex, multifaceted process, research that has examined factors associated with the prediction of sports injury has generally been mono-disciplinary in nature, and has not addressed the potential interaction between the sports science disciplines. Therefore, the central purpose of this thesis was to adopt a novel approach to explore the multifaceted nature of the relationships between stress and athletic injury. Specifically, how psychological and physiological markers of stress may act synergistically to increase the risk of sustaining an injury. As such, the current programme of research adopted an interdisciplinary approach using a range of markers from across the sports science disciplines to examine their combined and interactive influence on sports injury occurrence. The thesis comprised two empirical studies:

Study 1: Interdisciplinary examination of stress-related markers of sports injury.

The purpose of the first study was to explore the relationships between psychological sources of stress, stress-related physiological markers and injury occurrence in athletes. Specifically, the study aimed to address several limitations outlined in previous research, including the need for; (a) an interdisciplinary approach using both psychological and physiological stress-related markers; (b) the use of a repeated measures design; (c) the inclusion of both male and female participants from a range of different individual and team-based sports; and (d) the use of an appropriate analysis method that addresses the complex nature of the interactions between different markers of stress and injury occurrence. To address these limitations, 351 non-injured athletes were recruited from the university where the study was conducted, and from sports clubs in the local area. In a repeated measures design, data collection occurred at four time points over a 12-month period, and comprised measures of; major life event stress, personality, muscle stiffness, heart rate variability and postural stability. In addition, injury status was assessed at each time point. Bayesian networks were used to examine the relationships between variables, and to model the changes between time points across the 12-month period. Two Bayesian networks were used for the analysis; the first network examined the relationships between variables, and the second network modelled the changes between time points in the study. Findings from the first network revealed muscle stiffness to have the strongest relationship with injury occurrence, with "High" levels of stiffness increasing the probability of sustaining an injury compared to "Low" stiffness ("Low" = 0.16, "High" = Pr = 0.31). In addition, poor balance, low heart rate variability and low scores on the fight-flight-freeze subcomponent of the Reinforcement Sensitivity Theory Personality Questionnaire (RST-PQ) were all found to increase the probability of injury (Table 9). Surprisingly, negative life events (NLE) was not found to only marginally increase the probability of injury ("Low" Pr = 0.24, "High" Pr = 0.26) despite strong evidence from previous research to the contrary (Ivarsson et al., 2017; Williams & Andersen, 2007). In the second network that modelled changes between time points, muscle stiffness was again found to be related to injury, with increases resulting in a higher probability of injury. In contrast to the first network, *increases* in NLE were found to increase the probability of injury. To clarify, those athletes who had the greatest increases in NLE between time points in the study were more likely to sustain an injury than those who had minor increases or no changes to their NLE scores. This finding reflected the dynamic nature of life event stress and further emphasised the need for repeated measurements. The second network also revealed that the combination of increases in muscle stiffness and NLE resulted in the greatest probability of injury, supporting the need for an interdisciplinary approach.

Study 2: The relationship between major life events, muscle stiffness, cortisol and injury occurrence and severity in team sport athletes.

The purpose of the second study was to build on the findings of the first study and explore the role of additional markers of stress in the stress-injury relationship. Specifically, Study 2 examined: (a) whether the stress hormone cortisol was also associated with negative major life events and injury; (b) whether an alternative approach to evaluating negative major life events would be related to injury; and (c) how these measures related to both injury occurrence and severity. Participants, who were a sub-sample ($n = 51$) from the first study, were members of the university men's football and men's rugby teams where the research took place. In addition to the measures outlined in Study 1, participants also provided saliva samples pre-and post-high intensity training sessions at the first three time points in the study. The change in concentration of cortisol in the samples pre-and post-training session was used as a marker of the stress response to the training session (cf. Perna & McDowell, 1995). In contrast to the first study where the original method of scoring NLE was used (cf. Petrie, 1992), a modified method was used in Study 2 where each participant's total NLE score was divided by the number of events they had experienced, resulting in an average NLE response. This method enabled the distinction between athletes who experienced several minor events, from those that experienced fewer, but more major events. Under the original scoring method these athletes would receive the same life event score despite the potential for very different stress responses. For the analysis, a Bayesian hurdle regression model was used to explore the relationships between the predictors and injury, and Bayesian linear regression models were used to explore the relationships between the predictors. Results revealed that higher levels of both average NLE score and muscle stiffness increased the probability of injury occurrence and increased the number of days lost due to injury; however, the estimates had large credible intervals implying uncertainty regarding the observed relationships (Table 21). With regards to changes in cortisol, a positive relationship between increases in cortisol and number of days lost due to injury was found for the rugby team; however, the relationship was not clear for the football team (football, estimate = 0.10, 95% CrI = [-0.43, 0.62]; rugby, estimate = 0.54, 95% CrI = [0.05, 1.05]). The results from the study complement the findings from the first study, and offer several avenues for further research to explore, including the application of the modified scoring of major life events to other cohorts, and further clarification regarding the relationship between cortisol and injury.

Conceptual and measurement issues

This section highlights the key conceptual and measurement issues related to the current programme of research. Conceptual issues are discussed in relation to interdisciplinary research in sport injury research, and how the current research programme fits within the theoretical frameworks that underpin the thesis. Measurement issues and challenges faced by the researcher are discussed in relation to the implications of the choice of measures when using an interdisciplinary approach, and the additional complexities that arose with the repeated measures design.

Conceptual issues.

Interdisciplinary approach to sports injury. Interdisciplinary research can be defined as an integrative approach that involves the interaction of specialists across sub-disciplines working together to combine methods and ideas to generate new knowledge (Freedson, 2009). It differs from mono- and multi-disciplinary approaches due to the holistic nature of the approach to research. Since being advocated in the early 1990’s by Burwitz et al. (1994), interdisciplinary research within sport and exercise science has been relatively scarce, despite repeated calls for researchers to embrace it (Buekers et al., 2017). Recently, Piggott et al. (2018) identified only 25 studies since 1994 that had used an interdisciplinary approach to address sport performance-related research questions. The current research therefore adds to a small body of research that has adopted an interdisciplinary approach and extends it to the stress-injury relationship. Specifically, negative life event stress and personality characteristics were examined alongside stress-related physiological markers that have previously been found to be related to both psychological stress and injury occurrence. Such an approach is in line with Appaneal and Perna’s (2014) Biopsychosocial Model of Stress, Athletic Injury and Health (BMSAIH), which extends the widely cited Williams and Andersen (1998) model to include other behavioural and physiological markers of stress that will act synergistically with psychological sources of stress to exacerbate the risk of injury. Typically, however, these markers have been studied in isolation, thus the current study provides a contemporary, interdisciplinary approach to the research problem.

Recently, Bittencourt et al. (2016) echoed the need for an interdisciplinary approach to the sport injury problem and advocated concepts from complex systems to further advance our understanding of the multifaceted nature of injury occurrence. Indeed, complex systems theory provides a useful framework for interdisciplinary research, as it has several characteristics that can help bring together different areas of the sport injury literature. A central tenet of complex systems theory is that injury will arise from an interrelated "web of determinants" (Bittencourt et al., 2016, p. 1) that may be linked in a non-linear manner, with small changes in one area potentially leading to large and unexpected consequences. This view differs from the traditional monodisciplinary approach that sport injury research has adopted, typically using reductionist analysis techniques for linear combinations of isolated predictive factors (Devantier, 2011; Galambos et al., 2005; Vacek et al., 2016) The current research has attempted to address this issue by using analytical techniques that are more closely aligned with the complex system approach and reflect the uncertain nature of injury occurrence. The first study adopted a Bayesian network model that was inspired by the "web of determinants" outlined by Bittencourt et al. (2016; p. 1). In the network, the relationship between variables as well their effect on injury was examined, and relationships were uncovered and explored using both prior knowledge and data driven approaches. In the second study, Bayesian regression models where used to investigate the relationships between predictor variables and both injury occurrence and severity. Although these models were linear in nature, using a Bayesian approach allows for the uncertainty to be estimated, and improves upon the maximal likelihood methods commonly used within the frequentist paradigm (Heino et al., 2018). Such methods are particularly useful for sport injury research which typically suffers from small samples sizes, and small effects that can be problematic when using frequentist techniques (Mengersen, Drovandi, Robert, Pyne, & Gore, 2016).

Theoretical links. The current body of research was underpinned by Williams and Andersen's (1998) model of injury prediction and Appaneal and Perna's (2014) extension of Williams and Andersen (1998) model. The stress-injury model proposed by Williams and Andersen (1998) is one of the most widely cited models of injury within the sport injury literature, and there has been considerable support for the role of several of the psychological variables proposed in the model in the stress-injury literature, including; negative life events (Gunnoe et al., 2001; Ivarsson & Johnson, 2010; Maddison & Prapavessis, 2005; Rogers & Landers, 2005), personality characteristics (Junge, 2000; Lavallée & Flint, 1996; Petrie, 1993) and coping resources (Hardy, 1992; Petrie, 1993). A major criticism of Williams and Andersen's (1998) model, however, is the focus on the cognitive stress response and the absence of additional sources of stress, such as environmental and physiological factors, that are also likely to influence injury occurrence (Ivarsson et al., 2017). To address this issue, Appaneal and Perna (2014) proposed the BMSAIH, which extended the original model to include other behavioural, environmental and physiological factors that are also likely to contribute to the occurrence of injury. In particular, the BMSAIH proposed that the synergistic action of both psychological stress and physiological stress, such as that sustained in response to training, will exacerbate the stress response and increase the risk of injury. However, despite providing a framework for research to extend the findings of Williams and Andersen (1998) the BMSAIH has received relatively little attention within the sport injury literature, potentially because of the insufficient detail offered regarding additional predictive variables that could be included alongside the psychosocial characteristics proposed in the original model. While the BMSAIH alludes to autonomic nervous systems (ANS) and hypothalamic pituitary adrenal (HPA) axis activity being responsible for the physiological response to stress, as well as hormonal markers including cortisol and testosterone as potential mediating pathways for the stress-injury relationship, few specific predictive variables are offered beyond this.

