FACTORS INFLUENCING UPPER RESPIRATORY TRACT SYMPTOM RISK IN ELITE TEAM-SPORT ATHLETES

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ABSTRACT

The occurrence of illness, particularly upper respiratory tract symptom (URTS) episodes, is common in elite team-sport athletes. Recurrent or severe URTS is known to impair performance; despite this, research in elite team-sport athletes is lacking, with most exercise immunology studies focusing on endurance athletes. Therefore, this thesis used a multifaceted/holistic approach to investigate which factors (if any) meaningfully influence URTS risk in elite team-sport athletes during real-life training and competition periods.

A series of four related studies were conducted involving three groups of elite team-sport athletes; namely, rugby union, rugby league and field hockey players. In the first study (Chapter 5), URTS incidence and possible predictors of URTS were compared between elite rugby union and league players during an intensive pre-season training period. URTS incidence was similar between the rugby codes; however, predictors of URTS risk differed. Strongest predictors of URTS risk were reduced salivary secretory immunoglobulin (SIgA) (Hazard ratio (HR): 0.997, p = 0.094) in rugby union players, and decreased total wellness (HR: 0.731, p = 0.004) and sleep quality (HR: 0.345, p = 0.001) in rugby league players. These findings suggest that factors influencing URTS risk are perhaps sport and/or cohort specific.

The second study (Chapter 6) examined potential factors influencing URTS risk in elite rugby union players during an entire sporting season. Rest weeks were identified as periods of increased risk for URTS; whereas URTS risk was reduced during weeks involving international travel. Household illness incidence was found to be the strongest predictor of URTS risk (HR: 2.902, p = 0.002), and a trend for an inverse association between SIgA concentration and URTS incidence was also observed (HR: 0.998, p =

0.070). These findings suggest that self-reported household illness can be measured in surrogate of SIgA to predict URTS risk.

In the third study (Chapter 7), mucosal immunoendocrine, self-reported wellness, hydration and URTS data were repeatedly measured in elite field hockey players during a 3-week overseas tour, involving a congested competition period, long-haul travel, extreme environmental conditions and hypohydration. Despite multi-stressor exposure, all measures remained stable and only one player experienced an URTS episode. The low URTS incidence may be explained by the unique traits of elite athletes' immune systems, and/or the practitioners' management of tour stressors to prevent URTS.

Finally, in the fourth study (Chapter 8), factors influencing URTS risk were examined in elite field hockey players during an 8-week training and competition period that simulated the expected preparatory and competition phases of the 2020 Tokyo Olympics. Illness in players' households (HR: 4.9; $p < 0.001$) and increased self-reported stress (HR: 0.63; $p = 0.043$) predicted greater URTS risk. Additionally, low baseline resting SIgA concentration predicted players 'potential' URTS risk ($p = 0.021$). It is therefore recommended that practitioners screen SIgA and regularly monitor self-reported lifestyle and behavioural data to predict URTS risk in athletes.

In conclusion, the findings add to the body of knowledge with regards to factors influencing URTS risk in elite team-sport athletes. Specifically, lifestyle and behavioural factors outside of the team environment may influence URTS risk to a greater extent than sport-related stressors. Non-biological self-reported data were also found to be more effective than biomarkers in predicting team-sport athletes URTS risk. Therefore, monitoring of self-reported data, specifically household illness incidence and wellness indicators (i.e., sleep quality and stress), offer a practical and accessible method that team-sport practitioners can use in surrogate of biomarkers to identify elite athletes' risk for URTS.

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ATTESTATION OF AUTHORSHIP

“I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of university or institution of higher learning.”

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PUBLICATIONS AND PRESENTATIONS

Publications

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ETHICS APPROVAL

Ethical approval for all research within this thesis was obtained through the Auckland University of Technology's Ethic Committee (AUTEC). Each study and corresponding AUTEC Ethics Approval Number is outlined below.

- Studies 1 (Chapter 5) and 2 (Chapter 6): Ethics approval number 16/319
- Studies 3 (Chapter 7) and 4 (Chapter 8): Ethics approval number 18/196

LIST OF COMMON ABBREVIATIONS

AB	Arbitrary unit
ANOVA	Analysis of variance
AQUA	Allergy Questionnaire for Athletes
CD4+	T-helper cells
CD8+	T-cytotoxic cells
CHO	Carbohydrate
CMV	Cytomegalovirus
CV	Coefficient of variation
°C	Degrees celsius
DASS-21	Depression, anxiety, stress scale
EBV	Epstein Barr Virus
ELISA	Enzyme-linked immunosorbent assay
HA	Heat acclimation
HR	Hazard ratio
HRT	Heat response testing
ICP	Intensified competition period
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
IOC	International Olympic Committee
K_2EDTA	Dipotassium ethylenediamine tetra-acetic acid
LEA	Low energy availability
NFO	Non-functional overreaching
NT	Normal training
OTS	Overtraining syndrome
pIgR	Polymeric immunoglobulin receptor
Rest-Q-Sport-52	Recovery stress questionnaire

RH	Relative humidity
RPE	Rate of perceived exertion
SIgA	Salivary secretory immunoglobulin A
Treg cells	Regulatory T-cells
T_{re}	Rectal temperature
T_{sk}	Skin temperature
URTS	Upper respiratory tract symptoms
USG	Urine specific gravity
UV-B	Ultraviolet B exposure
WBC	White blood cell counts
WBGT	Wet bulb globe temperature
(Δ%)	Percentage variation

CHAPTER 1 INTRODUCTION

1.1 Theoretical background

Exercise can influence immunity and susceptibility to upper respiratory tract symptom (URTS) episodes (Nieman & Wentz, 2019). It is generally accepted that regular bouts of short-lasting (i.e., ≤ 45 min) moderate intensity exercise improve immune functions and lower URTS risk (Matthews et al., 2002; Nieman, 2011; Nieman & Wentz, 2019). However, repeated bouts of intense (> 70% VO_2 peak) (Nieman, 1997) or prolonged (≥ 90 min) (Diment et al., 2015; Nieman, 1997) exercise can be immunosuppressive, resulting in an increased risk of URTS (Nieman & Wentz, 2019; Walsh, Gleeson, Shephard, et al., 2011). As such, the relationship between exercise and susceptibility to upper respiratory tract infection has been modelled in the form of a J-shaped curve, whereby moderate intensity exercise decreases the relative risk of infection, below that of a sedentary individual, but high intensity/volume exercise is associated with an above average risk of infection (Nieman, 1994).

In accordance with the J-shaped curve hypothesis, athletes who regularly undertake high intensity/volume exercise may be more susceptible to URTS than their less active counterparts. However, the J-shaped curve is not always found to hold true, particularly in elite athletes (Schwellnus et al., 2016). In fact, during intensive training and competition periods, elite athletes have been found to experience a lower incidence of URTS episodes than sub-elite athletes (Hellard et al., 2015; Raysmith & Drew, 2016). These findings support the S-shaped curve hypothesis (Figure 1.1) which suggests that elite athletes are less URTS-prone as they possess or develop immune systems capable of withstanding infections even during severe psychophysiological stress (Malm, 2006). However, lower URTS incidence in elite athletes may also be explained by better

management and use of illness prevention strategies in elite settings (Williams et al., 2018).

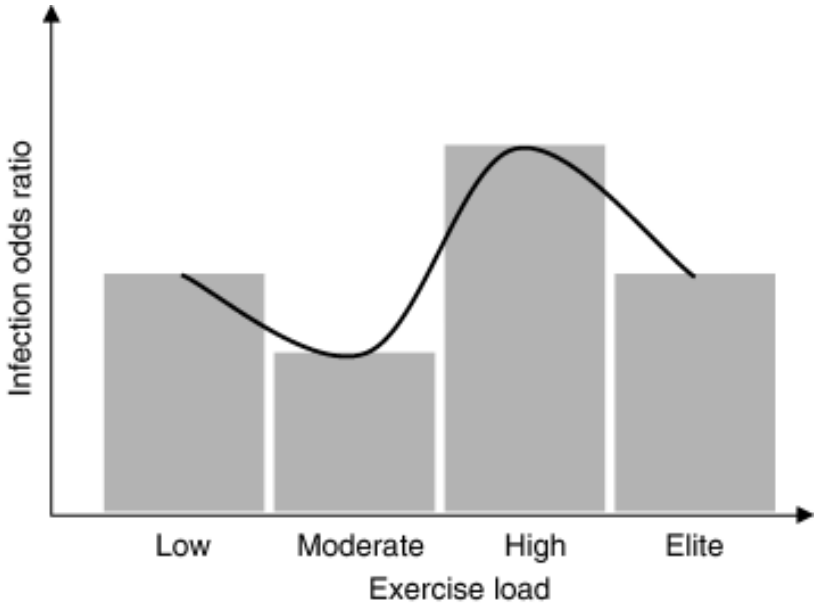

Figure 1.1 S-shaped relationship between training status and infection rate. Adapted from (Malm, 2006)).

URTS are the most common form of illness experienced by elite athletes; with severe and recurrent URTS episodes known to detract from athletes training availability, performance and success (Gleeson & Pyne, 2016). However, the majority of research conducted to establish current understanding of URTS risk and incidence in athletes, including J-shaped (Nieman, 1994) and S-shaped (Malm, 2006) hypotheses, has been completed in endurance athletes, with limited consideration of team-sport athletes (Schwellnus et al., 2016). Team-sport athletes are an important population to investigate as they are exposed to a greater range of physical stressors in training and competition, compared to endurance athletes. Firstly, while endurance exercise is prolonged and continuous, team-sport exercise is intermittent and multi-directional, often with short bouts of anaerobic activity (<5 sec) (e.g., sprinting and jumping), interspersed with lower intensity activities (e.g., walking, jogging and backwards running) (Taylor et al., 2017). Secondly, some team-sports such as rugby union, also involve significant physical

contact including whole-body tackles, scrummaging and rucking for possession of the ball (Gabbett et al., 2011; Hendricks et al., 2012; Taylor et al., 2017). Thirdly, competition format also differs whereby team-sport athletes often compete in congested competition schedules (e.g., tournaments), where recovery time between matches is limited (Vescovi & Watson, 2019), compared to endurance athletes who tend to compete in one-off events (e.g., marathon, ironman). Finally, the team-sport environment itself presents unique practices and demands including bottle sharing, shaking hands and close-proximity to team-mates and opponents. As such, it is possible that factors influencing URTS risk differ between team- and endurance-sport athletes.

URTS susceptibility in athletes is suggested to have a multifactorial underpinning in which exercise stress and additional stressors (i.e., stress, sleep, nutrition, etc.) directly impact or contribute to impaired immunity and URTS risk (Simpson et al., 2020; Walsh, 2018). In elite team-sport athletes, research has focused on the exercise component, with increased risk for immunosuppression and URTS demonstrated with intensified training blocks (e.g., pre-season training, training camps) and congested competition schedules (Cunniffe et al., 2011; Moreira et al., 2014). However, additional stressors that team-sport athletes are exposed to such as long-haul travel (Duffield & Fowler, 2017), environmental extremes (Pryor et al., 2019), psychological stress (Drew et al., 2017), sleep deprivation (Fullagar, Duffield, et al., 2015) and inadequate nutrition (Drew et al., 2017; Kampouri et al., 2019) have often not been accounted and/or controlled for in previous studies. Consequently, it is unclear how these additional stressors affect immunity and risk for URTS in elite team-sport athletes.

To evaluate athletes' susceptibility for URTS episodes, exercise immunology research has sought to identify which aspects of the immune system are responsible for increased URTS risk (Gleeson & Bishop, 2013). Baseline measurement of salivary secretory immunoglobulin A (SIgA), multi-antigen stimulated cytokine responses and cytomegalovirus (CMV) have been found to predict endurance athletes potential URTS

risk (Gleeson et al., 2012; He, Handzlik, Muhamad, et al., 2013). In team-sport athletes, SIgA has received the most attention (Jones et al., 2016); however, discrepant findings have been reported, with an inverse- (Fahlman & Engels, 2005; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012; Tiernan et al., 2020; Yamauchi et al., 2011b) or no- (Cunniffe et al., 2011; Moraes et al., 2017; Moreira et al., 2008; Morgans et al., 2014) association found between SIgA and URTS incidence. Moreover, the relationship between multi-antigen stimulated cytokine responses, CMV and URTS has not yet been explored in elite team-sport athletes. Nevertheless, it is acknowledged that measurement of biomarkers may be limited in an elite team-sport setting because they can be invasive, expensive and require laboratory expertise. As recommended by Thorpe and colleagues, team-sport monitoring tools need to be simple, cheap, non-invasive and quick to administer (Thorpe et al., 2017), and therefore further investigation of measures that meet these criteria is warranted.