Based on Williams and Andersen's (1998) and Appaneal and Perna's (2014) models, and the wider sport injury literature, the current research identified several related markers that were in line with the ideas proposed in both frameworks and provided an interdisciplinary perspective that united the psychological characteristics with the stress-related physiological markers alluded to in the BMSAIH. To help visualise how these markers fit within the original model, Figure 24 provides an updated version of Williams and Andersen's (1998) model to demonstrate how the ideas presented in the BMSAIH can be integrated. In Figure 24, the stress-related physiological markers are proposed to act between the psychosocial factors and the stress response from the original model. In addition to the direct effect of psychosocial characteristics on the stress response, stress-related physiological markers are proposed to provide a pathway between the psychosocial sources of stress and the stress response. Depending on the severity of the stress response, the physiological activation may also have longer lasting effects and consequently influence the state of the stress-related physiological markers. In particular, the arrows from the stress response, through the stress-related markers and to the psychosocial characteristics demonstrate how the stress response is likely to contribute to an athlete's "history of stressors" and play a role in their perception of similar stressful events in the future. The dashed arrow from stress-related physiological markers to injury indicates how the accumulation of stress and fatigue can contribute to injury occurrence through overuse or burnout mechanisms, which the proposed mechanisms in the original model does not adequately explain. The arrows towards injury are dashed to indicate that injury is not always a certainty, and that an athlete can face a stressful situation and recover, improving their tolerance for future similar stressful situations. Meeuwisse et al. (2007) identified the linear approach to most injury models as unrealistic and Figure 24 attempts to integrate the dynamic, recursive nature of injury occurrence highlighted by Meeuwisse et al. (2007).

With regards to the physiological markers themselves, HRV, muscle stiffness, postural stability and cortisol were selected on the basis that they have been examined in a variety of different stress-related disciplines including psychopathology, lifestyle and geriatric research, as well as the sport injury literature (Bailey et al., 2013; Gervasi et al., 2017; Ockenburg et al., 2015; Rath & Wade, 2017). Consequently, they provided markers of stress alongside the psychosocial characteristics proposed by Williams and Andersen (1998) to provide additional insights into the stress-injury relationship. In the current research, many of the markers were associated with injury occurrence to varying degrees, with both muscle stiffness and HRV also showing connections with NLE and personality characteristics respectively. Specifically, high levels of muscles stiffness were found to be

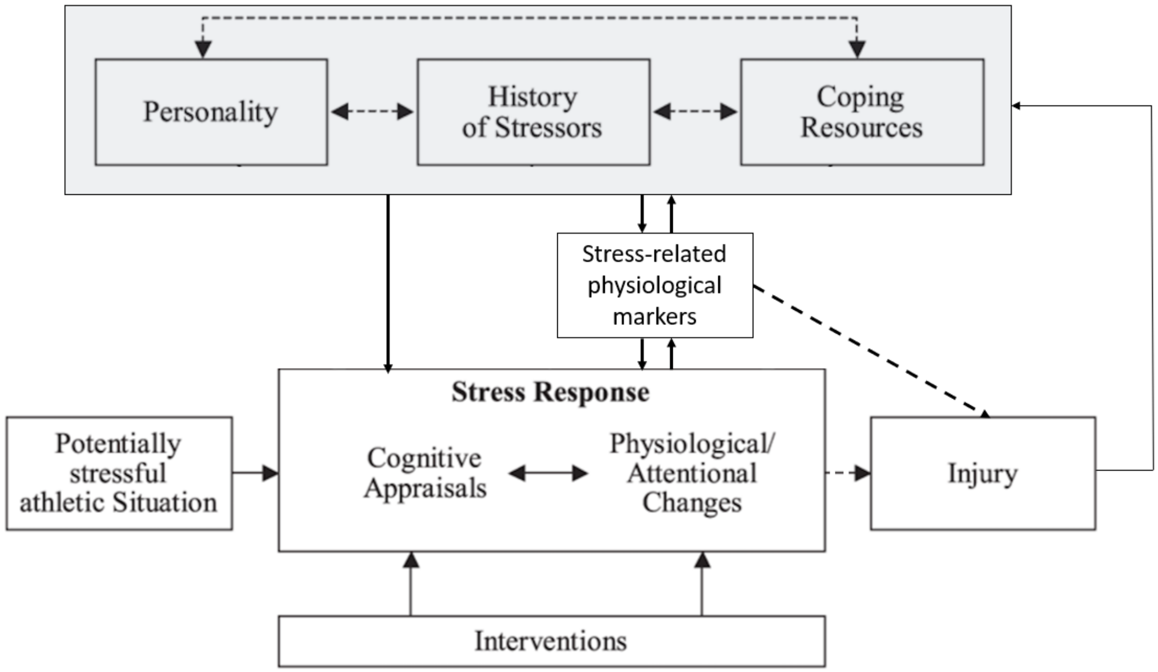

Figure 24. Modified version of Williams and Andersen (1998) model of stress and injury.

the strongest predictor of injury across both studies in the thesis. This finding is consistent with a small body of research that has investigated the link between stiffness, as measured with the MyotonPRO, and injury (Morgan et al., 2018; Pickering-Rodriguez et al., 2017). In the second network in Study 1, the combined effect of increases in both muscle stiffness *and* NLE resulted in the highest probability of injury, supporting Appaneal and Perna's (2014) proposed synergistic action of both psychological and physiological markers of stress interacting to exacerbate the risk of injury. A weaker relationship was observed for both poor balance and low HRV, which were found to increase the probability of injury. Despite the weak relationships with injury, the findings were in agreement with the relevant literature for both HRV (Lima-Borges et al., 2018; Williams et al., 2017) and postural stability (Romero-Franco et al., 2014; Trojian & McKeag, 2006), and further demonstrate the importance of using a range of stress-related markers. In the second study, a weak positive relationship between the change in cortisol following high intensity training and the number of days lost to injury was observed for the rugby team (estimate = 0.54, 95% CrI = [0.05, 1.05]), however the relationship was less clear for the football team (estimate

= 0.10, 95% CrI = [-0.43, 0.62]) making the results inconclusive. There was also a weak positive relationship between the change in cortisol and muscle stiffness (estimate = 0.07, 95% CrI = [-0.25, 0.40]), highlighting an interesting relationship for future research to explore further.

One characteristic that all the markers share is their relationship with the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis. Appaneal and Perna (2014) proposed the ANS, and subsequent HPA activity, as one of the mediating pathways through which psychological stress will combine with other physiological effects of training-related stress to exacerbate the overall stress response. Indeed, both the ANS and HPA axis are extremely sensitive to all forms of "stress" and, either directly or indirectly, will cause the modification of many systems within the body to prepare, react, or cope with, increasing levels of stress (Bellenger et al., 2016; Chrousos, 2009; Yaribeygi, Panahi, Sahraei, Johnston, & Sahebkar, 2017). These notions are central to the concept of allostasis, or stability through change, which has surprisingly received little attention in the sports injury literature despite a large body of research linking the psychological and physiological effects of stress that would be of value to the stress-injury relationship (Galambos et al., 2005; Ganzel et al., 2010; McEwen, 2007; Sterling & Eyer, 1988). Of particular relevance to sport injury is the concept of allostatic load, which has been defined as the "long-term carry-forward of the sequelae of stress and adversity" (Rutter, 1994, p. 373). A healthy athlete may experience negligible allostatic load, resulting in a symptom-free health profile reflected by adequate recovery and positive responses to training. However, allostatic load can accumulate as a result of daily low levels of stress in the environment and discrete major life events, both of which are proposed to be related to increased risk of injury (Appaneal & Perna, 2014). Furthermore, excessive increases in training volumes or intensity can further contribute to the allostatic load, leading to a more symptomatic profile with a greater risk of athletic injury. This global view of stress helps to demonstrate how the effects of both psychological and physiological sources of stress are related and highlights the need for stress-related injury research to continue to be addressed from an interdisciplinary perspective. The current programme of research has therefore provided the sport injury literature with an initial basis to move toward a more inclusive, holistic approach to injury prediction research that brings together disparate disciplines to address the sport injury problem.

In addition to the inclusion of stress-related physiological markers, the current research has also provided novel contributions to our understanding of the role of well-established psychosocial variables included in the original Williams and Andersen (1998) model, particularly with regard to the measures used to assess them. For example, the current research sought to integrate Reinforcement Sensitivity Theory (RST; Corr, 2008), which attempts to explain the multidimensional nature of personality. Initially proposed by Gray (1982), the RST was revised by Gray and McNaughton (2000) to include three systems that are proposed to govern an individual's behaviour, specifically the behavioural activation system (BAS), behavioural inhibition system (BIS) and fight-flight-freeze system (FFFS). According to the RST, the FFFS and BAS are responsive to all punishing and threatening stimuli (e.g., physical pain from training or competition) and all rewarding and appetitive stimuli (e.g., prize money from winning a major competition) respectively. When there is conflict between these two systems, BIS is activated and engages in risk assessment and threat identification that may inhibit or result in a more cautious approach or avoidance behaviour initially considered by the BAS or FFFS systems. Excessive BIS activation can elicit the emotional state of anxiety, which provides a link to the original Williams and Andersen (1998) model where high levels of anxiety have previously been found to be related to injury occurrence (Ivarsson & Johnson, 2010; Lavallée & Flint, 1996; Petrie, 1993)

Several measures of the revised theory have been developed (cf. Walker & Jackson, 2017), and a recent version proposed by Corr and Cooper (2016) was validated by Young (2019) using a sample of athletes from Study 1. The newly validated questionnaire was used within Study 1 and the analysis revealed the FFFS to be the only factor that was directly linked to injury. Specifically, low levels of FFFS were associated with a greater probability of injury occurrence. RST states that FFFS reflects the emotion of fear, and these findings suggest that those athletes who were less fearful potentially engaged in more risk-taking behaviours and consequently were exposed to a greater risk of injury. Indeed, when coupled with high BAS and low BIS activation, low FFFS is thought to result in “reckless” or “striving” behaviours (Corr et al., 2016), which could be considered “high risk, high reward” behaviours in a sporting environment. For example, a 1500 m runner may have this particular combination of characteristics (high BAS, low BIS and low FFFS) and begin their sprint finish with 600 m still left to run. Their lack of fear (low FFFS) and anxiety (low BIS) coupled with a strong motivation to succeed (high BAS) mean they are feeling positive about making the move from a reasonably long way still left to run, and may contrast to an athlete who has opposing characteristics (e.g., low BAS, high BIS and high FFFS). If they are able to sustain their effort, they may end up winning the race (high reward), however if they time their effort poorly and begin to fatigue too early, they may end up being caught and finish outside of the medals, or potentially sustain an injury due to the increased physical demand from sprinting so far out (high risk). Such an example, derived from principles outlined by Corr et al. (2016), demonstrates how the components of RST may interact and apply to a sporting context, and the current research provides the first support for the relationship between RST and sports injury.