Non-biological self-reported data offers a more accessible method than biomarkers to assess athletes risk for URTS. In team-sport athletes, a positive association between internal training load and URTS incidence has been demonstrated (Putlur et al., 2004; Thornton et al., 2016; Watson et al., 2016). Additionally, changes in self-reported wellness indicators, namely muscle soreness, wellbeing (Thornton et al., 2016), stress (Drew et al., 2017; Hamlin et al., 2019) and sleep quantity (Fitzgerald et al., 2019), have been linked to URTS incidence in team-sport athletes. In contrast, previous team-sport studies have found no relationship between URTS risk and internal training load (Anderson et al., 2003; Fitzgerald et al., 2019) or self-reported wellness indicators (Ahmun et al., 2019; Watson et al., 2017). Therefore, the efficacy of these self-reported measures in predicting URTS risk remains inconclusive. Self-reported household illness is another measure that may have the potential to predict athletes URTS risk. Household illness has been extensively examined in the general population, with close-contact between household members known to facilitate respiratory virus transmission (Tsang et al., 2016). However, its impact on URTS incidence in athletic populations remains

unknown. Given self-reported measures offer a more convenient, instantaneous and cost-effective approach than biomarker monitoring, further research is required to better elucidate their effectiveness in predicting elite team-sport athletes' URTS risk. Finally, an important limitation associated with the elite team-sport literature to date, is that researchers have tended to examine either biomarker (Cunniffe et al., 2011; Tiernan et al., 2020) or self-reported (Drew et al., 2017; Thornton et al., 2016) measures as possible predictors of URTS risk, but not concurrently. Therefore, it is currently unclear whether one approach is more effective than the other.

1.2 Purpose statement and significance of research

Existing exercise immunology studies have predominately focused on endurance athletes, as endurance exercise provides a simple model to understand the effects of acute and chronic exercise on URTS risk. Research on team-sport athletes is comparatively lacking with many unanswered research questions. Elite team-sport athletes are an important yet complex population to examine as they are exposed to multiple physical demands and different training and competition stressors compared to most endurance athletes. Given the substantial physical, personal, and economic investment that is required to optimally prepare elite team-sport athletes for competition, a real demand exists to broaden and develop further understanding of URTS risk in this population. This thesis therefore set out to take a multifaceted/holistic approach to better understand the complex and often interrelating factors that may impact upon team-sport athletes risk for URTS episodes. As such, the main aim of the thesis was to investigate which factors (if any) meaningfully influence URTS risk in elite team-sport athletes during real-life training and competition periods. This information could enable team-sport practitioners to target illness prevention strategies to maintain athlete health and optimise performance.

The following research questions underpinned the thesis aim and focus of each study/chapter:

- Are elite team-sport athletes at greater risk of URTS during specific periods of training and/or competition? (Chapters 5, 6, 7 and 8)
- Is URTS risk in elite team-sport athletes influenced by exposure to sport-related stressors, specifically:
    - Intensive training? (Chapters 5 and 6)
    - Congested competition? (Chapters 7 and 8)
    - Long-haul travel? (Chapters 6 and 8)
    - Hot and humid environmental conditions? (Chapters 7 and 8)
- Do sport-related stressors influence mucosal immunoendocrine responses? (Chapters 5, 6 and 7)
- Can biomarkers and/or self-reported measures 'predict' URTS risk in elite team-sport athletes? (Chapters 5, 6 and 8)
- Do factors influencing URTS risk differ between elite sporting teams, namely rugby union and league? (Chapter 5)

1.3 Thesis organisation

This thesis is composed of nine chapters within three primary sections (Figure 1.2). A summary of each chapter is provided below.

The literature review of this thesis (Chapter 2) examined URTS in team-sport athletes. First, the effect of stressors (i.e., exercise, international travel, extreme environmental conditions, international travel, lifestyle and behaviour factors and the team environment) on URTS risk is described. Second, the potential for biomarkers and/or self-reported measures to predict team-sport athletes' risk for URTS is explored. Based on the current state of the literature, factors influencing URTS risk in elite team-sport athletes have not

been thoroughly understood. Therefore, subsequent chapters of this thesis fill the gaps in the literature to offer a way forward for researchers and team-sport practitioners alike.

The next section of the thesis (Chapter 3) is a short review that was published in the *Journal Frontiers in Physiology* special issue titled *'Towards Tokyo 2020: What will contribute to Optimal Olympic athlete performance?'*. The Olympic and Paralympic games are a pinnacle event for many elite sporting teams (e.g., basketball, rugby, football etc.). As such, this short review identified risk factors for URTS in Olympic and Paralympic athletes and described which athletes may be more susceptible to URTS at the Summer Games, along with outlining suggested strategies to maintain and protect athlete health.

Chapter 4 outlines the general methodology that was used in the four original studies of the thesis (Chapters 5-8). The original studies examined several stressors and measures in a range of settings (i.e., different team sports and periods of the season) in an attempt to better elucidate factors influencing URTS risk in elite team-sport athletes during real-life multi-stressor training and competition periods.

The final chapter of the thesis (Chapter 9) provides a summary of the main findings and discusses the results of the thesis as a cohesive whole under key themes: 1) URTS incidence in elite team-sport athletes; 2) periods of increased risk/risk factors for URTS; 3) predictors of URTS risk; and 4) strategies to minimise team-sport athletes risk for URTS. This chapter then describes research limitations, directions for future research and practical recommendations.

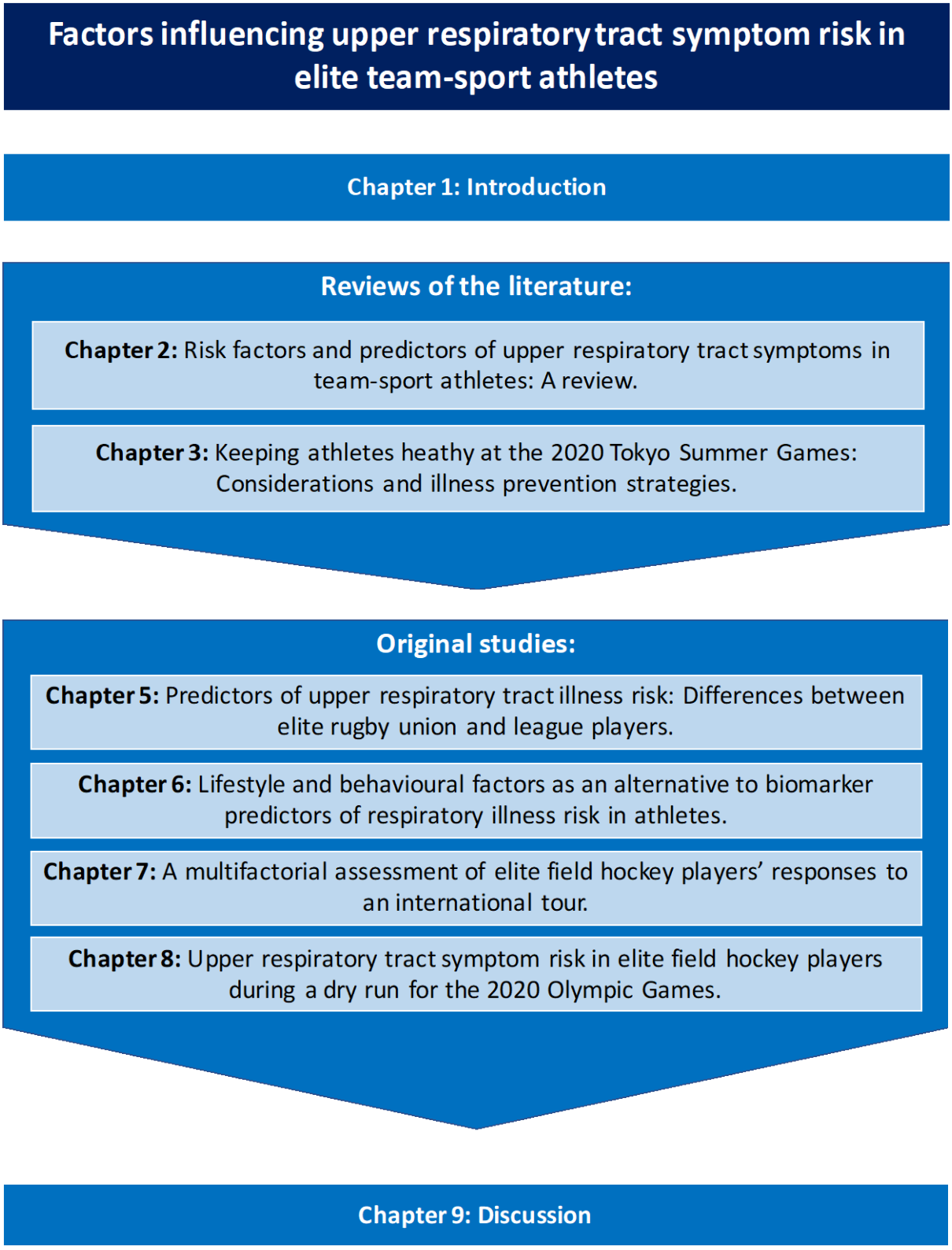

Figure 1.2 Overview of the thesis structure.

CHAPTER 2 RISK FACTORS AND PREDICTORS OF UPPER RESPIRATORY TRACT SYMPTOMS IN TEAM-SPORT ATHLETES: A REVIEW.

2.1 Introduction

URTS episodes are the most common non-injury related medical condition in elite athletes, accounting for 35-65% of all illness presentations (Fricker, 1997). The prevention of URTS is a key objective in elite athlete management, given recurrent or severe URTS episodes can detract from athletes' training availability and competition performance (Gleeson & Pyne, 2016; Hellard et al., 2015). URTS risk in athletes is suggested to be multifactorial, with stressors of intensified training- and competition-periods, long-haul travel, increased psychological stress, sleep deprivation and inadequate nutrition causing or contributing to increased risk for URTS episodes (Schwellnus et al., 2016; Simpson et al., 2020; Spence et al., 2007; Walsh, 2018). To assess athletes' susceptibility to URTS, exercise immunology research has sought to identify biomarker and self-reported data that can be measured to predict URTS risk.

However, the majority of research used to establish current understanding of factors influencing URTS risk has been completed in endurance-sport athletes, with relatively fewer studies conducted in team-sport athletes. As previously discussed in Chapter 1, demands and stressors differ between endurance-sports and team-sports; therefore, findings cannot necessarily be extrapolated. Elite team-sport athletes are an important population to examine because they are continually exposed to stressors that have the potential to increase URTS risk. Additionally, the team-sport environment itself presents unique demands and practices (e.g., close proximity to teammates and opponents, shaking hands, bottle sharing, contact sports) that can increase exposure to infection-

causing pathogens (Mela & Whitworth, 2014; White & Grant-Kels, 1984). It is, therefore, possible that factors influencing URTS risk in team-sport athletes differ to those found in endurance-sport athletes. As such, the purpose of this review was twofold: part 1) to describe how stressors impact upon team-sport athletes' URTS risk; and part 2) to explore whether biomarkers and/or self-reported measures have the potential to predict team-sport athletes' risk for URTS.

URTS in athletes

At least one acute illness is experienced by ~90% of athletes during a sporting season (Cunniffe et al., 2011; Fricker et al., 2000). In elite athletes, most illnesses have been found to affect the respiratory system (41-47%), followed by the gastrointestinal system (16-21%) and the skin and subcutaneous systems (9-11%) (Engebretsen et al., 2013; Soligard et al., 2017). Given the high prevalence of respiratory illness, exercise immunology research has focused on URTS episodes. Common URTS include sore throat, headache, fatigue, runny or blocked nose, repetitive sneezing and coughing (Fricker et al., 2005). Early epidemiologic evidence suggested that athletes experience more URTS episodes than the general population (Heath et al., 1991; Peters, 1983); however, this has been disputed by more recent studies reporting a similar distribution of URTS episodes between athletic and general populations (Fondell et al., 2011; Fricker et al., 2000; Hellard et al., 2015). Nevertheless, the association between URTS incidence and seasonal variation does not always hold true in elite athletes; rather, URTS episodes tend to cluster around sport-related stressors (e.g., intensified training periods, long-haul travel) (Gleeson & Pyne, 2016).

In athletic populations, infectious URTS episodes are predominantly caused by viruses (~35-65%), including rhinovirus and reactivation of herpes viruses such as CMV and Epstein Barr Virus (EBV) (Gleeson & Pyne, 2016). Whereas, bacterial and fungal infections only account for a small proportion (~1-5%) of athletes URTS episodes (Gleeson & Pyne, 2016). More recently, however, is has been acknowledged that URTS

episodes are not all infectious in nature; URTS can also result from non-infectious and inflammatory causes, including allergic responses to aeroallergens, asthma and respiratory epithelial membrane damage (Gleeson & Pyne, 2016). Undiagnosed or inappropriately treated allergies (~20-40%) and asthma (~10-20%) account for most non-infectious and inflammatory URTS presentations in athletes (Gleeson & Pyne, 2016). The aetiology of URTS is rarely determined in athletes as pathology testing is expensive and logistically difficult. Previous studies have tended to use self-reported illness data to establish URTS episodes (Cox et al., 2008). However, due to the limited number of pathology investigations, the proportion of infectious- versus non-infectious inflammatory caused URTS episodes remain uncertain, with 30% (Spence et al., 2007), 57% (Cox et al., 2008) and 75% (Valtonen et al., 2019) of URTS episodes found to have an infectious aetiology in elite athletes.