Although not related to injury prediction, the second Bayesian network in Study 1 revealed a relationship between changes in BIS, FFFS and heart rate variability (HRV). In this instance, increases in BIS were related to both increases in FFFS and decreases in HRV. The positive relationship between BIS and FFFS is supported by RST and is likely to result in moving from “apathetic” to “cautious” behaviour when BAS is low or from “reckless” to “volatile” behaviour when BAS is high (Corr et al., 2016). Each of the latter behaviours is related to higher anxiety, and the relationship with HRV provides physiological evidence supporting this association. For example, high levels of anxiety and anticipation of stressful situations are known to result in decreased high frequency HRV, as measured by the root mean square successive difference (RMSSD) between heart beats (Chalmers et al., 2014; Pulopulos et al., 2018). As such, the findings demonstrate a link between the constructs and associated behaviours proposed in RST and physiological response expected with such behaviours. Further research should continue to explore how the RST can expand this area of the sports injury literature. To summarise, the current body of research has both integrated elements from Appaneal and Perna’s (2014) BMSAIH by including stress-related physiological markers, and also made new contributions regarding the psychosocial factors proposed in Williams and Andersen (1998) model. The effect of these individual psychological factors and stress-related markers, in addition to interactions *between* the different variables were explored to investigate how they act in a synergistic fashion to exacerbate the stress response. The analysis drew inspiration from Bayesian principles and complex systems theory to provide an approach that offers an alternative to the commonly used frequentist multivariate regression-based techniques and captures the holistic nature of the research. The result is a novel coalescence of traditional theories and contemporary ideas, supported by an innovative analysis, that together answer the calls for an interdisciplinary perspective within sports injury research.

Analysis. Throughout this thesis Bayesian statistics have been used in favour of traditional frequentist methods of analysis in an attempt to address several issues associated with the frequentist approach. Indeed, a shift towards Bayesian statistics has occurred across the academic landscape, due in part to greater recognition of the issues surrounding the *p*-value and null hypothesis testing paradigms (Wasserstein, Schirm, & Lazar, 2019), and also an improvement in the accessibility to both hardware and software which can enable effective Bayesian analysis (Carpenter et al., 2017). While other fields such as clinical psychology (Heino et al., 2018) and medicine (Bittl & He, 2017) have started to embrace Bayesian statistics, their use within the sports science literature remains sparse, despite several characteristics which make Bayesian analysis particularly suited to sport science (Bernards, Sato, Haff, & Bazyler, 2017). For example, a Bayesian approach can help overcome issues when small sample sizes are present, and provide a solution to some of the difficulties of evaluating small effects, both of which are common issues within sport science research (Mengersen et al., 2016).

Despite the advantages offered by Bayesian statistics, there are a number of concerns with using Bayesian methods. Indeed, Gelman (2008a) provides a comprehensive list of issues that are commonly raised against Bayesian analysis[2]. One issue in particular that may

[2] Gelman also provides a comprehensive counter arguments to these issues in his follow up paper (Gelman 2008b).

raise concern is the use of subjective prior information within a model. While the idea of including subjective information in a model that is supposed to be concerned with objective knowledge may initially seem surprising, there are justifiable reasons for doing so. For example, the prior allows information from previous experiments, historical data or expert opinion to be included in the model, which can improve both the sampling and inference. However, if no information is available, non-informative priors can be used, allowing inferences to be driven by the data alone (Mengersen et al., 2016). Even if no information is available, it is almost always possible to determine a range of values which are plausible, compared to using a completely non-informative prior (Bernards et al., 2017). While it is possible to use strong informative priors to influence results (akin to p-hacking), authors should be able to justify the use of any particular prior. Indeed, transparency in the decisions made when conducting Bayesian analysis is vital for both validity of findings and reproducibility of results.

A second common issue that is the idea that Bayesian methods are presented as an "automatic inference engine" (Gelman 2008a, p. 2). However, inference is only one part of the process for conducting a Bayesian analysis. Before inference can occur, a model must first be formulated. This step requires careful thought about what model is most realistic to the data. After the model is is fit, several steps must be taken to check and evaluate the model fit. Model convergence metrics and posterior predictive checks are examples of necessary steps to ensure a robust model has been fit, and can help identify when there may be issues with the model (Heino et al., 2018). These steps are far from automatic, requiring careful thought and consideration, and are equally important to the model building process as a whole (Gelman 2008b). Similarly to the choice of prior, it is vital that the checks performed, and any adjustments to the model are recorded, to help provide a transparent model building process.

To aid the necessary transparency in Bayesian analysis, Depaoli & van de Schoot (2017) provided the WAMBS-checklist (When to worry and how to Avoid the Misuse of Bayesian Statistics). By following the checklist, authors can ensure that each decision made is defensible, and the Bayesian model that is used for analysis is robust and justified. In this thesis, Study 2 implemented a number of checks recommended by the Depaoli & van de Schoot (2017), and the full analysis script is openly available for other researchers to access (Appendix F). By adhering to a clear and transparent approach to Bayesian analysis, the thesis aimed to address many of the potential issues cited by Gelman (2008a), and add to the small, but growing, number of studies using a fully Bayesian approach within the sports science literature and benefit from the numerous strengths a Bayesian approach has to offer.

Measurement issues. The additional complexity of interdisciplinary research presents researchers with several challenges in terms of measurement. To help researchers with these challenges, Tobi and Kampen (2018) have recently proposed the Methodology for Interdisciplinary Research (MIR) framework (Figure 25) that highlights several issues that were present in the current research, particularly when choosing variables of interest, and how they should be measured. Specifically, Tobi and Kampen (2018) discuss the impact that instrument selection and associated reliability have on the execution of a data collection plan and the quality of data that can be collected. While the data collection was planned and executed prior to the publication of Tobi and Kampen's (2018) framework, the points discussed are particularly salient in the current research, which combined questionnaire-based data collection with measurement of stress-related physiological markers in an interdisciplinary setting. A clear difference between these types of data was that questionnaires could be completed with relatively low input from the researcher, as participants were simply given a set of instructions to follow and were able to complete the questionnaire at their own pace during the data collection session. In contrast, each of the physiological measures required the researcher to prepare and/or manually perform each measurement. Due to the large-scale of the study, measures that had a high time cost were therefore not suitable for the data collection procedure. Ease of administration of the measures was favoured over the respective "gold standard" for each measure, which would have been more time consuming and impractical for such a large scale data collection. In line with Tobi and Kampen's (2018) framework, a considerable amount of time was spent during the variable identification, instrument selection and pilot testing stages (Chapter 2), to ensure the measures that were chosen were robust and reliable, and that the quality of the data was not compromised by the chosen procedures and measures.