Irrespective of the cause, URTS episodes can have detrimental effects on athletic performance (Schwellnus et al., 2016). In a three year prospective cohort study in 322 Olympic athletes, ~30% of illnesses (most commonly URTS) resulted in training modification (e.g., reduced volume and/or intensity of training), while ~70% of illnesses (most commonly URTS) caused athletes to miss training and/or competition (Palmer-Green et al., 2013). Training interruptions due to illness (and/or injury) have been associated with a significantly lower chance of achieving pre-defined performance goals in elite track and field athletes (Raysmith & Drew, 2016). Similarly, more successful elite endurance athletes (i.e., athletes who had won world championship and/or Olympic medals) have been found to experience a lower incidence of URTS episodes than less successful elite athletes (Svendsen et al., 2016). As such, maintaining and protecting athletes' health is paramount, given URTS can disrupt athletes' training and competition availability, and therefore sporting success.

2.2 Part one: Risk factors for URTS in team-sport athletes

Modern day elite team-sport athletes are being increasingly exposed to a variety of stressors, as summarised in Figure 2.1. Given the substantial investment that goes into preparing team-sport athletes for competition, a real demand exists to identify periods of increased risk/risk factors for URTS. In the next section of this review stressors will be separated for clarity, although it is appreciated that some stressors are inherently combined.

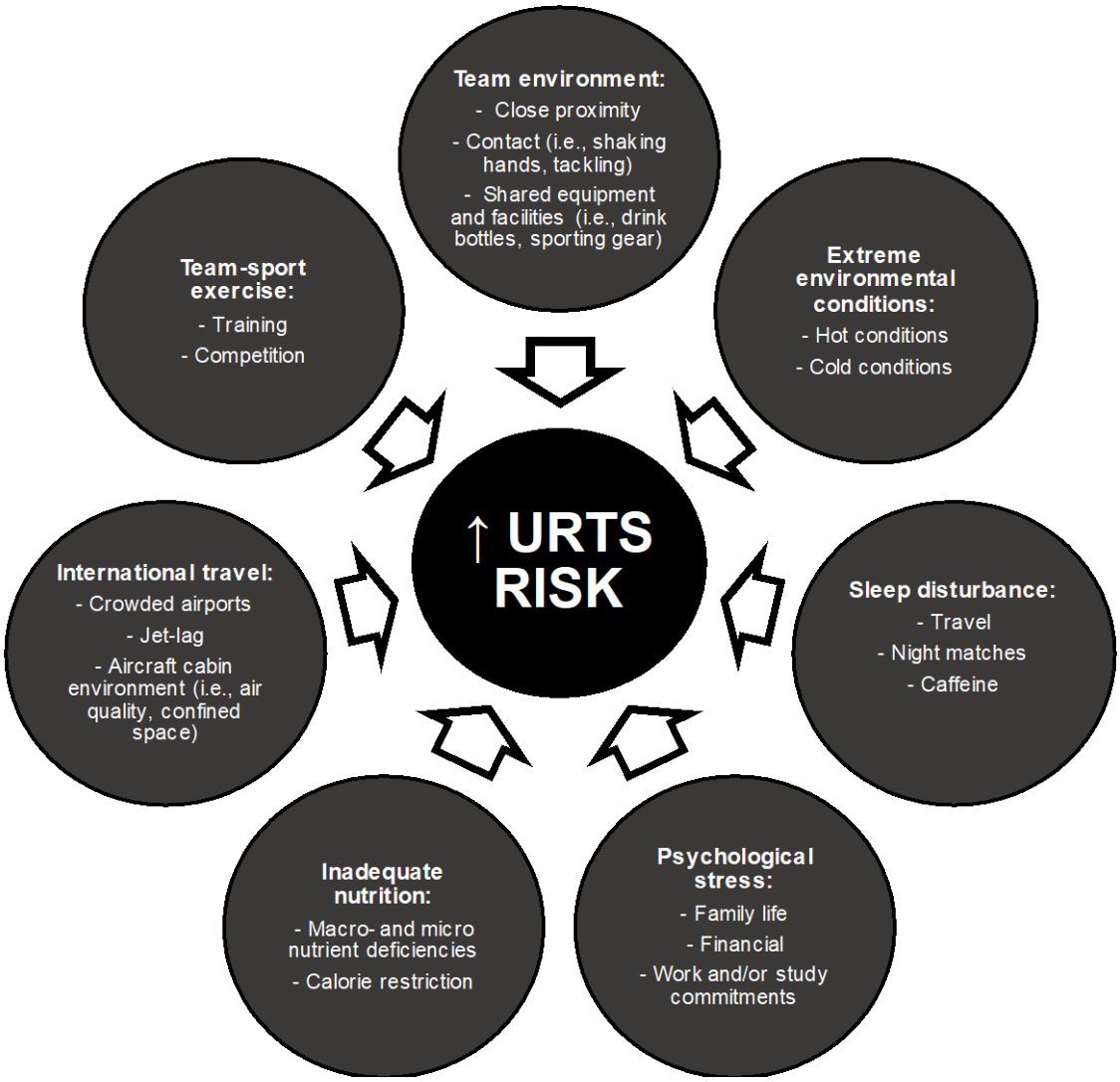

Figure 2.1 Team-sport stressors that have the potential to increase risk for URTS.

Team-sport exercise

Training

Increased risk for URTS has been observed in team-sport athletes during pre-season training and intensified training periods, which are typically characterised by high training loads over a specified amount of time (weeks-months). In elite rugby union and youth soccer players, a greater number of URTS episodes were observed during intensive pre-season training compared to off-season and recovery periods (Cunniffe et al., 2011; Moreira et al., 2014). Moreover, at an amateur level, intensive pre-season football training has been shown to modulate URTS risk, regardless of the time of year. For example, in comparison to the off-season where only 5% of players experienced URTS, 32% and 56% of the players reported URTS during autumn and spring pre-season training periods, respectively (Fahlman & Engels, 2005). In support of these findings, several studies have demonstrated a positive association between training load and URTS incidence in team-sport athletes (Putlur et al., 2004; Thornton et al., 2016; Watson et al., 2017). However, URTS risk is not always increased with intensified training periods, with other studies reporting no association between training load and URTS incidence in elite athletes (Fitzgerald et al., 2019; Hellard et al., 2015; Svendsen et al., 2016). Therefore, as outlined in a systematic review, the evidence in athletes pertaining to training load and URTS incidence is conflicting (Drew & Finch, 2016).

Competition

Competition periods have been found to increase URTS risk in team-sport athletes (Cunniffe et al., 2011; Fahlman & Engels, 2005; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012). In American college football players, the highest incidence of URTS was observed during competition periods, compared with recovery and pre-season training periods (Fahlman & Engels, 2005). Similarly, in elite rugby union players, URTS incidence was doubled during the competition period versus the pre-season training period (30 vs 15 URTS episodes) (Thornton et al., 2016). These findings suggest competition may be a more prominent risk factor for URTS than pre-

season training. During competition, risk for URTS appears to be exacerbated when fixtures are congested, and recovery time is limited (Cunniffe et al., 2011). Indeed, in elite rugby union players, significantly higher URTS rates were observed during an intensified competition period (nine games in eight weeks) compared with a less competitive period (two games in four weeks) (Cunniffe et al., 2011). In support of these findings, heightened URTS incidence with a congested competition schedule has been demonstrated in sub-elite footballers, with 35% of players reporting URTS episodes over a 20 day period involving 7 matches (Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012). In contrast, no URTS episodes were reported in English premier league footballers during a less congested competition period involving 7 matches over 30 days (Morgans et al., 2014). As such, these findings suggest that team-sport athletes' health may be maintained when competition periods are less congested.

International travel

Along with exercise stress, the requirement for long-haul travel is common for elite team-sport athletes. International travel is associated with a range of challenges that may increase URTS risk. For example, confined space, close proximity to others, limited ventilation and recirculating dry air—all common in an aircraft cabin—can increase risk for infection transmission (Pipe, 2011). Jet-lag and disruption to sleep following long haul travel may also heighten URTS risk (Walsh, 2018). In the general population, increased URTS incidence has been demonstrated following air travel, with approximately 20% of individuals found to experience URTS within one week of flying (Zitter et al., 2002). Furthermore, increased URTS susceptibility has been observed in flight attendants compared with the general population (Whelan et al., 2003). In support, international travel has been identified as a risk factor for URTS in athletes (Walsh, 2018). Research in elite rugby players found that changing time zones was associated with increased illness incidence, not the direction of travel or flying per se, whereby travelling to international destinations that were more than a 5 hour time zone difference away from a player's home country were associated with a 2–3 fold increased risk of illness

(Schwellnus et al., 2012). In support, long haul travel across 11 times zones was found to exacerbate URTS in elite rugby league players (Fowler et al., 2016). Similar findings have also been reported in elite endurance athletes, with cross country skiers shown to be five times more likely to experience URTS within the 2-3 days following international travel (Svendsen et al., 2016). However, more work is needed, given research on this topic is still in its infancy.

Environmental extremes

Elite team-sport athletes are often required to train and compete in challenging environmental conditions including extreme heat, humidity and cold. Exercising in such conditions imposes greater physiological strain than thermoneutral conditions (Walsh & Whitham, 2006); although, exercise in hot (air: 28–38.7 °C, 45–76% relative humidity (RH)) and cold (water: 18–23 °C or air: −6.4–5 °C) environments does not appear to cause further immunosuppression than exercise in a thermoneutral environment (air: 18–22 °C, 30–60% RH or water: 35 °C) (Laing, Gwynne, et al., 2005; Mylona et al., 2002; Walsh et al., 2002; Walsh & Whitham, 2006). However, there are several factors that limit the applicability of these study findings to elite team-sport athletes. Firstly, most studies used aerobic steady state exercise protocols (Laing, Gwynne, et al., 2005; Mylona et al., 2002; Walsh et al., 2002), with only one study to date examining athletes' immune responses to team-sport specific intermittent exercise in the heat (Sari-Sarraf et al., 2011). Secondly, these studies all examined a one-off bout of exercise in extreme environmental conditions (Laing, Gwynne, et al., 2005; Mylona et al., 2002; Sari-Sarraf et al., 2011; Walsh et al., 2002). In reality, elite team-sport athletes are often subjected to multiple hot or cold exposures; for example, during heat acclimation (HA) and multi-day competitions (i.e., tournaments). Finally, URTS could not be examined in pervious acute laboratory studies (Laing, Gwynne, et al., 2005; Mylona et al., 2002; Walsh et al., 2002; Walsh & Whitham, 2006). To summarise, as many major team-sport events are scheduled in potentially adverse environments, more research is needed to determine

whether acute and/or repeated exposure to exercise in extreme environmental conditions is a risk factor for URTS.

Lifestyle and behaviour factors

To date, exercise immunology studies have focused on sport-related stressors (as discussed above). However, team-sport athletes are also frequently exposed to lifestyle and behavioural factors that have the potential to influence URTS risk, including psychological stress, sleep disruption and inadequate nutrition.

Psychological stress

Elite team-sport athletes are regularly exposed to competitive stressors (e.g., pressure to perform, issues with form, injury etc.) and personal stressors (e.g., financial issues, traumatic life events, work and/or study commitments etc.) that can increase psychological stress (McKay et al., 2008). It is well established that psychological stress influences illness susceptibility (Pedersen et al., 2010). In the general population, rates of URTS episodes have been found to increase in a dose-response manner with increases in the degree of psychological stress (Cohen et al., 1991). In support, a positive association between perceived stress and illness incidence has been reported in Australian Olympic athletes (Drew et al., 2018; Drew et al., 2017), elite youth soccer players (Brink et al., 2010) and university athletes (Hamlin et al., 2019). However, more work in elite team-sport athletes is needed as there is a paucity of research investigating the impact of psychological stress on URTS risk in this population.

Sleep disturbance

Elite team-sport athletes are also frequently exposed to situations and conditions that can disturb sleep quantity and quality, including: international travel, caffeine consumption, night matches and morning trainings (Fullagar, Duffield, et al., 2015). Sleep can influence URTS susceptibility; landmark general population research demonstrated a threefold increased risk of developing a common cold in individuals who

slept less than seven hours per night compared to those who slept over eight hours per night (Cohen et al., 2009). In support, an association between reduced sleep quality and/or quantity and increased risk for URTS episodes has been demonstrated in highly trained triathletes (Hausswirth et al., 2014) and professional Australian footballers (Fitzgerald et al., 2019). However, given research is still in its infancy, more work is needed to fully elucidate the effect of sleep on URTS risk in elite team-sport athletes.