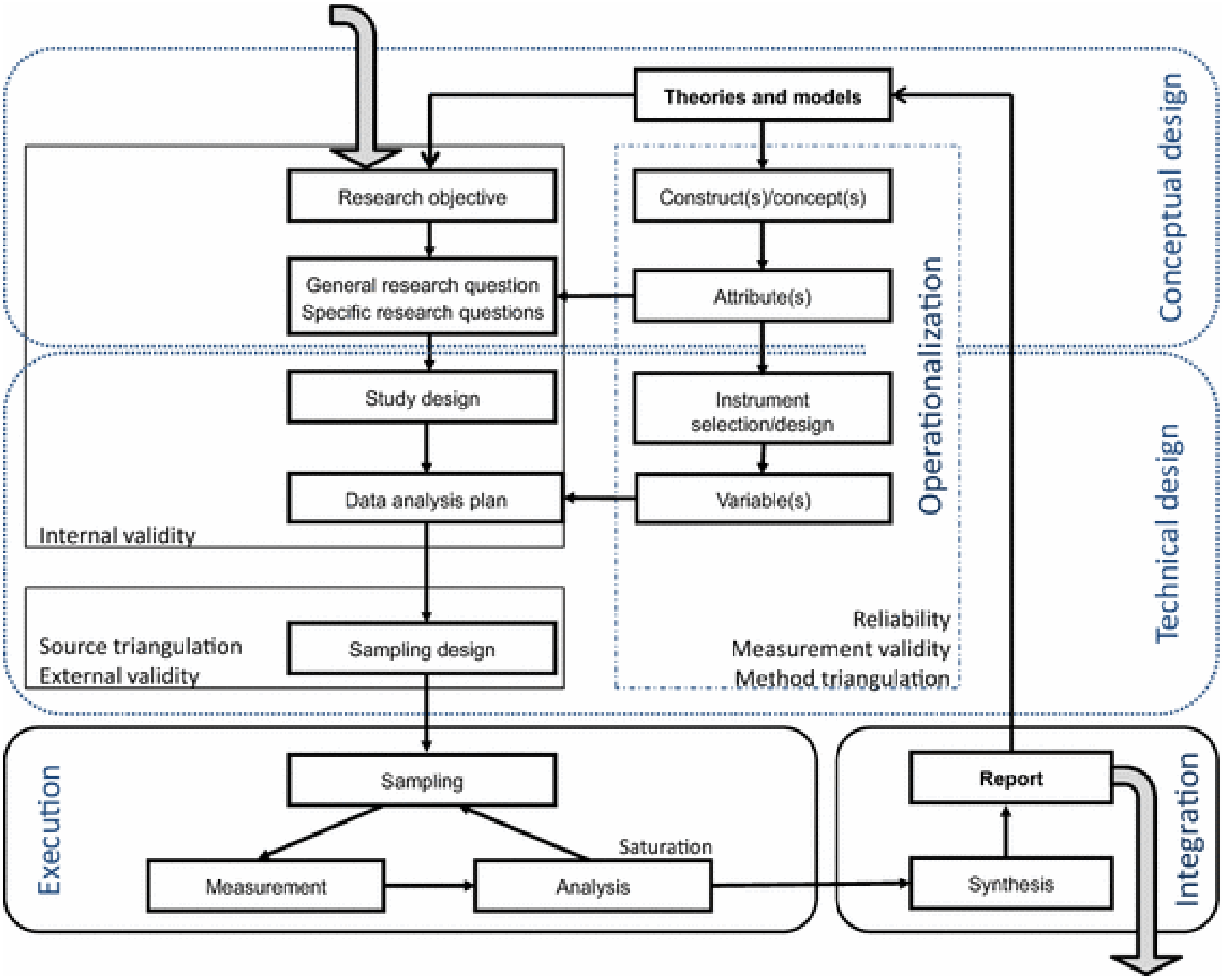

Figure 25. Methodology for Interdisciplinary Research (MIR) framework (Tobi & Kampen, 2018).

During the execution of the data collection, the identified measures and instruments generally performed as expected, and no *major* challenges were faced. Occasionally, however, there were particular challenges with both the muscle stiffness measurement, where results occasionally did not save and measurements had to be repeated; and heart rate variability, where chest strap sensors failed to pick up heart rate signals and time was spent adjusting or reconnecting chest straps. Given the rigorous planning prior to data collection, these issues were quickly resolved and did not significantly impact the overall execution of the data collection.

While the selection of variables of interest and choice of measurement instruments from an interdisciplinary perspective presented considerable challenges, the complexities of measurement were further confounded due to the repeated measures design of the current research. Specifically, the chosen measures also needed consideration with regard their suitability over repeated measurement occasions. While efforts were made to ensure the choice of measures were appropriate for a repeated measures design (Chapter 2), one issue that became apparent was the use of the LESCA questionnaire over a shortened time interval. The instructions in the original LESCA questionnaire ask participants to report major life events that have occurred over the previous 12 months (Petrie, 1992), however in the current study, participants completed the LESCA at approximately 4-monthly intervals after the first data collection. To avoid inflated scores caused by reporting the same event on multiple occasions, they were asked to report any events which had occurred *since the previous data collection session.* An unintended consequence of this approach was particularly low scores in the second and third data collection sessions, with 26% of participants reporting 0 negative life events for the preceding four-month period. This result suggests that the items on the LESCA may be less suitable for repeated measurements with durations shorter than the original 12-months, and a measure that captures more minor life events in addition to major events may be more appropriate (cf. Fawkner et al., 1999).

A further consideration that was made regarding the repeated measures design was how demanding and time consuming each measure would be for each participant. While obtaining reliable and valid data across all variables was important, using arduous or uncomfortable measures may have exacerbated participant drop-out which is a known limitation of longitudinal, repeated measures research designs (Abshire et al., 2017). Therefore, a goal of the current study was to establish the feasibility of valid and reliable measures that would capture the variables of interest but also safeguard retention of participants in a repeated measures design. Indeed, the field-based nature of the chosen measures meant that it was possible to collect data in different locations, which was highly convenient for completing testing with the local sports teams who were not based at the university. In this instance, the choice of measures had a positive impact on participant retention, and the measures chosen achieved the goal of being both valid and reliable, yet feasible within the large scale, longitudinal, repeated measures design adopted in the current research.

With regard to the specific measures used in the research, there are several points that warrant discussion, including:

- The scoring of the LESCA.
- The novelty of the MyotonPRO in sports injury research.
- The combined strength of the measures in an interdisciplinary study.

The LESCA has been widely used as the measure of life event stress for collegiate athlete, with a preponderance of findings supporting the link between negative life events and injury. (Gunnoe et al., 2001; Ivarsson et al., 2017; Maddison & Prapavessis, 2005; Williams & Andersen, 2007). However, the current research did not support this relationship (Rider & Hicks, 1995). Instead, it was the first study to highlight a potential issue with the original scoring method. The original scoring method of the LESCA does not differentiate between athletes who may experience few minor events and several major events. To clarify, an athlete who reports one major event receives the score of -4, which is the same as an athlete who reports four minor events (scored at -1 each). These two profiles, and the associated responses are unlikely to be equivalent given their potential impact, and medium to longer term consequences (Tosevski & Milovancevic, 2006). By using the average life event response, it was possible to distinguish between these two profiles, which revealed some promising findings. In Study 2, higher average NLE response was found to both increase the risk of injury and was also associated with greater injury severity; a finding that offers several possible avenues for future research.

The use of the MyotonPRO within sports science research has recently started to gain traction, with several studies using the device to assess musculoskeletal characteristics such as stiffness in athletes (Gervasi et al., 2017; Kalkhoven & Watsford, 2017; Pruyn et al., 2015). However, these studies have typically focused on performance, and relatively few studies have used the MyotonPRO to investigate the relationships between musculoskeletal characteristics and sports injury (Pickering-Rodriguez et al., 2017). The current research found muscle stiffness to have the strongest relationship to injury, with high levels of stiffness increasing the probability of sustaining an injury. Given the ease with which the data could be collected when using the device, the MyotonPRO could be included in a variety of study designs to further investigate the relationship between stiffness and injury. Indeed, in contrast to other measures available to assess muscle stiffness, the MyotonPRO presents an objective, non-invasive, cost effective method to obtain muscle stiffness measurements that would be particularly useful for coaches and sports practitioners to obtain data in a field-based environment. Data of this type would enable them to make more informed decisions about an athlete's muscle characteristics. As such, using a device such as the MyotonPRO could become a vital part of a holistic assessment of an athletes readiness to train and compete.

A major point of contention for interdisciplinary researchers is how to integrate measures from different disciplines into a coherent data collection plan that addresses the research question (Tobi & Kampen, 2018). In the current study, considerable time was spent identifying, evaluating and pilot testing different measures to decide which ones would be suitable for the planned data collection. Specifically, measures for the stress-related physiological markers presented the most challenges, as these required the most amount of time from the researcher. For example, postural stability was initially planned to be assessed with a balance task using a force plate, however, this more complex mode of data collection would have had a negative impacted on the other measures given the additional time needed to complete the balance task. Instead, the Balance Error Scoring System (BESS; Riemann et al., 1999) was identified as a reliable alternative that required significantly less time to complete. Similarly, a specialised Bluetooth sensor was initially planned to collect both heart rate and breathing rate data. While the data collected using this sensor could have been used for a more sensitive analysis in the frequency domain, participants would have needed to complete the collection individually, requiring almost 100 hours to record the necessary data, which was beyond the scope of the present research programme. Instead, the PolarV800 was identified as a reliable and valid alternative that did not measure breathing rate but was significantly lower in cost, enabling multiple units to be used to collect heart rate data. This significantly reduced the time cost of collecting heart rate data and allowed groups of participants to be tested at the same time. Combined with the psychological questionnaires, benefits of using the MyotonPRO for muscle stiffness assessment and ease of collecting saliva using field based sampling kits, the measures adopted in the current study enabled a truly interdisciplinary approach that captures the combined strengths of multiple disciplines in a holistic data collection procedure that outweighs the strengths of any one of the measures in isolation.

Practical implications

Several practical implications have emerged from the current research, with relevance for athletes, coaches, practitioners and researchers interested in sports injury. For athletes and coaches, awareness regarding the additive and interactive effects of multiple sources of stress needs to be emphasised. Study 1 demonstrated how the combined effect of psychological and physiological characteristics can increase the probability of injury to a greater extent than any characteristic in isolation. As such, multiple related risk factors for injury need to be considered when assessing an athlete's training plan, readiness to engage in, and recovery from, training. For example, in addition to monitoring training loads and using tools to determine an athlete's physiological status, coaches need to also consider an athlete's psychological state. In particular, when an athlete is facing significant life event stress, training intensity and volume may need to be adjusted to help the athlete cope with the additional duress they are experiencing. This holistic approach centred around the athlete has recently been emphasised by Dijkstra, Pollock, Chakraverty, and Alonso (2014), who advocate an integrated model regarding athlete's performance, health and coaching. Such an approach prioritises a balanced approach to training and competition, incorporating information from several sources (i.e., psychological and physiological markers of stress) to ensure the optimal health and well-being of the athlete. These recommendations are supported and echoed by the current research that found when an athlete is experiencing psychological stress due to exposure to negative life events in conjunction with physiological characteristics that are associated with an increased risk of injurious events, injury risk may be exacerbated further. Specifically, the identification of a "high risk" profile may help to reduce the risk of injury for athletes. For example, while high muscle stiffness is important for optimal performance, the risk of injury also increases with high muscle stiffness. This risk is likely to be exacerbated when there are also increases in the negative life events experienced by an athlete. In addition, findings from the current study also revealed poor balance, low HRV and low levels of fear to all be associated with increased risk of injury. It is therefore important to acknowledge the breadth of these characteristics and be receptive to changes in both the training and life experiences of an athlete to understand how the risk of injury may increase over time.