Inadequate nutrition

Inadequate nutrition is another lifestyle and behavioural factor that can increase risk for URTS in athletes, as it is well established that energy restriction and deficiencies of macro- and micro- nutrients impair immunity (Bermon et al., 2017). Team-sport athletes may be at an increased risk for nutritional deficiencies when purposefully calorie restricting. Such dietary behaviours typically occur during pre-season periods as athletes seek to improve body composition (Argus et al., 2010). Calorie restriction, whether it be purposeful or inadvertent, will likely increase team-sport athletes' risk for illness, given low energy availability has been associated with increased incidence of illness in Australian Olympic athletes (Drew et al., 2018; Drew et al., 2017). However, due to the limited number of studies, more work is needed to understand the impact of elite team-sport athletes' nutritional practices on URTS risk.

Team-sport environment

The team-sport environment and practices including close proximity to teammates (e.g., team-huddles, small changing rooms etc.), shared use of equipment (e.g., drink bottles, towels, sporting gear and gym equipment) and shaking hands with the opposition can facilitate transmission of infectious URTS (Grosset-Janin et al., 2012; Mela & Whitworth, 2014; Passioti et al., 2014). Risk for infection transmission is further increased in contact team-sport athletes (e.g., rugby) due to the close physical contact and trauma inherent in playing these sports (Grosset-Janin et al., 2012). Despite it being known in the general

population that such practices increase infection transmission risk, limited studies have directly quantified their impact on URTS risk in elite team-sport athletes.

Interactive effect of stressors on immunity and URTS risk

To understand the impact of stressors on URTS risk, researchers have tended to examine individual stressors in isolation; however, in reality, elite team-sport athletes are often exposed to several stressors at one time. It is possible that the combination of stressors could have a compounding effect on URTS risk. Indeed, heightened URTS incidence has been demonstrated in military recruits with multi-stressor exposure including strenuous exercise, psychological stress, sleep deprivation, energy restriction and close proximity to others (Dimitriou et al., 2017; Gomez-Merino et al., 2005; Tiollier et al., 2005). Therefore, to better identify periods of increased risk and/or risk factors for URTS, further research is required to examine the independent and collective impact of stressors on URTS risk in elite team-sport athletes.

2.3 Part two: Predictors of URTS episodes

It is apparent that URTS risk is influenced by a number of stressors that team-sport athletes are exposed to on a regular basis. To assess athletes' susceptibility for URTS episodes, exercise immunology research has sought to identify relevant biomarker and self-reported data that can be used to predict athletes risk for URTS, as summarised in Figure 2.2. Identifying predictors of URTS may inform athlete management and treatment strategies to maintain athletes' health and, in turn, training and competition availability.

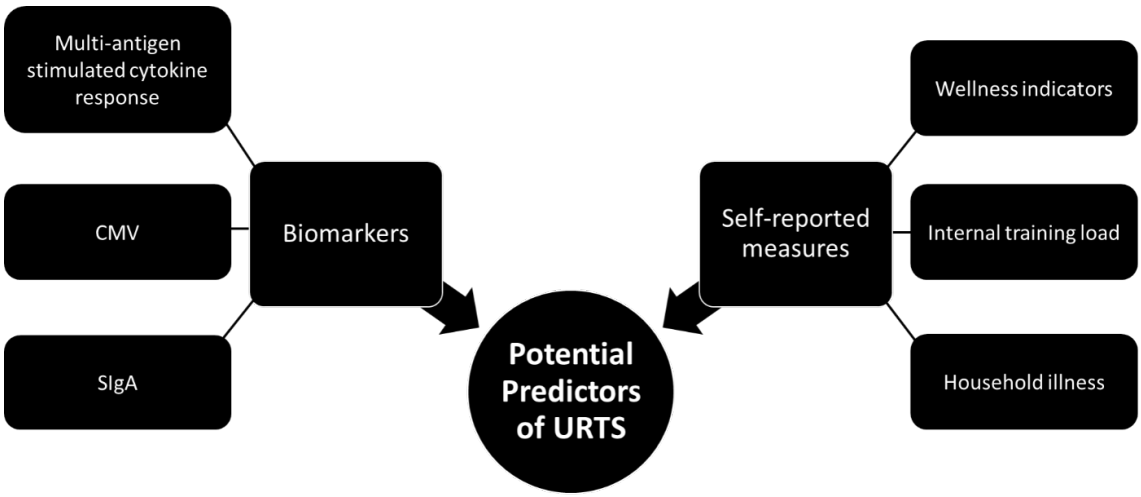

Figure 2.2 Biomarker and self-reported data that have the potential to predict elite team-sport athletes risk for URTS.

Biomarkers and URTS risk

The human body is under constant assault by bacteria, fungi, viruses and other infection-causing microorganisms (Parkin & Cohen, 2001). Fortunately, the immune system provides humans with an array of defence measures to resist such attacks (Parkin & Cohen, 2001). Humans have three functional divisions that defend the host from infection; the mucosal, innate and acquired immune systems (Figure 2.3) (Parkin & Cohen, 2001). Although these systems work independently, they are inextricably linked and often work synergistically in the overall immune response (Parkin & Cohen, 2001). Exercise immunology research, to date, has examined the efficacy of numerous immune markers in predicting URTS risk. SIgA and T-cell cytokine responses, which belong to the mucosal and acquired immune systems, respectively, have been identified as predictors of URTS risk in athletes (Gleeson & Bishop, 2013). However, it is unclear whether innate immune markers can predict athletes risk for URTS as, in addition to defending the body from infection, these cells are involved in tissue damage repair and remodelling (Dias et al., 2017; Walsh, Gleeson, Shephard, et al., 2011). Therefore, only immune markers of SIgA and T-cell cytokine responses will be discussed in this review,

given research suggests they are more directly related to URTS risk in athletes (Gleeson & Bishop, 2013).

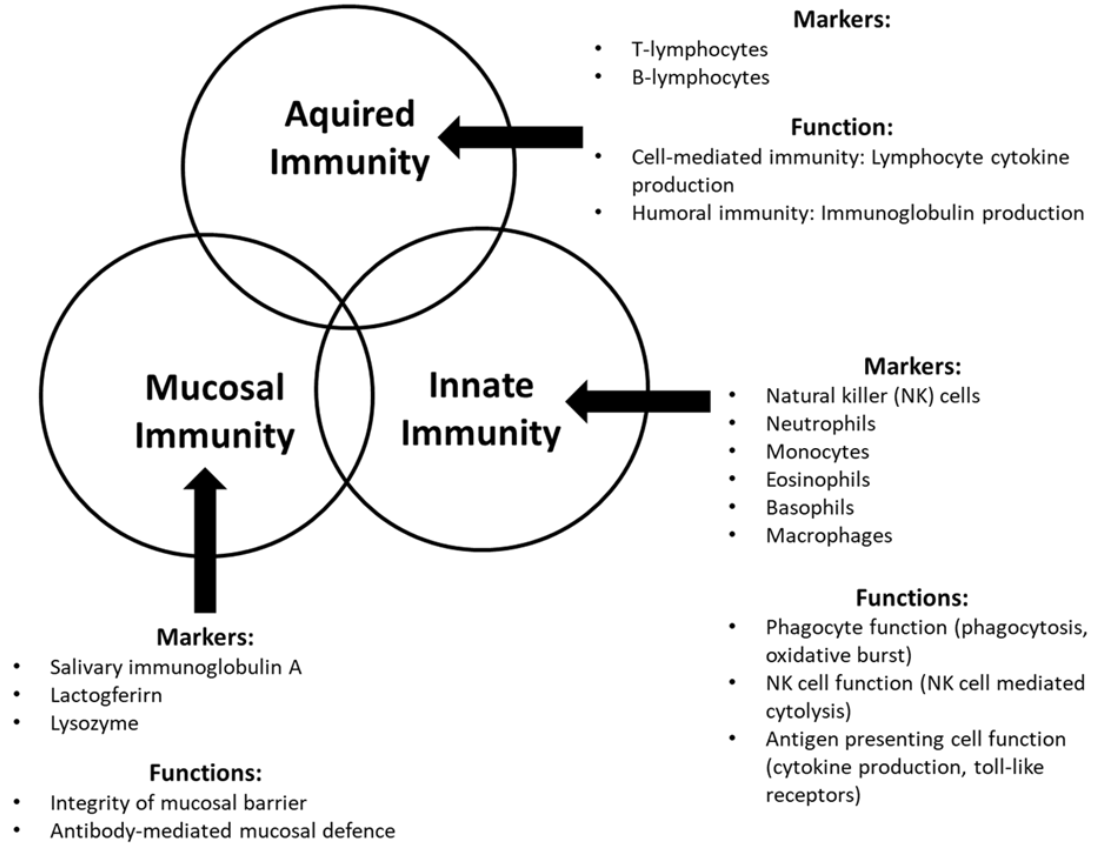

Figure 2.3 An overview of the mucosal, innate and acquired immune systems with examples of the immune functions most commonly examined in exercise immunology studies.

Salivary secretory immunoglobulin A (SIgA)

SIgA production and functional role

SIgA is the principle antibody in oral and nasal mucosal fluids, acting as the body's 'first line of defence' against pathogens (Bishop & Gleeson, 2009). The formation and secretion of IgA into saliva is summarised in Figure 2.4. IgA is produced locally by plasma cells (differentiated B-cells) residing in the basolateral surface and is transported by a transmembrane polymeric immunoglobulin receptor (pIgR) across the mucosal epithelial

(Asano et al., 2004; Korsrud & Brandtzaeg, 1980; Sakaguchi et al., 2013). After being transported through the cell, pIgR is cleaved, leaving its external domain, secretory component bound to IgA. The secretory component acts to stabilise IgA, making it more resistant to protease degradation in saliva secretion (Gleeson, Bishop, & Walsh, 2013). This form of IgA is SIgA (Asano et al., 2004; Korsrud & Brandtzaeg, 1980; Sakaguchi et al., 2013). SIgA exhibits a broad-spectrum of antimicrobial activity against a range of pathogens; specifically, SIgA inhibits pathogen adherence and penetration of the mucosal epithelium, neutralises viruses within epithelial cells, and binds pathogens for transport to the epithelial cell surface (Bishop & Gleeson, 2009).

The rate of SIgA secretion itself depends on the production of IgA by plasma cells and/or rate of transcytosis across the epithelial cell which is determined by pIgR availability (Bishop & Gleeson, 2009). Neuroendocrine responses can modulate IgA transcytosis (Bishop & Gleeson, 2009). Animal studies have reported increased IgA transcytosis in response to acute stimulation of beta-adrenoceptors (Carpenter et al., 2004; Proctor et al., 2003), via increased mobilisation of pIgR (Carpenter et al., 2004). This mechanism has not yet been demonstrated in humans, although, in support, a positive association between adrenaline and SIgA secretion rate has been reported in endurance athletes (Bishop et al., 2006). In contrast, cortisol appears to reduce SIgA translocation into saliva, with cortisol found to inhibit pIgR expression in animals (Rosato et al., 1995), and an inverse association between SIgA and S-cortisol demonstrated in elite team-sport athletes (Cunniffe et al., 2011; He et al., 2010). As such, stress hormones are often measured in the exercise immunology literature to elucidate possible mechanisms underlying alterations in SIgA secretion.

Although widely examined, methods of collecting, measuring and expressing SIgA vary considerably between studies (Pritchard et al., 2017). The choice of collection method (e.g., cotton swab, passive drool, stimulated, unstimulated) differs between studies, as do the quantitative bioassays that are used to measure SIgA (e.g., direct, indirect,

functional, ligand-binding) (Bishop & Gleeson, 2009). SIgA can also be expressed in various ways; for example, as a concentration ($\mu g \cdot mL^{-1}$), secretion rate ($\mu l \cdot min^{-1}$), ratio to osmolality (µg SIgA: mg osmoality$^{-1)}$ and ratio to protein (µg SIgA: mg protein$^{-1)}$ (Bishop & Gleeson, 2009). Moreover, SIgA can be expressed as absolute or relative to an athlete's mean healthy or baseline SIgA levels (Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012; Neville et al., 2008; Owen et al., 2014). These methodological differences make it difficult to directly compare SIgA results between team-sport studies.

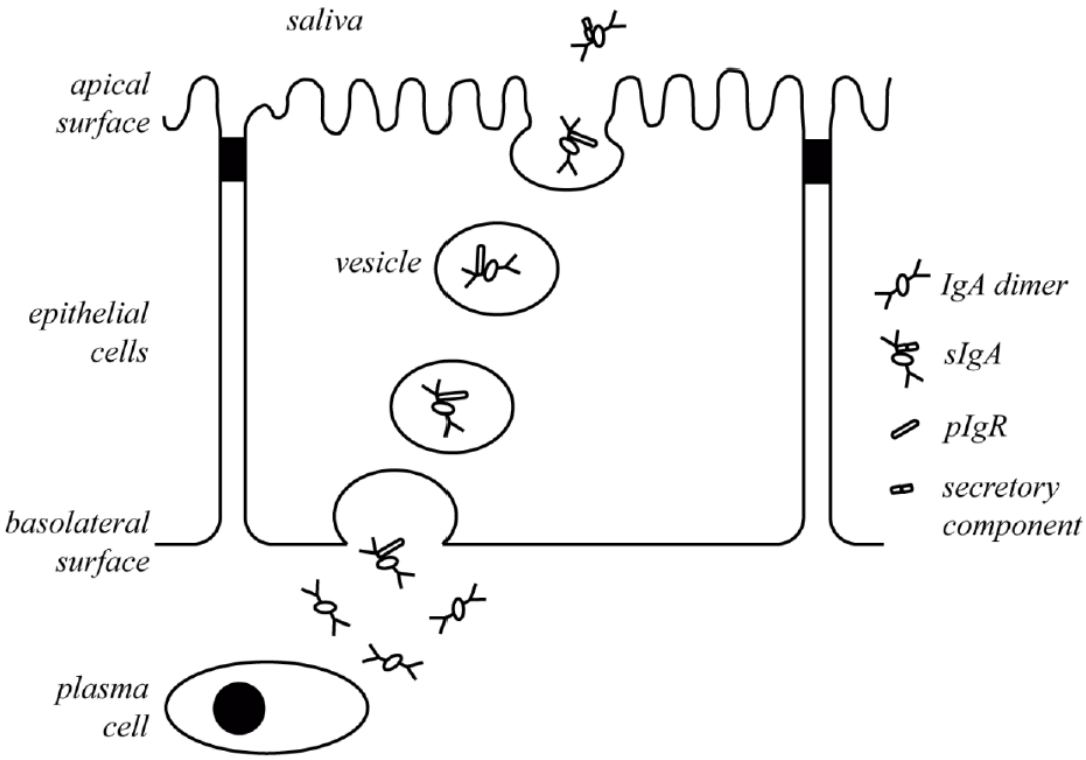

Figure 2.4 Epithelial transport of immunoglobulin A (IgA) into saliva. Adapted from (Bishop & Gleeson, 2009).

SIgA and URTS risk

SIgA is considered one of the most useful clinical biomarkers for predicting URTS risk in team-sport athletes, with low or declining SIgA levels associated with increased risk for URTS episodes (Fahlman & Engels, 2005; Mortatti, Moreira, Aoki, Crewther, Castagna,

de Arruda, & Jose Filho, 2012; Tiernan et al., 2020; Yamauchi et al., 2011b). In team-sport settings, SIgA measurement is more feasible than measurement of blood biomarkers, because saliva sampling is non-invasive, easy and quick to carry out (Lindsay & Costello, 2017). SIgA measurement has recently become even more accessible with the emergence of point-of-care devices (Dunbar et al., 2011), potentially meaning that assessment of an individual athlete's risk for URTS can occur in real time (Dulson et al., 2019).

To prevent URTS, researchers have attempted to identify the timeframe between lowered SIgA and the actual onset of an URTS episode. It has been suggested that a latency period exists between reduced SIgA and URTS development, with athletes who exhibit sustained suppression of SIgA (7-21 days) found to have a ~50% greater risk for URTS (Jones et al., 2016). However, shorter time frames have been reported in soccer players, with a preceding decrease observed within one week (Putlur et al., 2004) or just three days (Nakamura et al., 2006) before the appearance of URTS episodes. As such, the timeframe between reduced SIgA levels and URTS onset remains uncertain in team-sport athletes.

Along with establishing a time interval, researchers have sought to identify which SIgA concentrations and/or secretion rates may predispose athletes to URTS episodes. In endurance athletes, baseline SIgA concentrations of <40mg·L^{-1}, between 40-60mg·L^{-1} and >60mg·L^{-1} have been associated with high, moderate and low risk for URTS, respectively (Gleeson et al., 1999). In addition, significantly lower baseline SIgA secretion rate has been demonstrated in URTS-prone endurance athletes (49.3 ± 35.8 μg·min^{-1}) versus URTS-free endurance athletes (80.3 ± 52.5 μg·min^{-1}) (Gleeson et al., 2012). Similarly, in team-sport athletes, American football players' risk for URTS was increased when SIgA secretion rate dropped below 40 μg·min^{-1} (Fahlman & Engels, 2005). However, the usefulness of measuring absolute SIgA concentration and secretion rate is somewhat limited as high inter- and intra-individual variability in these markers

have been demonstrated (Bishop & Gleeson, 2009). Therefore, it may be more appropriate to express SIgA in relative terms (i.e., SIgA levels relative to an athlete's mean healthy SIgA or baseline SIgA levels) when trying to predict URTS risk. Indeed, in elite sailors, a SIgA concentration less than 40% relative to mean healthy SIgA indicated a 48% chance of developing URTS within three weeks (Neville et al., 2008). In support, a recent study in elite rugby union players found that a 65% or more reduction in SIgA concentration, compared relative to mean healthy SIgA, increased players risk of experiencing URTS within the following 2-weeks (Tiernan et al., 2020). However, SIgA does not always predict URTS risk, with no association between URTS incidence and absolute- (Cunniffe et al., 2011; Moraes et al., 2017; Moreira et al., 2008; Morgans et al., 2014) or relative- (Cunniffe et al., 2011) SIgA levels reported in team-sport athletes.

In addition to potentially predicting URTS risk, SIgA appears to be a sensitive tool for monitoring athletes' responses to exercise stress. Significant reductions in SIgA have been demonstrated in team-sport athletes during and/or following intensified training (Cunniffe et al., 2011; Fahlman & Engels, 2005; Moreira et al., 2014) and competition (Coad et al., 2015; Cunniffe et al., 2011; Fahlman & Engels, 2005; Morgans et al., 2014; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012) periods. However, more research is needed, as the impact of additional stressors (e.g., travel, extreme environmental conditions, psychological stress etc.) on SIgA remains unclear in team-sport athletes.

In summary, measurement of SIgA may provide an assessment of a team-sport athletes susceptibility to URTS. However, there are discrepancies in the literature; thus, there is currently not enough evidence to suggest that measurement of SIgA alone can predict URTS risk in team-sport athletes. Additionally, while SIgA appears to be a sensitive tool to examine team-sport athletes' responses to exercise stress, further research is needed to understand the impact of additional stressors (i.e., long-haul travel, environmental extremes and lifestyle and behaviour factors) on SIgA.

Multi-antigen stimulated cytokine responses

Cytokine production and functional role

Cytokines are small signalling molecules that play an important role in directing the immune response (Paul & Seder, 1994). Cytokines are produced by numerous cells in the immune system, including T-cell subpopulations of T-helper (CD4+), T-cytotoxic (CD8+) and Regulatory T-cells (Treg cells) (Andersen et al., 2006; Cosmi et al., 2014). CD4+ and CD8+ cells can be further subdivided into type 1 (T1) or type 2 (T2) T-cells based on, in part, the cytokines they produce (Lancaster et al., 2004). T1 cells produce pro-inflammatory cytokines including interferon gamma (IFN-γ), interleukin-12 (IL-12) and IL-2 to promote cell mediated immunity. Cell mediated immunity is the arm of the immune system responsible for defending the body from intracellular pathogens like viruses (Abbas et al., 1996). T2 cells produce various anti-inflammatory cytokines (IL-4, IL-5, Il-9, IL-13) that are essential for the development of humoral immunity (Paul & Seder, 1994). The humoral arm of the immune system primarily targets bacterial and fungal infections (Abbas et al., 1996). Treg cells are also an important T-cell subpopulation as these cells are central anti-inflammatory regulators of the immune response and prevent the development of autoimmunity (Weinhold et al., 2016). Treg cells primarily produce IL-10 to suppress the effector functions of various immune cells (O'garra & Vieira, 2004). The balance between T1, T2 and Treg cell immunity needs to be maintained for optimal immune defence, when it is tipped to favour T2 and/or Treg cell immunity, T1 antiviral activity is impaired (Smith, 2003). This is a concern considering most infectious URTS presentations in athletes are of viral origin (Gleeson & Pyne, 2016).

Cytokine responses and URTS risk

Cytokine responses have been shown to predict endurance athletes' 'potential' URTS risk (Gleeson & Bishop, 2013), with URTS-prone athletes found to exhibit lower IFN-γ production (Clancy et al., 2006) and higher multi-antigen stimulated IL-4 and IL-10 production at rest (Gleeson et al., 2012; Gleeson & Bishop, 2013). Cytokines IL-4 and

IL-10 are both antagonistic to IFN-γ, and suppression of IFN-γ may predispose athletes to viral infection and recurrent URTS (Brink et al., 2010). Training load appears to influence resting antigen stimulated cytokine production, with higher anti-inflammatory cytokine production reported in endurance-based athletes completing high training loads compared with those who were only moderately active (Gleeson, Bishop, Oliveira, et al., 2013). However, the nature of training itself may also affect antigen stimulated cytokine production; increased IL-10 production has been found in endurance versus sprint trained athletes, despite no differences in training load. As such, previous findings in endurance athletes are not necessary applicable to team-sport athletes given training loads and the nature of training differ between the sports.

There appears to be only one study to date that has examined the relationship between cytokine production and URTS risk in team-sport athletes (Orysiak et al., 2016). In contrast to endurance athletes, no difference in resting IL-10 cytokine production was found between URTS-prone and URTS-free ice hockey players (Orysiak et al., 2016). In this study, cytokine production was not stimulated (Orysiak et al., 2016), which may explain the conflicting result. The relevance of unstimulated cytokine production to assess URTS risk is unclear, while the capacity of leukocytes to produce cytokines in response to an appropriate challenge (e.g., multi-antigen) is considered a more clinically relevant measure as it provides an indication of the functional capacity of the immune system to defend the body against infectious microorganisms (Gleeson & Bishop, 2013). Therefore, further research is required to determine whether baseline screening of cytokine responses to a multi-antigen challenge can predict 'potential' URTS risk in elite team-sport athletes.

Cytomegalovirus

Cytomegalovirus infection and prevalence

Cytomegalovirus is a ubiquitous β-herpes virus that is transmitted through body fluids, such as saliva, urine, blood and tears (Landolfo et al., 2003). CMV infects 45-100% of

the general population (Cannon et al., 2010), while only 22-25% of endurance athletes have been found to be CMV seropositive (Gleeson et al., 2016; He, Handzlik, Muhamad, et al., 2013). However, the prevalence of CMV is uncertain in team-sport athletes. Once an individual becomes infected with CMV, it persists in a latent state in the body for life (Landolfo et al., 2003). CMV is kept under control by the immune system, primarily natural killer cells and CD8+ cells (Landolfo et al., 2003); although, when these cell functions are suppressed and/or compromised, latent CMV can reactivate and elicit URTS (Dupont & Reeves, 2016).

Cytomegalovirus and URTS risk

In the exercise immunology literature, herpes viral (e.g., CMV, EBV) reactivation is thought to account for 25-55% of all URTS episodes experienced by athletes (Gleeson & Pyne, 2016), with researchers speculating that latent CMV may become reactivated and elicit URTS during periods of immunosuppression (e.g., intensified training or competition periods) (Simpson et al., 2016). Nevertheless, in a study conducted in university endurance athletes, CMV seropositive athletes were shown to experience fewer URTS episodes than CMV seronegative athletes during 4-months of winter training (He, Handzlik, Muhamad, et al., 2013). These findings suggest CMV seropositivity might in fact promote protective immunosurveillance and, in turn, reduce athletes' risk for URTS (He, Handzlik, Muhamad, et al., 2013). However, the influence of CMV on URTS risk remains unknown in team-sport athletes. CMV is a particularly interesting marker to assess in athletes involved in contact team-sports (e.g., rugby), given previous research has shown that the close physical contact and trauma inherent in playing these sports can facilitate the transmission of herpes viral infections (White & Grant-Kels, 1984). Therefore, further research is required to determine whether CMV can predict risk for URTS episodes in team-sport athletes.

Limitations associated with biomarker measurement in an elite team-sport athletes'

Biomarker measurement is expensive and often requires a specialist to administer or analyse results. In addition, rigorous pre-sample standardisation is required to get a valid result, because biomarkers can be influenced by several factors including circadian rhythm, caffeine ingestion, hydration status, recent infection, and exercise. Therefore, it is acknowledged that screening and/or regular measurement of biomarkers, particularly invasive blood biomarkers, may be limited in a team-sport setting, especially considering the squad size (i.e., n=20-40 players).

Potential self-reported predictors of URTS risk

A more accessible method to predict team-sport athletes' risk for URTS relies on non-biological self-reported data including internal training load, and lifestyle and behavioural factors such as wellness indicators and household illness.

Internal training load

Internal training load is commonly measured in elite sport to monitor athletes' psychophysiological responses to trainings and matches, and to reduce incidence of illness and injury (Drew & Finch, 2016; Impellizzeri et al., 2019). Internal training load can be calculated by multiplying an athlete's self-reported session rating of perceived exertion (sRPE) by the given training session or match duration (Foster et al., 2001). To date, equivocal evidence has been presented on the relationship between internal training load and URTS incidence in team sport-athletes (Jones et al., 2016). For example, increases in internal training load have been linked to elevated illness incidence in rugby league (Thornton et al., 2016) and soccer players (Putlur et al., 2004; Watson et al., 2016, 2017); whereas in Australian football rules (Fitzgerald et al., 2019) and basketball players (Anderson et al., 2003) this positive association was not supported. Therefore, the utility of measuring internal training load to predict URTS risk remains inconclusive in team-sport athletes.