For sport injury researchers, both studies in this programme of research have highlighted the importance of taking an interdisciplinary approach to further advance the understanding of the complex and multifaceted stress-injury relationship. Furthermore, capturing how markers of stress change over time in response to prolonged exposure to training, and changes in major events that may occur in athletes' lives, is vital for the development of the sport injury literature. For example, in the second network in Study 1, the combination of increases in negative life events and muscle stiffness resulted in the highest probability of injury occurrence. Given that stress is a dynamic, contextual phenomenon, repeated measures designs are required to adequately understand how fluctuating levels of stress, whether psychological or physiological, will impact injury risk. As such, the author echoes the recommendations made by Johnson et al. (2014) and Ivarsson et al. (2017) with regards to how future sport injury research should be designed to further advance the field. In particular, an interdisciplinary approach combining related factors from different areas of sports science combined with multiple measurements over a period of time will yield the most productive and insightful findings.

In addition to adopting an interdisciplinary approach, there is also scope to explore the implications of the individual markers in greater detail. Specifically, through more regular monitoring of muscle stiffness it may be possible to determine what types of training are likely to increase stiffness, compared to those sessions that have less of an effect. This knowledge would be valuable for coaches who could use such information to help plan training and competition cycles, with an objective indicator of an athlete's muscle health. In addition to muscle stiffness, researchers may also find further development of the RST in a sports injury setting useful in the identification of “at risk” psychological profiles in athletes. In doing so, it may be possible to develop interventions that address the undesirable characteristics and address injury prevention from a psychological perspective alongside the physical preventative measures that are often used by coaches and athletes. This would provide a more holistic approach to injury prevention in line with the recommendations and benefits of interdisciplinary research discussed in the sports injury literature.

Future research directions

In addition to the points already alluded to regarding future research directions, there are several key lines of enquiry that would be beneficial;

- Further development of the LESCA questionnaire based on the issues raised in both Study 1 and Study 2.
- Examination of other factors within the Williams and Andersen (1998) and Appaneal and Perna (2014) models of injury.
- Examination of additional markers of stress.
- Continued development of the analytical techniques used to examine the complex relationship between stress and injury.

While the LESCA remains the most widely used measure of life event stress with collegiate level athletes, both Study 1 and Study 2 raised some potential issues with the measure. Specifically, (a) the suitability of the LESCA for repeated measures designs where the time interval between collections may be shorter than the original 12-month period proposed by Petrie (1992); (b) many of the events listed on the original LESCA are unlikely to occur repeatedly over relative short periods of time, thus a way of quantifying more minor events is necessary. While previous research has used the daily hassles scale to capture more minor life events (Fawkner et al., 1999; Ivarsson & Johnson, 2010), the scale is not designed with athletes in mind and therefore lacks the construct validity of the LESCA. Consequently, the development of a scale that can capture the impact of more minor events in athletes would therefore be desirable. In addition, the issues around the original scoring of the LESCA that similarly weights multiple minor events and a single or smaller number of major events is potentially problematic in relation to the stress response that might be elicited. Study 2 addressed the issue by calculating an average response and found that there was a weak relationship between higher average responses and increase risk of injury. Future studies could examine this relationship in greater detail or develop a scale that is better able to distinguish between and account for both minor and major life events.

A second line of enquiry advocated is for researchers to explore other factors that have been proposed by both the Williams and Andersen's (1998) and Appaneal and Perna's (2014) models, particularly in relation to an interdisciplinary perspective. For example, Williams and Andersen (1998) proposed coping resources (e.g., coping behaviours, social support systems and stress management) and Appaneal and Perna (2014) proposed behavioural mechanisms (e.g., impaired self-care and poor sleep quality) as factors that are likely to be related to injury occurrence. However, comparatively few studies have investigated these factors in comparison to other psychosocial factors such as major life events and personality characteristics (Ivarsson et al., 2017). Indeed, how these coping and behavioural factors contribute alongside the physiological markers of stress, such as those used in the current research, may provide a more holistic understanding of the stress-injury relationship.

To extend the proposed variables in both Williams and Andersen (1998) and Appaneal and Perna (2014), future research should also continue to explore other factors that may be related to injury occurrence. While the variables proposed by Williams and Andersen (1998) and Appaneal and Perna (2014) provide a suitable starting point, there are many other possibilities that may provide additional insight into the stress-injury relationship. Indeed, allostatis may provide sports injury researches with several new avenues to examine in addition to variables that have not yet been widely used in the sports injury literature. For example the Allostaic Load Index has been proposed as a basis for assessing the wear and tear on the body caused by chronic stress and combines 10 variables including; cortisol, epinephrine, norepinephrine and dehydroepiandrosterone sulphate (DHEAS), systolic and diastolic blood pressure, waist-hip ratio, high-density lipoprotein

(HDL) and total cholesterol ratio and glycosylated haemoglobin (Seeman, Singer, Rowe, Horwitz, & McEwen, 1997). A score is calculated based on the number of markers an individual has in the highest risk quartile (Seeman et al., 1997). While other combinations of markers have also been used, and there is not yet consensus regarding the best combination of markers to measure allostatic load (Daniel, Li, Schmidt, Angerer, & Jarczok, 2014), researchers could use these examples to generate new ideas for how to integrate different markers of stress alongside commonly used measures in sports injury research to extend our understanding of the stress-injury relationship.

Finally, the continued development of appropriate analysis techniques that address the complex nature of the stress-injury relationship is required to increase our understanding of the contributory factors and their interactions. Typically, multivariate regression-based techniques have been widely used within the sports injury literature, however in line with Bittencourt et al. (2016) recommendations regarding complex systems theory, research now needs to explore the interactions *between* different variables and not just variables studied in isolation. The current study addressed this issue by using Bayesian networks which offer several advantages, including the ability to incorporate both expert knowledge and empirical data, and the ability to investigate probabilistic dependences between any combination of variables in the network. However, there are several other techniques that may also be of use for researchers. For example, Johnson et al. (2014) recently proposed latent growth curve modelling as an appropriate method to capture how psychosocial stress change over time, and how changes in psychosocial stress are related to injury occurrence. Johnson et al. (2014) also highlighted the need for intra-individual based analysis, given the highly individualised responses to stress and complex nature of injury occurrence. A further possibility is to explore machine learning (ML) based techniques that concentrate on prediction by finding patterns in often large and unwieldy data sets (Bzdok, Altman, & Krzywinski, 2018). As greater quantities of data become available, traditional statistical methods can become intractable as the number of variables per participant increases and suffer from issues such as multicollinearity and over-fitting (Iniesta, Stahl, & McGuffin, 2016). In contrast, ML techniques such as Classification and Regression Trees (CART), random forests and gradient boosted trees greatly benefit from a large number of variables and may be used alongside traditional statistical methods (Bittencourt et al., 2016). Indeed, Rossi et al. (2018) recently used a decision tree method to forecast injury occurrence in soccer players using GPS data from training session with promising results. The predictive ability of ML techniques may therefore have important implications for sports injury researchers and help greatly in the identification of athletes who are at risk of injury, allowing timely interventions to be put in place and injury to be averted.

Strengths

The current programme of research had several strengths that have been discussed in earlier chapters. The points below provide a summary of the main strengths of the programme of research.

- The interdisciplinary approach to the research and examination of both psychological and physiological markers of stress and their relationship to injury (Ivarsson et al., 2017).
- The prospective, longitudinal, repeated measures design of the research (Johnson et al., 2014) .
- The use of an analytical approach that captures the complex nature of injury occurrence (Bittencourt et al., 2016), including:
  - A novel application of Bayesian networks to sports injury data in Study 1.
  - The use of Bayesian hurdle regression and linear regression models in Study 2, offering several advantages over traditional frequentist methods (Kruschke, 2013).
- Inclusion of a wide range of athletes from different sports, including both males and females from individual and team-based sports (Johnson et al., 2014)
- Use of a novel device to measure muscle stiffness which added to the body of literature linking muscle stiffness to injury (Pickering-Rodriguez et al., 2017)

- The identification and proposed solution to a potential issue with the original LESCA scoring system.
- The use of contemporary personality measure that has yet to be used within sports injury literature (RST-PQ; Corr & Cooper, 2016)
- The field-based nature of the measures used in the research means that coaches and practitioners can easily replicate the data collection procedure.

Limitations

Many of the research limitations of this research programme have also been identified in the discussions of each individual study, and within earlier sections of this chapter. These have been addressed in some detail and will only be listed here.

- Participant drop-out rate during the study (Abshire et al., 2017).
- Use of the LESCA questionnaire in a repeated measures design.
- The field-based nature of the cortisol collection and lack of control over the session intensity.
- The field-based measures may have lacked sensitivity compared to the "gold-standard" measures available.

Conclusions

The purpose of this thesis was to explore the multifaceted nature of the stress-injury relationship. Findings from this programme of research have demonstrated that several psychological and physiological factors combine and interact to exacerbate the risk of injury. Specifically, muscle stiffness and *increases* in negative life event stress were identified as strong predictors of injury, while other factors including personality characteristics and postural stability were also found to contribute to the probability of injury occurrence. Taken together, the interdisciplinary approach coupled with a complex systems framework has provided a novel examination of the stress-injury relationship that has addressed many of the limitations identified in previous research. Furthermore, the analytical techniques used have reflected the complex and uncertain nature of injury occurrence and provide a contemporary approach to the research. As such, it is believed that this thesis has achieved its purpose, as it presents athletes, coaches, practitioners and researchers with valuable insights into the risk factors associated with the stress-injury relationship, along with the importance of adopting an holistic approach to training and competition to mitigate against the risk of injury.

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Appendix A

Life Event Survey for Collegiate Athletes

Data collection 1

Life Event Survey

Instructions: Listed below are 69 events that sometimes occur in the lives of college athletes. These events often produce change within an individual's life that require some adjustment by the individual. For each event that you have experienced within the last year (12 months), indicate what kind of effect it had on your life when the event occurred.

A rating of -4 would indicate that the event had an extremely negative effect on you.

A rating of +4 would indicate that the event had an extremely positive effect on you.

For those events that have happened more than once, indicate the average effect across all occurrences. If you have not experienced an event within the last year, leave that item blank. The events are listed in no particular order, and there are no right or wrong answers. Please respond to each event honestly as applies to you.

If you have NOT experienced **an event listed below (e.g., Marriage) in the past 12 months, leave that item blank.**
ONLY respond to items you have experienced.