Wellness indicators

Psychometric wellness indicators are also routinely monitored in elite team-sport as a method of understanding athletes' physical and mental status (Buchheit et al., 2016; Buchheit et al., 2013; Buchheit et al., 2013; Thornton et al., 2016). In the literature, there are several established sport-specific psychometric questionnaires, including the Recovery-Stress Questionnaire (Kellmann & Kallus, 2001), Recovery-Cue (Kellmann, 2002), Athlete Burnout Questionnaire (Raedeke & Smith, 2001) and Daily Analysis of Life Demands for Athletes (Rushall, 1990). However, these questionnaires do not tend to be used in elite team-sport settings as they are often considered too lengthy to foster compliance, non-specific and impractical for daily use (Saw et al., 2016). As such, team-sport practitioners tend to use customised, shortened questionnaires to assess wellness indicators, including: stress, mood, fatigue, muscle soreness, motivation, sleep quality and quantity (Buchheit et al., 2016; Buchheit et al., 2013; Fitzgerald et al., 2019; Thornton et al., 2016). Indeed, a survey of Australian and New Zealand high-performance sport practitioners on trends of wellness monitoring demonstrated that 80% of respondents used custom self-reported questionnaires (Taylor et al., 2012).

Recently, researchers have examined the efficacy of customised self-reported wellness questionnaires in predicting team-sport athletes' risk for URTS. Changes in self-reported wellness indicators, including decreased wellbeing (Thornton et al., 2016), reduced sleep quantity (Fitzgerald et al., 2019) and increased stress (Drew et al., 2018; Drew et al., 2017) have been linked to increased incidence of URTS in team-sport athletes. In contrast, no association between self-reported wellness indicators and URTS incidence has been reported in soccer (Watson et al., 2017) and cricket players (Ahmun et al., 2019). The discrepant findings may be explained by age or maturity of athletes assessed. Elite adult team-sport athletes were examined in studies supporting an association between self-reported wellness measures and illness (Drew et al., 2018; Drew et al., 2017; Fitzgerald et al., 2019; Thornton et al., 2016); whereas in the studies reporting no association (Ahmun et al., 2019; Watson et al., 2017), adolescent team-sport athletes

were assessed. Self-reported wellness measures are open to cognitive (e.g., lack of understanding) and conscious bias (e.g., responding with the answer the athlete perceives is correct/desirable rather than how they feel) (Saw et al., 2015). It is possible that such biases are more common in adolescent athletes, which may explain why self-reported wellness measures were not predictive of URTS risk in this population.

In addition to potentially predicting athletes' risk for URTS, self-reported wellness data may provide an indication of an athletes' immune status. In elite sailors, a relationship between a simple self-reported fatigue assessment and SIgA was demonstrated, with worse than normal and better than normal self-reported fatigue associated with lower and higher relative SIgA concentration, respectively (Neville et al., 2008). However, the relationship between self-reported wellness indicators and immune markers is uncertain in team-sport athletes. Therefore, more work in this area is needed, given monitoring of self-reported wellness could provide a simple, non-invasive, and cost-effective tool to assess immunity and risk for URTS in team-sport athletes.

Household illness

In the general population, it is well established that households play a significant role in spread of respiratory infection because of the frequency and intensity of contacts between household members (Tsang et al., 2016). As outlined in reviews, once illness in the household is present, risk for infection transmission to another household member can be up to 40%, and this typically occurs within 2-4 days (Lau et al., 2012; Tsang et al., 2016). Children appear to be the main introducers of respiratory infections into households (Longini et al., 1982), and infection transmission has been shown to be almost twofold higher in households with young children (i.e., 0-5 years) (Viboud et al., 2004). Household respiratory infection transmission risk is also influenced by the household environment (e.g., crowding) and the age, health and influenza vaccination status of occupants (Petrie et al., 2013). Therefore, household illness monitoring may be an effective tool to assess URTS risk in team-sport athletes. However, the relationship

between self-reported household illness and URTS incidence has not yet been examined in athletic populations.

Biomarkers or self-reported measures to predict URTS risk?

To date, an important limitation of the team-sport literature is that researchers have tended to examine either biomarkers (Fahlman & Engels, 2005; Morgans et al., 2014; Tiernan et al., 2020) or self-reported measures (Fitzgerald et al., 2019; Thornton et al., 2016; Watson et al., 2017) as potential predictors of URTS risk, but not concurrently. Therefore, it is currently unclear whether one approach is more effective than the other.

2.4 Conclusion

Factors influencing URTS risk have been extensively examined in endurance athletes, while a growing body of work is beginning to amount in team-sport athletes. To date, team-sport studies have focused on exercise stress, with intensified training and congested competition periods shown to increase URTS risk. Little attention, however, has been given to additional stressors and their impact on URTS risk, including long-haul travel, extreme environmental conditions, psychological stress, sleep disturbance, inadequate nutrition and the team sport environment itself. Moreover, previous studies have tended to investigate each stressor in isolation, when in reality elite team-sport athletes are frequently exposed to multiple stressors at one time. It is possible the combination of stressors could have a compounding effect on URTS risk. Therefore, further research examining the independent and collective effect of stressors on URTS incidence is needed to identify potential periods of increased risk/risk factors for URTS in elite team-sport athletes.

Exercise immunology literature has sought to identify biomarker and self-reported data with the potential to predict athletes' susceptibility to URTS. Monitoring of SIgA appears to provide an assessment of team-sport athletes' risk for developing URTS, although equivocal evidence on the association between SIgA and URTS risk has been reported.

Baseline measurement of stimulated cytokine responses and CMV has been found to predict ‘potential’ URTS risk in endurance athletes. However, the association between these blood biomarkers and URTS risk remains unknown in team-sport athletes. More recent team-sport studies have examined the efficacy of self-reported measures in predicting URTS risk. Research in this area is still in its infancy and, to date, discrepant findings have been reported on the association between URTS incidence and self-reported measures of internal training load and wellness indicators. In addition, the relationship between self-reported household illness and URTS incidence remains unknown in team-sport athletes. More research examining self-reported measures is required, given they could provide a simple and cost-effective tool to assess elite team-sport athletes’ risk for URTS. Finally, further studies simultaneously examining biomarker and self-reported data is needed to determine whether one approach is more effective than the other at predicting URTS risk in elite team-sport athletes.

CHAPTER 3 KEEPING ATHLETES HEALTHY AT THE 2020 TOKYO SUMMER GAMES: CONSIDERATIONS AND ILLNESS PREVENTION STRATEGIES.

Prelude

Chapter 2 described factors influencing URTS risk in team-sport athletes during general training and competition periods. The Olympic and Paralympic Games (Summer Games) are a pinnacle event for many elite sporting teams and keeping team-sport athletes healthy in the lead up to- and during- the Summer Games is important for optimal performance. This short review described the impact of stressors associated with the Summer Games on athletes' URTS risk, along with outlining stressor-specific strategies to maintain and protect athletes' health.

3.1 Introduction

Acute illness is one of the single biggest factors that can prevent athletes from successful performance at pinnacle events (Raysmith & Drew, 2016). URTS are the most common illness reported by elite athletes at the Olympic and Paralympic Games (Derman et al., 2013; Derman et al., 2017; Engebretsen et al., 2013; Soligard et al., 2017). Common URTS include sore throat, headache, runny nose and coughing (Walsh, Gleeson, Shephard, et al., 2011). URTS can negatively impact training availability, reduce exercise performance and even result in athletes missing a major competition (Gleeson & Pyne, 2016). The importance of maintaining athlete health has been highlighted by studies demonstrating that World and Olympic winning medal athletes experience fewer URTS than less successful athletes (Raysmith & Drew, 2016; Svendsen et al., 2016). As such, keeping athletes healthy in preparatory and competition phases of the 2020 Tokyo Olympic and Paralympic Games (hereinafter “Summer Games”) will contribute toward optimal performance. The purpose of this short review is to describe how stressors impact upon an athletes’ risk for illness and to identify which athletes may be more susceptible to illness at the Summer Games, along with outlining strategies to maintain and protect athlete health.

3.2 Preparatory phase of the Summer Games

Keeping athletes healthy in the lead up to the Summer Games is important for optimal performance. In elite track and field athletes, having fewer illnesses (and injuries) and completing more than 80% of planned training sessions in the 6-months prior to a major event increases the likelihood of achieving pre-defined performance goals (Raysmith & Drew, 2016). Nevertheless, maintaining athlete health during the preparatory phase may prove to be a challenging task, as both Northern and Southern hemisphere athletes will be exposed to different stressors that can challenge immunity and increase URTS risk, including seasonal specific stressors, limited ultraviolet B exposure (UV-B), heat acclimation and travel.

Seasonal specific stressors

Seasonal influenza

It is well established that the incidence of influenza exhibit seasonal fluctuations, with peak incidence occurring during the winter months (Doyle & Cohen, 2009). Therefore, compared to Northern hemisphere athletes, athletes residing in the Southern hemisphere will be at an increased risk for infectious URTS episodes during the preparatory phase of the Summer Games.

Strategies to minimise seasonal influenza risk

- Vaccination: Advise Southern hemisphere athletes to have the influenza vaccine in autumn (April), before the influenza season, as it usually takes 5–7 weeks to take effect (Schwellnus et al., 2016). Administration of the vaccine should occur during a non-competition period, or at least 2 weeks prior to competition to allow time for the development of a specific adaptive immune response and any potential side effects (Daly & Gustafson, 2011). There may be some benefit in performing moderate intensity exercise prior to vaccine administration as it has been shown to facilitate vaccine efficacy (Edwards et al., 2007; Edwards et al., 2012) and reduce adverse reactions (Lee et al., 2018). This adjuvant strategy seems to be most successful in immunocompromised individuals (e.g., elderly) (Ranadive et al., 2014). Therefore, it is reasonable to suggest it may be worthwhile in elite athletes. It is considered a harmless strategy where the potential benefits could be crucial to preparation; however, further research in athletes is required to determine optimal pre-vaccine exercise protocols.
- Hygiene: Maintain good hygiene (refer to Table 3.1 for guidelines).
- Illness monitoring: Use the Jackson Common Cold Scale to monitor illness in athletes (Jackson et al., 1958), and enable early detection and application of appropriate illness prevention strategies. Consider monitoring household illness by adding a question alongside the Jackson Common Cold Scale (Bermon et al.,

2017). Household illness monitoring is a promising strategy; although, further research in this area is required.

- Probiotic supplementation: Supplement throughout the preparatory phase (3 months) (refer to Table 3.1 for further guidelines).
- Zinc acetate supplementation: Supplement athletes experiencing acute URTS with zinc acetate lozenges (75 mg/day) to decrease the duration of URTS (Note: zinc must be taken <24 hours after onset of URTS and can be taken for 1–2 weeks) (Maughan et al., 2018). Excessive zinc supplementation (>150 mg/day) should be avoided as it can impair immune cell functions (Maughan et al., 2018).

Cold environmental conditions

URTS can result from infectious (viral, bacterial or fungal etiology) or non-infectious and inflammatory causes (e.g., caused by allergies, asthma and trauma to respiratory epithelial membranes) (Gleeson & Pyne, 2016). Southern hemisphere athletes training during winter will be exposed to cold dry air. Inhalation of cold dry air can damage airway epithelium and lead to non-infectious URTS episodes (Koskela, 2007). Athletes with asthma and allergies may be at higher risk for URTS, as winter training has been shown to increase URTS incidence among individuals with these conditions (Gleeson & Pyne, 2016; Hyrkäs et al., 2014).

Strategies to minimise cold air mediated non-infectious URTS:

- Diagnose asthma and allergies: Administer the validated questionnaire Allergy Questionnaire for Athletes (AQUA) to identify athletes with asthma and allergies (Bonini et al., 2009). Confirm the diagnosis with a physician.
- Control asthma and allergies: Ensure appropriate therapeutic control of asthma and allergies in compliance with the World Anti-Doping Agency (WADA) regulations (Helenius & Haahtela, 2000).
- Protect airways: When practical, take extra precautions to avoid inhalation of cold dry air (below 0°C). For example, train indoors or for outdoor training use facial

masks to protect airways (Walsh, 2018). It is unknown if facial masks reduce URTS incidence; however, they can attenuate cold air exercise-induced asthma which is known to elicit non-infectious URTS (Beuther & Martin, 2006).

Summer allergies and asthma

Northern hemisphere athletes will be exposed to environmental factors and high periods of allergen load during the preparatory phase of the Summer Games, including heat and humidity, pollen, grasses, weed, mould and dust. During exercise, high ventilation rates combined with increased exposure to environmental factors and allergens can exacerbate asthma and allergies. Prevalence of asthma and allergies is high in elite athletes (Silva & Moreira, 2017). Exacerbations of asthma and allergies may elicit non-infectious URTS, such as runny nose, repetitive sneezing and coughing, all of which can disrupt training and performance (Gleeson & Pyne, 2016).

Strategies to minimise summer allergy and asthma mediated non-infectious URTS

- Diagnose and control asthma and allergies (see section “Strategies to Minimise Cold Air Mediated Non-infectious URTS” for details).
- Allergen avoidance: When practical, avoid exposure to allergens (e.g., clean room and change bed lining regularly to reduce house dust mite exposure; and follow pollen forecasts and consider adapting training venues and schedules during high pollen periods) (Silva & Moreira, 2017).

Low Ultraviolet B Exposure

Vitamin D is predominately obtained through sunlight UV-B exposure (Backx et al., 2017). Consequently, vitamin D status is known to exhibit seasonal variation in athletes, with lowest and highest vitamin D concentrations typically present in summer and winter months, respectively (Backx et al., 2017). Athletes training during the Southern hemisphere winter may, therefore, be at an increased risk of vitamin D deficiency. Indeed, previous research suggests that up to 50% of athletes could be considered to

have an inadequate vitamin D status during winter training months (He, Aw Yong, et al., 2016; He, Handzlik, Fraser, et al., 2013). However, some athletes residing in the Northern hemisphere may also be at risk for vitamin D deficiency, such as indoor athletes, athletes with dark skin tone, athletes who live and train in northern latitudes (<30° or >70°) and athletes residing in countries with a poor summer season with limited sun exposure (i.e., sun exposure <20 min/day) (He, Aw Yong, et al., 2016). Vitamin D deficiency appears to be an important determinant of URTS risk in athletes, with an optimal circulating 25(OH)D of 75 nmol/L^{-1} suggested to enhance immunity and prevent URTS episodes (He, Aw Yong, et al., 2016).

Strategies to maintain vitamin D levels

- Vitamin D recommendations for Southern hemisphere and at-risk Northern hemisphere athletes: There may be some benefit in measuring athletes' serum 25(OH)D concentration to allow more targeted vitamin D supplementation. As outlined in recent guidelines, athletes with serum 25(OH)D concentrations less than 75 nmol/L^{-1} should be supplemented with 2000-4000 IU vitamin D3/day (Owens et al., 2018). However, the measurement of serum 25(OH)D is not always feasible (cost approximately US$255 per athlete) and may not be the most appropriate measure of an athlete's vitamin D status (Allison et al., 2018; Owens et al., 2018). Therefore, rather than measuring serum 25(OH)D, the most practical approach may be to supplement all Southern hemisphere and at-risk Northern hemisphere athletes with 1000 IU vitamin D3/day (comply to WADA anti-doping regulations) (He, Aw Yong, et al., 2016). There is some risk for toxicity when supplementing with exogenous vitamin D; however, previous reports suggest 1000 IU vitamin D3/day is a safe dosage (He, Aw Yong, et al., 2016).
- General vitamin D guidelines for Northern hemisphere athletes: Aim to acquire 15 minutes of non-protected (i.e., no sunscreen) sun exposure per day (He, Aw Yong, et al., 2016).

Heat acclimation

The Summer Games are expected to be hot (>30°C) and humid (>70% relative humidity). Therefore, heat acclimation (HA) will be an integral component of the preparatory phase. Exercise immunology research suggests that heat does not pose a challenge to the immune system. Indeed, performing a one-off bout of exercise in hot conditions [28–38.7°C, 45–76% RH] does not appear to exacerbate exercise-induced immune perturbations, compared to temperate conditions (Laing, Blackwell, et al., 2005; Laing, Gwynne, et al., 2005; McFarlin & Mitchell, 2003; Mitchell et al., 2002; Niess et al., 2003). Similarly, HA has been shown to have negligible effects on immunity. For example, no change in white blood cell counts (Willmott et al., 2016) or inflammatory cytokines (Amorim et al., 2011; Barberio et al., 2015) has been demonstrated following HA. However, the current limitation to the HA studies discussed is that illness reports were not measured alongside immune markers. Therefore, it remains unclear how HA may impact upon athletes' risk for URTS. Consideration may want to be given to the acclimation status of athletes performing HA. During the preparatory phase, it is possible that acclimation status will differ between Northern and Southern hemisphere athletes; athletes residing in the Northern and Southern hemisphere will likely be seasonally acclimated and unacclimated, respectively. Future studies should assess the baseline acclimation status of athletes engaging in HA to understand whether it is associated with URTS risk.

Strategies to maintain athlete health during HA

The health status of athletes should be considered when implementing HA. Athletes experiencing illness symptoms should not participate in HA as it may exacerbate illness (Casadio et al., 2017).

- Hygiene: Maintain good hygiene (e.g., remove wet clothing and have a warm shower immediately following HA sessions) (refer to Table 3.1).
- Training load and recovery management: Heat stress adds to athletes' overall training load. Carefully manage training load when training with additional heat

(Walsh, 2018). Ensure adequate recovery between HA sessions, particularly if HA protocols involve prolonged exercise (≥90 min) as it can cause more severe immune perturbations than shorter duration exercise (<60 min) (Diment et al., 2015).

- Daily wellness monitoring and management: Monitor wellness to understand how individual athletes tolerate HA. A customised psychometric questionnaire (Hooper & Mackinnon, 1995) utilising Likert scales can be used to assess indicators of wellness (e.g., sleep quality, stress, fatigue, mood and muscle soreness) (Buchheit et al., 2013; Gallo et al., 2017). In addition to monitoring wellness during the HA period, the questionnaire should be administered during normal training weeks to establish baseline wellness data. High stress/anxiety levels and sleep deprivation have been linked to increased URTS incidence (Cohen et al., 2009; Cohen et al., 1991). Over the HA period, apply strategies listed in Table 3.1 (i.e., minimise stress and anxiety and improve sleep). Moreover, if athletes' wellness scores are substantially reduced consider adjusting the HA protocol (e.g., reduce load) (Schwellnus et al., 2016).
- Carbohydrate (CHO) intake: Maintain day-to-day CHO availability over the HA period, aim for >50% daily energy intake as CHOs (Walsh, 2018).
- Hydration: Permissive dehydration is often used during HA sessions to accelerate the adaptation process (Garrett et al., 2014). Exercising in a dehydrated state does not appear to cause further exacerbation of immune perturbations, compared to euhydrated exercise (Killer et al., 2015; Svendsen et al., 2014). Therefore, permissive dehydration can be used during HA as it is unlikely to impair immunity. However, during recovery from HA, fluid replacement should be prioritised, as per current rehydration guidelines (Thomas et al., 2016).
- Probiotic supplementation: Begin supplementation at least 2-weeks before the HA block is due to commence and supplement throughout HA (refer to Table 3.1 for guidelines).

Long-haul travel

During the preparatory phase, many Northern and Southern hemisphere athletes will undertake international travel to competition, heat camps and the Summer Games itself. Travel has been identified as a prominent risk factor for URTS in athletes (Walsh, 2018). Indeed, increased URTS incidence and severity has been demonstrated in team-sport athletes travelling to international destinations that were >5 or 11 time zones from their home country (Fowler et al., 2016; Haywood et al., 2014). Moreover, increased incidence of URTS episodes has been reported in elite endurance athletes following air travel (Svendsen et al., 2016). However, the current limitation to the studies discussed is that immune markers were not measured alongside illness reports, and this limits our understanding of how travel may impact upon the immune system to subsequently increase risk of URTS. Nevertheless, in the general population, simulated long-haul travel has been found to induce transient immune changes that may contribute to increased URTS susceptibility (Wilder-Smith et al., 2012). In contrast, a recent study in master-level athletes showed that long-haul travel did not impair mucosal immune responses (Stevens et al., 2018). Given research in this area is still in its infancy, further studies in elite athletes are required to better elucidate the impact of long-haul travel on immunity and URTS risk.

Strategies to maintain athlete health during long-haul travel

- Travel vaccines: Consult a physician and update athlete and support staff vaccines.
- Hand hygiene: Apply alcohol-based hand gel after touching potentially contagious objects. For example, hand gel should be used after handling airport plastic security screening trays as a recent study identified that they have the highest frequency of respiratory viruses, compared to other airport surfaces (e.g., toilets, handrails) (Ikonen et al., 2018).
- Avoid ill people: If possible, seat athletes away from ill passengers. Increased risk for infection transmission has been associated with sitting within two rows of

a contagious passenger for >8 hours (Mangili & Gendreau, 2005). If it is not possible to change seats, athletes should wear a disposable face mask (Walsh, Gleeson, Pyne, et al., 2011).

- Hydration: Encourage athletes to drink plenty of water to keep well hydrated and potentially prevent mucosal membranes from drying out.
- Optimise sleep hygiene: Pre-departure, improve sleep quantity and quality (refer to Table 3.1 for guidelines). After long-haul travel, the greatest sleep disruption has been reported in the first 48 hours (Stevens et al., 2018). Therefore, optimise sleep hygiene (Table 3.1) to improve sleep, particularly on the first 2 nights after arrival (Stevens et al., 2018).
- Recovery: Avoid flying on the same day as competition or intensive training. Delay travel until at least the subsequent day (Svendsen et al., 2016).
- Probiotic supplementation: Begin supplementation at least 2-weeks before scheduled travel (refer to Table 3.1 for guidelines).

3.3 Competition phase of the Summer Games

Stressors associated with the Summer Games

During the Summer Games, both Northern and Southern hemisphere athletes will be exposed to a range of stressors. Such stressors include intensive competition, hot and humid environmental conditions, dehydration, psychological stress and sleep deprivation (Keaney et al., 2018; Walsh, 2018). The effect of these stressors on immunity and URTS risk has been summarised in recent reviews (Keaney et al., 2018; Walsh, 2018; Williams et al., 2018). However, previous exercise immunology studies are limited in that they have tended to examine each stressor in isolation; when in reality, athletes will be simultaneously exposed to all stressors at the Summer Games. The synergism of these stressors could potentially have a compounding effect on immunosuppression, resulting in higher incidence of URTS episodes than if each stressor were applied alone. Further

research is needed to understand how multiple stressors affect athletes' immunity and URTS risk.

At the Summer Games, medals are often won by the smallest of margins, so even a mild URTS episode could negatively affect results. To keep athletes healthy and minimise the potential immunosuppression evoked by Summer Games stressors, athletes should consider adhering to the five key illness prevention strategies listed in Table 3.1. These strategies have been selected on the assumption that Summer Games athletes will adhere to fundamental principles of nutrition and sport science (e.g., macro- and micro-nutrient intake, hydration, recovery protocols, training load management, etc.). Illness prevention strategies should not replace fundamentals, but work alongside them to keep athletes healthy. In addition to these strategies, other reviews exist which provide detailed recommendations on avoiding infection and maintaining immune health in athletes (Schwellnus et al., 2016; Walsh, 2018).

Table 3.1 Summary of five key illness prevention strategies that athletes should consider adhering to during the Summer Games

Strategy	Proposed rationale	Practical recommendations	References
1. Hygiene practices	Minimise risk for infection transmission	- Hand hygiene: Wash hands regularly (rub hands with soap >20 sec and dry hands thoroughly with clean towel) and carry alcohol-based hand gel - Regularly clean sporting equipment and clothing - Isolate sick athletes and support staff (e.g., move out roommates) - Avoid self-inoculation by touching eyes, nose and mouth - Avoid shaking hands with other athletes and support personal - Where possible, avoid crowded areas, sick people and young children. If avoidance is not possible, wear face masks	(Keaney et al., 2018; Schwellnus et al., 2016; Walsh, 2018)
2. Maintain day-to-day CHO availability	Preventing low CHO availability may minimise the exercise induced rise in stress hormones (cortisol and catecholamines) which, in turn, may attenuate immune perturbations	- Total CHO intake should match daily training and competition requirements - Athletes engaging in prolonged continuous exercise or high intensity intermittent team-sport exercise should aim to consume 30–60 gCHO/h	(Bermon et al., 2017; Burke et al., 2011)
3. Probiotic supplementation	Probiotics may help to reduce the incidence, severity and duration of URTS	-Type: Non-refrigerated (travel friendly), multi-strain probiotic combining Lactobacillus and Bifidobacterium, ensure selected probiotic complies with WADA anti-doping regulations - Dosage: 1×10^{10} colony forming units per day -Timing: Commence probiotic supplementation at least 2-weeks before travelling to Tokyo, to allow adequate time for colonisation - Potential side effects: In first 2-weeks athletes may experience gastrointestinal issues (e.g., stomach rumbles, increased flatulence). Athletes experiencing these symptoms should take their probiotic on an empty stomach. If side effects persist (>2-weeks) try reducing the dosage by half and gradually increase dosage as symptoms ease	(Pyne et al., 2015; Williams et al., 2018)