2. P1

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Marriage	○	○	○	○	○	○	○	○
Death of mate (boyfriend, girlfriend, spouse, significant other)	○	○	○	○	○	○	○	○
Major change in sleeping habits (increase or decrease in amount of sleep)	○	○	○	○	○	○	○	○
Death of a close family member(s) - Specify below:	○	○	○	○	○	○	○	○
Father	○	○	○	○	○	○	○	○
Mother	○	○	○	○	○	○	○	○
Brother	○	○	○	○	○	○	○	○
Sister	○	○	○	○	○	○	○	○
Grandfather	○	○	○	○	○	○	○	○
Grandmother	○	○	○	○	○	○	○	○
Other	○	○	○	○	○	○	○	○
Death of close friend(s)	○	○	○	○	○	○	○	○
Outstanding personal achievement	○	○	○	○	○	○	○	○
Male: mate pregnant	○	○	○	○	○	○	○	○
Female: becoming pregnant	○	○	○	○	○	○	○	○

Life Event Survey

3. P2

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Sexual difficulties	○	○	○	○	○	○	○	○
Being fired from job	○	○	○	○	○	○	○	○
Being apart from mate (boy/girlfriend, spouse, etc) due to sport	○	○	○	○	○	○	○	○
Serious injury or illness to close family member(s) - Specify below:	○	○	○	○	○	○	○	○
Father	○	○	○	○	○	○	○	○
Mother	○	○	○	○	○	○	○	○
Brother	○	○	○	○	○	○	○	○
Sister	○	○	○	○	○	○	○	○
Grandfather	○	○	○	○	○	○	○	○
Grandmother	○	○	○	○	○	○	○	○
Other	○	○	○	○	○	○	○	○
Major change in the number (more/less) of arguments with mate	○	○	○	○	○	○	○	○
Major personal injury or illness	○	○	○	○	○	○	○	○
Major change in the frequency (increased or decreased) of social activities due to participation in sport	○	○	○	○	○	○	○	○
Serious injury or illness to close friend	○	○	○	○	○	○	○	○

Data collection 1

Life Event Survey

4. P3

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Breaking up with mate (boy/girlfriend, etc)	◯	◯	◯	◯	◯	◯	◯	◯
Beginning a new school experience (beginning university, transferring university etc)	◯	◯	◯	◯	◯	◯	◯	◯
Engagement	◯	◯	◯	◯	◯	◯	◯	◯
Academic probation/ineligibility	◯	◯	◯	◯	◯	◯	◯	◯
Being dismissed from halls or other residence	◯	◯	◯	◯	◯	◯	◯	◯
Failing an important exam	◯	◯	◯	◯	◯	◯	◯	◯
Major change in relationship with coach (better or worse)	◯	◯	◯	◯	◯	◯	◯	◯
Failing a course	◯	◯	◯	◯	◯	◯	◯	◯
Major change in the length and/or conditions of practice/training (better or worse)	◯	◯	◯	◯	◯	◯	◯	◯
Financial problems concerning school	◯	◯	◯	◯	◯	◯	◯	◯
Major change in relationship with family member(s) (better or worse)	◯	◯	◯	◯	◯	◯	◯	◯
Conflict with roommate	◯	◯	◯	◯	◯	◯	◯	◯
Male: mate having an abortion	◯	◯	◯	◯	◯	◯	◯	◯
Female: having an abortion	◯	◯	◯	◯	◯	◯	◯	◯
Major change in the amount (more or less) of academic activity (home work, class time, etc)	◯	◯	◯	◯	◯	◯	◯	◯

Data collection 1

Life Event Survey

5. P4

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Pressure to gain/lose weight-due to participation in sport	○	○	○	○	○	○	○	○
Discrimination from teammates/coaches	○	○	○	○	○	○	○	○
Major change in relationship(s) with team-mate(s) (better/worse)	○	○	○	○	○	○	○	○
Suspended from team for non-academic reasons	○	○	○	○	○	○	○	○
Trouble with academic counsellor	○	○	○	○	○	○	○	○
Major change in use of alcohol/drugs (increased or decreased)	○	○	○	○	○	○	○	○
Beginning sexual activity	○	○	○	○	○	○	○	○
Major change in relationship(s) with friend(s) (better or worse)	○	○	○	○	○	○	○	○
Recovery from illness/injury/operation	○	○	○	○	○	○	○	○
Major change in level of athletic performance in actual competition (better or worse)	○	○	○	○	○	○	○	○
Divorce or separation of your parents	○	○	○	○	○	○	○	○
Major change in level of responsibility on team (increased/decreased)	○	○	○	○	○	○	○	○
Receiving an athletic scholarship	○	○	○	○	○	○	○	○

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Not attaining personal goals in sport	○	○	○	○	○	○	○	○
Major change in playing status on team	○	○	○	○	○	○	○	○

Data collection 1

Life Event Survey

6. P5

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Injury to team-mates	◯	◯	◯	◯	◯	◯	◯	◯
Being absent from university (classes) because of participation in sport	◯	◯	◯	◯	◯	◯	◯	◯
Troubles with athletic association and/or athletic director	◯	◯	◯	◯	◯	◯	◯	◯
Difficulties with trainer/physician	◯	◯	◯	◯	◯	◯	◯	◯
Major change in playing time (playing more or less) – due to injury	◯	◯	◯	◯	◯	◯	◯	◯
Major errors/mistakes in actual competition	◯	◯	◯	◯	◯	◯	◯	◯
Losing your athletic scholarship	◯	◯	◯	◯	◯	◯	◯	◯
No recognition/praise of accomplishments from coaching staff	◯	◯	◯	◯	◯	◯	◯	◯
Pressure from family to perform well	◯	◯	◯	◯	◯	◯	◯	◯
Loss of confidence due to injury	◯	◯	◯	◯	◯	◯	◯	◯
Unable to find a job	◯	◯	◯	◯	◯	◯	◯	◯
Change in coaching staff	◯	◯	◯	◯	◯	◯	◯	◯
Female: menstrual period/PMS	◯	◯	◯	◯	◯	◯	◯	◯
Major change in level of academic performance (doing better or worse)	◯	◯	◯	◯	◯	◯	◯	◯
Making career decisions (applying for Masters degree, interviewing for jobs, etc)	◯	◯	◯	◯	◯	◯	◯	◯

Data collection 1

Life Event Survey

7. P6

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Being cut/dropped from the team	◯	◯	◯	◯	◯	◯	◯	◯
Continual poor performance of team	◯	◯	◯	◯	◯	◯	◯	◯
Change in graduation schedule	◯	◯	◯	◯	◯	◯	◯	◯
Major change in family finances (increased or decreased)	◯	◯	◯	◯	◯	◯	◯	◯
Major change in attitude toward sport (like/enjoy more or less)	◯	◯	◯	◯	◯	◯	◯	◯
Victim of harassment/abuse (sexual, emotional, physical)	◯	◯	◯	◯	◯	◯	◯	◯
Victim of personal attack (rape, robbery, assault, etc)	◯	◯	◯	◯	◯	◯	◯	◯

8. P7 - Other events might have occurred to you in the past year (and affected you in a positive or negative manner) but were not included in the questionnaire. If there were such events, please list them below and rate them accordingly.

	Extremely Negative -4	Negative -3	Moderately Negative -2	Somewhat Negative -1	Somewhat Positive +1	Moderately Positive +2	Positive +3	Extremely Positive +4
Other A	○	○	○	○	○	○	○	○
Other B	○	○	○	○	○	○	○	○
Other C	○	○	○	○	○	○	○	○
Other D	○	○	○	○	○	○	○	○
Other E	○	○	○	○	○	○	○	○

Other A — Other (please specify)

Other B — Other (please specify)

Other C — Other (please specify)

Other D — Other (please specify)

Other E — Other (please specify)

Appendix B

Reinforcement Sensitivity Theory Personality Questionnaire

Data collection 1

Personality Questionnaire

Below are a list of statements about everyday feelings and behaviours.

Please rate how accurately each statement describes you in general.

Select only one response per question.

Do not spend too much time thinking about the questions and please answer honestly. Your answers will remain confidential.

Make sure to answer all of the questions.

* 9. P1

	Not at all	Slightly	Moderately	Highly
I feel sad when I suffer even minor setbacks.	◯	◯	◯	◯
I am often preoccupied with unpleasant thoughts.	◯	◯	◯	◯
Sometimes even little things in life can give me great pleasure.	◯	◯	◯	◯
I am especially sensitive to reward.	◯	◯	◯	◯
I put in a big effort to accomplish important goals in my life.	◯	◯	◯	◯
I sometimes feel ‘blue’ for no good reason.	◯	◯	◯	◯
When feeling ‘down’, I tend to stay away from people.	◯	◯	◯	◯
I often experience a surge of pleasure running through my body.	◯	◯	◯	◯
I would be frozen to the spot by the sight of a snake or spider.	◯	◯	◯	◯

	Not at all	Slightly	Moderately	Highly
I have often spent a lot of time on my own to “get away from it all”.	◯	◯	◯	◯
I am a very active person.	◯	◯	◯	◯
I’m motivated to be successful in my personal life.	◯	◯	◯	◯
I am always ‘on the go’.	◯	◯	◯	◯
I regularly try new activities just to see if I enjoy them.	◯	◯	◯	◯
I get carried away by new projects.	◯	◯	◯	◯
Good news makes me feel over-joyed.	◯	◯	◯	◯
The thought of mistakes in my work worries me.	◯	◯	◯	◯
When nervous, I sometimes find my thoughts are interrupted.	◯	◯	◯	◯
I would run quickly if fire alarms in a shopping mall started ringing.	◯	◯	◯	◯
I often overcome hurdles to achieve my ambitions.	◯	◯	◯	◯

Data collection 1

Personality Questionnaire

* 10. P2

	Not at all	Slightly	Moderately	Highly
I often feel depressed.	○	○	○	○
I think I should 'stop and think' more instead of jumping into things too quickly.	○	○	○	○
I often feel that I am on an emotional 'high'.	○	○	○	○
I love winning competitions.	○	○	○	○
I get a special thrill when I am praised for something I've done well.	○	○	○	○
I take a great deal of interest in hobbies.	○	○	○	○
I sometimes cannot stop myself talking when I know I should keep my mouth closed.	○	○	○	○
I often do risky things without thinking of the consequences.	○	○	○	○
My mind is sometimes dominated by thoughts of the bad things I've done.	○	○	○	○
I get very excited when I get what I want.	○	○	○	○
I feel driven to succeed in my chosen career.	○	○	○	○
I'm always finding new and interesting things to do.	○	○	○	○
I'm always weighing-up the risk of bad things happening in my life.	○	○	○	○

	Not at all	Slightly	Moderately	Highly
People are often telling me not to worry.	○	○	○	○
I am very open to new experiences in life.	○	○	○	○
I always celebrate when I accomplish something important.	○	○	○	○
I find myself reacting strongly to pleasurable things in life.	○	○	○	○
I find myself doing things on the spur of the moment.	○	○	○	○
I would instantly freeze if I opened the door to find a stranger in the house.	○	○	○	○
I'm always buying things on impulse.	○	○	○	○
I am very persistent in achieving my goals.	○	○	○	○
When trying to make a decision, I find myself constantly chewing it over.	○	○	○	○
I often worry about letting down other people.	○	○	○	○
I would go on a holiday at the last minute.	○	○	○	○
I would run fast if I knew someone was following me late at night.	○	○	○	○
I would leave the park if I saw a group of dogs running around barking at people.	○	○	○	○
I worry a lot.	○	○	○	○
I would freeze if I was on a turbulent aircraft.	○	○	○	○

Data collection 1

Personality Questionnaire

* 11. P3

	Not at all	Slightly	Moderately	Highly
My behavior is easily interrupted.	◯	◯	◯	◯
It’s difficult to get some things out of my mind.	◯	◯	◯	◯
I think the best nights out are unplanned.	◯	◯	◯	◯
There are some things that I simply cannot go near.	◯	◯	◯	◯
If I see something I want, I act straight away.	◯	◯	◯	◯
I think it is necessary to make plans in order to get what you want in life.	◯	◯	◯	◯
When nervous, I find it hard to say the right words.	◯	◯	◯	◯
I find myself thinking about the same thing over and over again.	◯	◯	◯	◯
I often wake up with many thoughts running through my mind.	◯	◯	◯	◯
I would not hold a snake or spider.	◯	◯	◯	◯
Looking down from a great height makes me freeze.	◯	◯	◯	◯
I often find myself ‘going into my shell’.	◯	◯	◯	◯
My mind is dominated by recurring thoughts.	◯	◯	◯	◯
I am the sort of person who easily freezes-up when scared.	◯	◯	◯	◯
I take a long time to make decisions.	◯	◯	◯	◯

	Not at all	Slightly	Moderately	Highly
I often find myself lost for words.	◯	◯	◯	◯
I will actively put plans in place to accomplish goals in my life.	◯	◯	◯	◯

Appendix C

HRV analysis script

```r
setwd("/Users/HarryFisher/Downloads/ProjectR/phdthesis/data/hrv/complete/")
path <- "~/Downloads/ProjectR/phdthesis/data/hrv/complete/"
file.names <- dir(path, pattern = ".txt")

out.file <- "" # empty file for results
# setting loop to go through files
for (i in 1:length(file.names)) {
  rrTable <- read.table(file.names[i],
    sep = "",
    dec = ".",
    stringsAsFactors = FALSE
  )

  colnames(rrTable) <- c("time", "rr")

  # create HRV data analysis
  hrv.data <- CreateHRVData() %>%
    SetVerbose(FALSE) %>%
    LoadBeatVector(rrTable$time) %>%
    BuildNIHR()
  new.hrv <- Window(hrv.data, 180, 480) # extract 5 min segment
  originalbeat <- length(new.hrv$Beat$niHR)
  # adaptive threshold filter
  new.hrv <- FilterNIHR(new.hrv, long = 20, minbpm = 20, maxbpm = 140)
  acceptedbeat <- length(new.hrv$Beat$niHR)

  # time analysis not used in results
  new.hrv <- CreateTimeAnalysis(new.hrv, size = 60, interval = 7.8125) %>%
    # interpolate at 4hz using spline method
    InterpolateNIHR(method = "spline") %>%
    CreateFreqAnalysis() %>%
    CalculatePowerBand(indexFreqAnalysis = 1, size = 256, shift = 10) %>%
    # STFT
    CreateFreqAnalysis() %>%
    # FFT analysis
    CalculatePSD(1,
      method = "pgram", ULFmin = NULL, detrend = T,
      doPlot = FALSE
    )

  # collect results
  results <- tibble(
    id = as.numeric(data.frame(str_sub(file.names[i],
      start = 1,
      end = 3
    ),
    stringsAsFactors = F
    )),
    time = as.numeric(data.frame(str_sub(file.names[i],
      start = 6,
      end = 6
    ),
    stringsAsFactors = FALSE
    )),
    meanHR = mean(new.hrv[["Beat"]][["niHR"]]),
    # time doamin measures
```

```
    sdnn = new.hrv[["TimeAnalysis"]][[1]][["SDNN"]],
    rmssd = new.hrv[["TimeAnalysis"]][[1]][["rMSSD"]],
    HFmean = mean(new.hrv[["FreqAnalysis"]][[1]][["HF"]]),
    # STFT
    artifacts = (originalbeat - acceptedbeat) / originalbeat * 100
    # % of artifacts
  )
  out.file <- rbind.data.frame(out.file, results) ## tidying up
  out.file <- lapply(out.file, as.numeric) %>%
    data.frame() %>%
    drop_na() %>%
    round(digits = 2)
  rownames(out.file) <- seq(length = nrow(out.file))
}

write_csv(out.file, "~/Downloads/ProjectR/phdthesis/data/hrv/hrvdata.csv")
```

Appendix D
Additional tables from Study 1

Table D1
All variables included in the initial network structure.

Variable	Definition	State 1	State 2
clevel	Current competitive level	Club_university_county	National_international
gender	Gender of the participant	Female	Male
hours	Number of hours spent training per week	0-9 (Low)	>9-35 (High)
ind_team	Participate in an individual or team based sport	Individual	Team
pi	Previous injury - Whether an injury had been sustained in the previous 12 months prior to the study	No Injury	Injury
nlebase	Untransformed NLE at TP 1	0-13 (Low)	>13-93 (High)
FFFS	Fight-Flight-Freeze System	8-16 (Low)	>16-30 (High)
BIS	Behavioural Inhibition System	17-38 (Low)	>38-68 (High)
RI	Reward Interest	4-10 (Low)	>10-16 (High)
RR	Reward reactivity	8-21 (Low)	>21-31 (High)
I	Impulsivity	7-16 (Low)	>16-27 (High)
GDP	Goal drive persistence	7-22 (Low)	>22-28 (High)
stiffness	Sum of all stiffness locations	1543-2330 (Low)	>2330-4518 (High)
rmssd	Root mean squared difference of successive RR intervals	2.03-4.02 (Low)	>4.02-5.94 (High)
sdnn	Standard deviation of RR series	20.2-83.5 (Low)	>83.5-432.42 (High)
bal_asym	Percentage difference between left and right leg balance score	0-1 (Low)	>1-14 (High)
balance	Total balance score	5-15 (Low)	>15-46 (High)
nlelg_1	Log NLE at TP 1	0-2.64 (Low)	>2.64-4.54 (High)
nlelg_2	Log NLE at TP 2	0-3.04 (Low)	>3.04-5.19 (High)
nlelg_3	Log NLE at TP 3	0-3.18 (Low)	>3.18-4.79 (High)
tlelg_1	Log TLE at TP 1	1.79-3.4 (Low)	>3.4-4.88 (High)
tlelg_2	Log TLE at TP 2	1.79-3.74 (Low)	>3.74-5.42 (High)
tlelg_3	Log TLE at TP 3	1.79-3.81 (Low)	>3.81-5.18 (High)

Table D2

All arc strengths greater than 0.3 included in the network.

from	to	strength	direction
nlebase	nlelg_1	1.00	1.00
nlebase	nlelg_2	1.00	1.00
nlelg_1	injured_1	1.00	1.00
nlelg_2	injured_2	1.00	1.00
BIS_1	FFFS_1	0.99	0.63
FFFS_1	BIS_1	0.99	0.37
ind_team	hours	0.88	0.50
hours	ind_team	0.88	0.50
nlebase	RI_1	0.83	1.00
ind_team	nlebase	0.82	1.00
gender	stiffness_1	0.76	1.00
BIS_2	FFFS_2	0.72	0.72
FFFS_2	BIS_2	0.72	0.28
gender	stiffness_2	0.64	1.00
pi	stiffness_1	0.55	1.00
injured_1	stiffness_1	0.55	0.32
stiffness_1	injured_1	0.55	0.68
clevel	balance_1	0.52	1.00
FFFS_1	BIS_2	0.52	1.00
nlelg_1	BIS_1	0.51	0.98
BIS_1	nlelg_1	0.51	0.02
injured_2	stiffness_2	0.50	0.66
stiffness_2	injured_2	0.50	0.34
gender	FFFS_1	0.45	1.00
hours	injured_1	0.42	1.00
FFFS_1	injured_2	0.40	1.00
pi	nlelg_2	0.39	1.00
pi	clevel	0.38	0.50
clevel	pi	0.38	0.50
nlebase	rmssd_1	0.37	1.00
gender	FFFS_2	0.37	1.00
clevel	RI_2	0.37	1.00
RI_1	BIS_2	0.37	1.00
pi	ind_team	0.36	0.46
ind_team	pi	0.36	0.54
hours	rmssd_1	0.36	1.00
injured_1	balance_1	0.36	0.70
balance_1	injured_1	0.36	0.30
nlebase	RI_2	0.34	1.00

Table D2 continued

from	to	strength	direction
pi	stiffness_2	0.32	1.00
BIS_1	FFFS_2	0.32	1.00
RI_1	BIS_1	0.32	0.55
BIS_1	RI_1	0.32	0.45
rmssd_1	nlelg_2	0.31	1.00
injured_2	rmssd_2	0.30	0.82
injured_2	balance_2	0.30	0.97
rmssd_2	injured_2	0.30	0.18
balance_2	injured_2	0.30	0.03
ind_team	FFFS_1	0.30	1.00

Table D3

Probabilities of injury conditional on the variables in the Markov blanket for injured_1.

prob	hours	nlelg_1	stiffness_1	balance_1
0.51	High	Low	High	High
0.40	High	Low	High	Low
0.38	Low	High	High	High
0.32	High	High	High	High
0.28	Low	High	Low	High
0.28	High	High	Low	High
0.27	Low	High	High	Low
0.23	High	High	High	Low
0.19	Low	High	Low	Low
0.19	High	High	Low	Low
0.16	Low	Low	High	High
0.15	High	Low	Low	High
0.10	Low	Low	High	Low
0.10	High	Low	Low	Low
0.07	Low	Low	Low	High
0.04	Low	Low	Low	Low

Table D4

Probabilities of injury conditional on the variables in the Markov blanket for injured_2.

prob	FFFS_1	nlelg_2	stiffness_2	rmssd_2	balance_2
0.53	Low	Low	High	Low	High
0.45	Low	High	High	Low	High
0.41	Low	Low	High	High	High
0.39	Low	Low	High	Low	Low
0.34	Low	High	High	High	High
0.32	Low	High	High	Low	Low
0.31	Low	Low	Low	Low	High
0.29	Low	Low	High	High	Low
0.28	Low	High	Low	Low	High
0.25	High	Low	High	Low	High
0.23	Low	High	High	High	Low
0.22	Low	Low	Low	High	High
0.21	High	High	High	Low	High
0.20	Low	Low	Low	Low	Low
0.19	Low	High	Low	High	High
0.18	Low	High	Low	Low	Low
0.18	High	Low	High	High	High
0.16	High	Low	High	Low	Low
0.14	High	High	High	High	High
0.14	Low	Low	Low	High	Low
0.13	High	High	High	Low	Low
0.13	High	Low	Low	Low	High
0.12	Low	High	Low	High	Low
0.11	High	High	Low	Low	High
0.11	High	Low	High	High	Low
0.09	High	High	High	High	Low
0.08	High	Low	Low	High	High
0.08	High	High	Low	High	High
0.07	High	Low	Low	Low	Low
0.07	High	High	Low	Low	Low
0.05	High	Low	Low	High	Low
0.04	High	High	Low	High	Low

Appendix E
High intensity training session

Rugby

Session 1 (pre season conditioning)

Total time = 55mins

Warmup / rehab drills 15 mins (light jogging, strectching, mobility)

3 work stations:

- Bike - 10 seconds flat out sprint - Row machine travel 100m fast - Ski travel 100m fast

- 60 seconds to complete distance / time and move to next station, repeat each station three times in a set (two sets in total).

- 2 minutes recovery between rounds in a set.

- 10 minutes between sets.

- Double distance on second set (20 sec bike, 200m row and ski).

Session 2

Total time = 1:30

warm up 15 mins / jogging / passing

Core skills roations 3 x 6 mins

- Handling - Contact - tackle bags scrum etc - Defense blitz

Team attack and defence 12min each (high intensity) - practicing skills from previous section

Match play 15 minutes (high intensity)

Session 3

Repeat of session 2

Football

Session 1

Total time = 1:55

- Strength and conditioning and warm-up: 25mins

- Technical passing drill 20 minutes (5 x 4 minute blocks)

- Strength and conditioning exercises (10mins)

- Possession based activity multi-directional activity. 4 teams, 2 resting, 2 works (30mins, 4 minute blocks)

- 7 vs 7 small sided game

- High intensity sprints – 30 seconds each

Session 2

Total time = 1:45

- Warm up 25 mins

- Technique – passing drills 30s x 6 reps cone – cone

- 1v1 / 2v2 possession games - Groups of 6 2 min rotations

- 3v3 possesion + overload 2 x 2 mins

- 2 touch / 1 touch game

- 2 minute games (high intensity)

Session 2

Total time = 2:00

- Warm up combined with rondos (15-20 minutes). 3 rounds which are progressively more intense.

- A circuit of 3 passing drills (repeated twice, 15mins)

- Possession based exercise with three teams (repeated twice, 20mins)

- 6 box-to-box runs

- 11 vs 11 practice match – frequently intervened by the coaches (25/30mins)

Appendix F
Bayesian analysis in Study 2

The following code was used for the Bayesian analysis in Study 2. The data and code can be downloaded from https://github.com/HarryFisher1/phd-thesis.

```r
# data available at:
# https://github.com/HarryFisher1/phd-thesis
sem <- read_csv("data/study2_cleandata.csv")

## First model

options(mc.cores = parallel::detectCores())

prior1 <- c(
  set_prior("normal(0, 10)", class = "Intercept"),
  set_prior("normal(0,2.5)", class = "b"),
  set_prior("cauchy(0,1)", class = "sd")
)

fit1 <- brm(
  bf(
    days_missed ~ negsev_z + delta_new_z:sportg + stiffness_z + (1 | id),
    hu ~ negsev_z + delta_new_z:sportg + stiffness_z + (1 | id)
  ),
  prior = prior1,
  data = sem,
  family = hurdle_negbinomial(),
  control = list(adapt_delta = 0.99)
)

## Summary
summary(fit1)

## Clean table
tidy_stan(fit1)

## Interactive model checks
launch_shinystan(fit1)

x <- marginal_effects(fit1, dpar = "hu", probs = c(0.2, 0.8))
y <- marginal_effects(fit1, probs = c(0.2, 0.8))

## Second model

bf1 <- bf(delta_new_z ~ negsev_z + stiffness_z + (1 | ID | id))
bf2 <- bf(stiffness_z ~ negsev_z + (1 | ID | id))

prior2 <- c(
  set_prior("normal(0, 10)", class = "Intercept"),
  set_prior("normal(0,2.5)", class = "b")
)

fullmod <- bf1 + bf2 + set_rescor(FALSE)

fmod <- brm(
  fullmod,
  data = sem,
  control = list(adapt_delta = 0.99),
```

```
  iter = 3000,
  prior = prior2
)

# Posterior predictive check
pp <- pp_check(fmod, resp = "stiffnessz", nsamples = 100)
pp
```

Appendix G
Salemetrics cortisol assay procedure

Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is recommended for the 24 mL of Assay Diluent used in Step 5 (conjugate dilution) to come to room temperature.
- Bring Microtitre Plate to room temperature before use. ***It is important to keep the foil pouch with the plate strips closed until warmed to room temperature, as humidity may have an effect on the coated wells.***
- Prepare 1X wash buffer by diluting Wash Buffer Concentrate (10X) 10-fold with room-temperature deionized water (100 mL of Wash Buffer Concentrate (10X) to 900 mL of deionized H_2O). ***Dilute only enough for current day's use and discard any leftover reagent.*** (If precipitate has formed in the concentrated wash buffer, it may be heated to 40°C for 15 minutes. Cool to room temperature before use in assay.)

Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout. Here is a suggested layout. (Standards, controls, and saliva samples should be assayed in duplicate.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.000 Std	3.000 Std	Ctrl-H	Ctrl-H								
B	1.000 Std	1.000 Std	Ctrl-L	Ctrl-L								
C	0.333 Std	0.333 Std	SMP-1	SMP-1								
D	0.111 Std	0.111 Std	SMP-2	SMP-2								
E	0.037 Std	0.037 Std	SMP-3	SMP-3								
F	0.012 Std	0.012 Std	SMP-4	SMP-4								
G	Zero	Zero	SMP-5	SMP-5								
H	NSB*	NSB*	SMP-6	SMP-6								

*NSB = Non-specific binding wells. These may serve as blanks. Use is optional.

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSB wells included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Cautions: 1. Extra NSB wells should not be used for determination of standards, controls, or unknowns.
2. Do not insert wells from one plate into a different plate

Step 3: Pipette 24 mL of Assay Diluent into the disposable tube. (Scale down proportionally if using less than the entire plate.) Set aside for Step 5.

Step 4:

- Pipette 25 µL of standards, controls, and saliva samples into appropriate wells.
- Pipette 25 µL of Assay Diluent into 2 wells to serve as the zero.
- Pipette 25 µL of Assay Diluent into each NSB well.

Step 5: Dilute the Enzyme Conjugate 1:1600 by adding 15 µL of the conjugate to the 24 mL tube of Assay Diluent. (Scale down proportionally if not using the entire plate.) Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted conjugate solution and add 200 µL to each well using a multichannel pipette.

Step 6: Mix plate on a plate rotator for 5 minutes at 500 rpm and incubate at room temperature for a total of 1 hour.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Add 200 µL of TMB Substrate Solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm and incubate the plate in the dark (covered) at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of Stop Solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.

  Caution: Spillage may occur if mixing speed exceeds 600 rpm.
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. (For best results, a secondary filter correction at 490 to 492 nm is recommended.)

Quality Control

The Salimetrics' High and Low Cortisol Controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Subtract the average OD for the NSB wells (if used) from the OD of the zero, standards, controls, and saliva samples.
3. Calculate the percent bound (B/Bo) for each standard, control, and saliva sample by dividing the OD of each well (B) by the average OD for the zero (Bo). (The zero is not a point on the standard curve.)
4. Determine the concentrations of the controls and saliva samples by interpolation using data reduction software. We recommend using a 4-parameter non-linear regression curve fit.
5. Samples with Cortisol values greater than 3.0 µg/dL (82.77 nmol/L) should be diluted with Assay Diluent and rerun for accurate results. If a dilution of the sample is used, multiply the assay results by the dilution factor.

A new Standard Curve must be run with each full or partial plate.