Strategy	Proposed rationale	Practical recommendations	References
4. Minimise stress and anxiety	Stress and anxiety are risk factors for illness; management may lower risk for URTS	- Identify athletes with high stress and anxiety using validated questions (e.g., Depression, Anxiety, Stress Scale (DASS-21), Recovery Stress Questionnaire (REST-Q-Sport-52)) - Monitor stress and anxiety using a wellness questionnaire (refer to 'strategies to maintain athlete health during HA' for details) - Consult a psychologist to provide education around stress and anxiety management techniques - Mindfulness practices (refer to 'strategies to maintain multi-competition/event athlete health' for details)	(Drew et al., 2017; Schwellnus et al., 2016; Walsh, 2018)
5. Improve Sleep	Sleep deprivation is a risk factor for illness; improving sleep may reduce URTS risk	- Use objective (e.g., wrist actigraphy) or subjective (e.g,. questionnaire) methods to identify sleep deprived athletes (<7 h per night) athletes - Aim for a minimum of 8 hours sleep per night - Apply sleep hygiene strategies to optimise sleep quantity and quality (e.g., maintaining a regular bed and wake time, ensuring a quiet, cool and dark bedroom environment (19-22°C), avoidance of stimulants (e.g., caffeine) prior to sleep, avoidance of light-emitting technology devices in the 30 min prior to sleep))	(Fullagar, Skorski, et al., 2015; O'Donnell & Driller, 2017)

3.4 Athletes at increased risk for illness during the Summer Games

With the aim of protecting the health of athletes, the International Olympic Committee (IOC) monitored illness incidence at the London (2012) and Rio (2016) Olympic and Paralympic Games (Derman et al., 2013; Derman et al., 2017; Engebretsen et al., 2013; Soligard et al., 2017). At previous Summer Games, 5-14% of athletes experienced at least one illness, with the highest incidence of illness affecting the respiratory tract (Derman et al., 2013; Derman et al., 2017; Engebretsen et al., 2013; Soligard et al., 2017). IOC reports demonstrated that the illness rates varied considerably between genders and sports (Derman et al., 2013; Derman et al., 2017; Engebretsen et al., 2013; Soligard et al., 2017). As summarised in Figure 3.1, it appears that some athletes may be more susceptible to illness during the Summer Games, namely: (1) female athletes; (2) Paralympic athletes; (3) water-sport athletes; and (4) multi-competition/event athletes (i.e., athletes who compete on >1 day) (Derman et al., 2013; Derman et al., 2017; Engebretsen et al., 2013; Soligard et al., 2017).

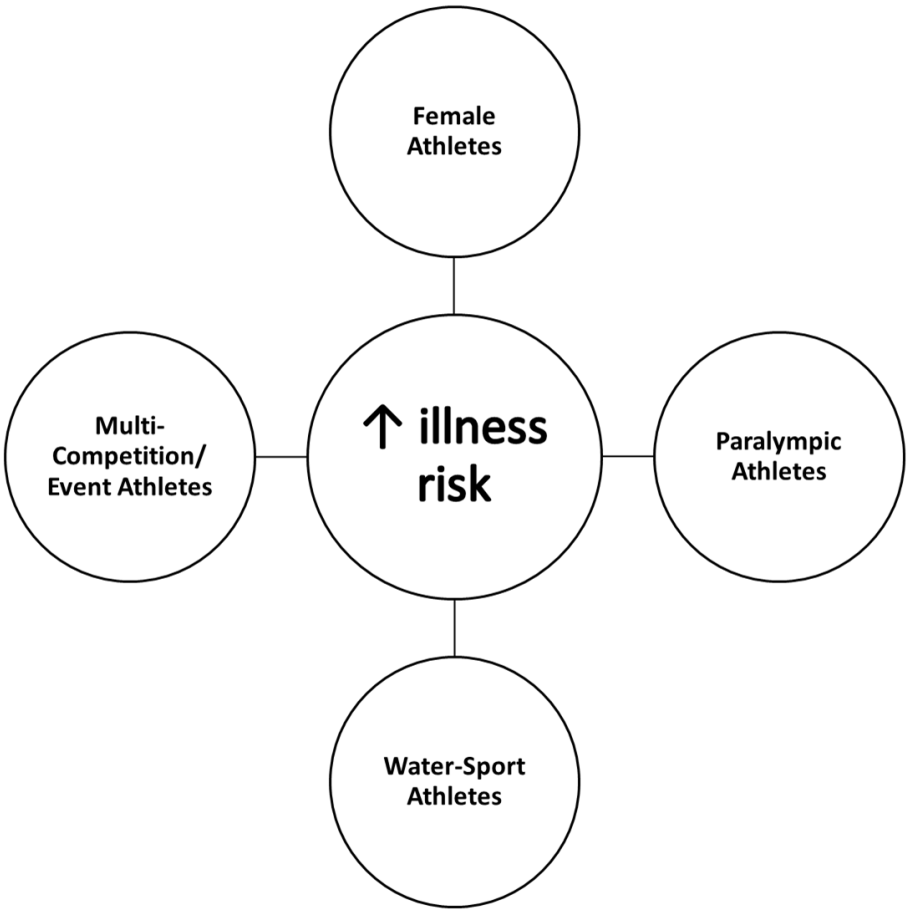

Figure 3.1 Athletes potentially at increased risk for URTS during the Summer Games.

Female athletes

Data obtained at the London and Rio Summer Games demonstrated significantly higher (40–60%) illness incidence in female compared to male athletes (Derman et al., 2017; Engebretsen et al., 2013; Soligard et al., 2017). In agreement with these findings, longitudinal studies have shown that female athletes tend to be at increased risk for URTS (Gleeson et al., 2011; He, Bishop, et al., 2014) and experience URTS episodes for a longer duration than male athletes (He, Bishop, et al., 2014). Sex differences in immune variables may explain the higher illness susceptibility observed in female athletes. Differences in immune responses between females and males have largely been attributed to sex hormones and their inherent immune modulatory functions (Klein & Flanagan, 2016). Furthermore, increased URTS susceptibility in female athletes may be associated with low energy availability (LEA). Higher rates of LEA have been demonstrated in female compared to male athletes (Logue et al., 2018), and LEA has

been identified as a key risk factor for illness in Olympic-level female athletes (Drew et al., 2017).

Strategies to maintain female athlete health

- Diagnose and treat LEA: In the preparatory phase, identify female athletes with LEA using the validated questionnaire Low Energy Availability in Females Questionnaire (LEAF-Q) (Melin et al., 2014). Athletes with LEA should work closely with a nutritionist to ensure daily energy intake matches training and competition demands (Logue et al., 2018).
- Supplementation: A number of supplements have been proposed to alter specific aspects of the immune system and reduce athletes' risk for URTS (Maughan et al., 2018). However, few supplements have convincing evidence supporting their use. Currently, probiotics (refer to Table 3.1 for guidelines), vitamin C (0.25–1.0 g/day) (Hemilä & Chalker, 2013) and quercetin (1 g/day) (Somerville et al., 2016) are the most promising supplements; although further research is needed to determine how the combined use of these supplements influence URTS risk. Athletes' need for these supplements should be assessed on an individual case-by-case basis, based on several factors (e.g., URTS history, sport, nutrient status, etc.). Supplements to be used at the Summer Games should be piloted (for acceptance/compliance/safety) in an off season/preparatory phase and the selected supplements should be batch tested and comply with WADA regulations.

Paralympic athletes

Paralympic athletes appear to be more susceptible to illness than able-bodied athletes. Paralympic athletes suffered almost double the amount of URTS episodes than able bodied athletes during previous London and Rio Summer Games (Paralympics: 12-14% vs. Able-bodied: 5-7%) (Derman et al., 2013; Derman et al., 2017; Engebretsen et al., 2013; Soligard et al., 2017). Research on Paralympic sport is limited compared to

investigations of able-bodied athletes (Van Rensburg et al., 2018); therefore, it is difficult to ascertain why Paralympic athletes are at a heightened risk for illness. Illness risk will differ between Paralympic athletes based on their disability type. Paralympic athletes with spinal cord injuries have altered autonomic control and immunity, and impaired immune function has been cited as the main reason for increased illness susceptibly in this population (Leicht et al., 2013). In addition, the use of wheelchairs by Paralympic athletes likely increases infection transmission risk, as wheelchairs pick up and carry high numbers of bacteria. Indeed, at the Rio Paralympics, the highest illness incidence rate was reported in wheelchair fencing, while wheelchair basketball was only behind Paralympic swimming in terms of illnesses sustained (Derman et al., 2017).

Strategies to maintain Paralympic athlete health

- Hygiene: Wheelchair athletes should regularly disinfect wheelchairs, wear gloves, ensure good hand hygiene and avoid self-inoculation by touching eyes, nose and mouth (Walsh, 2018).
- Supplementation: See section “Strategies to Maintain Female Athlete Health” for details.

Water-sport athletes

Athletes involved in water-sports may be at an increased risk for illness during the Summer Games. At previous Summer Games, the IOC identified the top 5 sports with the highest illness incidence; water-sports accounted for 2 out of 5 (sailing and synchronized swimming), and 4 out of 5 (diving, open water marathon, canoe slalom, and synchronized swimming) sports at the London (Engebretsen et al., 2013) and the Rio Olympics (Soligard et al., 2017), respectively. Furthermore, at the Rio Paralympics, para-swimming had the second highest illness incidence (Derman et al., 2017). There are two likely factors underpinning increased illness susceptibility in this population: (1) chlorine exposure for pool-athletes; and (2) water quality issues for open-water athletes. Airway disorders, including asthma and rhinitis, are prevalent in pool-athletes and are

often attributed to chlorine and chlorine by-products causing airway changes (Škrgat et al., 2018). As such, asthma and allergy mediated non-infectious URTS may explain why pool-sport athletes appear to be at an increased risk for illness. Alternatively, for open-water sport athletes, particularly at the Rio Summer Games, reports suggested that water quality issues (i.e., contamination with bacteria and viruses) were the primary cause of higher illness incidence (Keith, 2017).

Strategies to maintain water-sport athlete health

- Diagnose and control asthma and allergies: See section “Strategies to Minimise Cold Air Mediated Non-infectious URTS” for details.
- Supplementation: See section “Strategies to Maintain Female Athlete Health” for details.

Multi-competition/event athletes

Multi-competition/event athletes may be at an increased risk for illness. Indeed, data obtained at previous Olympic games demonstrated that of the top 5 sports with the highest illness incidence, the majority were multi-competition/event sports [5/5 in London: athletics, beach volleyball, football, sailing, and synchronized swimming (Engebretsen et al., 2013)] [4/5 sports in Rio: diving, canoe slalom, equestrian and synchronized swimming (Soligard et al., 2017)]. It is unclear why these athletes are more susceptible to illness; nonetheless, it may be explained by the psychological element of having to mentally prepare for multiple events. Recent research suggests that mental state influences immunity. For example, state-anxiety and perceived psychological stress before exercise has been shown to influence immune responses to a greater extent than exercise itself (Edwards et al., 2018). In addition, a significant association between mental health (i.e., perceived stress and depression) and illness incidence has been demonstrated in athletes preparing for the Rio Olympics (Drew et al., 2017).

Strategies to maintain multi-competition/event athlete health

- Manage stress and anxiety: Refer to Table 3.1 for guidelines.
- Mindfulness practices: Mindfulness interventions such as meditation, breathing awareness, walking and yoga have the potential to alleviate psychological stress and anxiety. Recent studies have reported significant improvements to athletes' mental state with 4–6 weeks of mindfulness training (Ajilchi et al., 2019; Chen et al., 2019). Furthermore, in wheelchair basketball players, 8-weeks of mindful mediation utilising a smart phone app attenuated the rise in cortisol associated with a competition period (MacDonald & Minahan, 2018). However, immune responses did not appear to be influenced (MacDonald & Minahan, 2018). It is currently unclear if mindful practices influence URTS risk in athletes. Nevertheless, in the general population, a reduction in URTS incidence has been demonstrated following 8-weeks of mindful meditation (Barrett et al., 2012). Mindfulness practices appear to be a promising strategy for athletes, although, further investigation is warranted. Athletes planning to use mindfulness interventions at the Summer Games should pilot and optimise practices in an off season/preparatory period.
- Supplementation: See section "Strategies to Maintain Female Athlete Health" for details.

3.5 Conclusions

It is apparent that athletes will be exposed to various stressors during both the preparatory and competition phases of the Summer Games. Athletes residing in the Southern hemisphere appear to be at increased risk for URTS episodes during the preparatory phase; while female, Paralympic, water-sport and multi-competition/event athletes may be more susceptible to illness during the competition phase of the Summer Games. To maintain athlete health, illness prevention strategies should be targeted to stressors and at-risk athletes. Keeping athletes healthy will contribute to optimal Olympic

and Paralympic athletic performance. While the considerations and strategies outlined in this short review are targeted for the Summer Games, many could be used for other major competitions and, as such, should be considered for future sporting success.

CHAPTER 4 GENERAL METHODOLOGY

A similar methodology was used in several studies of this thesis. To avoid repetition, the methods used in more than one of the chapters 5–8 are described in this section.

4.1 Saliva sampling and analysis

Saliva collection

Unstimulated whole saliva samples were collected by passive drool method. Athletes were instructed to dribble into a pre-weighed plastic container with minimal orofacial movement, eyes open and head tilted slightly forward.

SIgA analysis

SIgA concentration was analysed using a previously published in-house, sandwich enzyme-linked immunosorbent assay (ELISA) method (Leicht et al., 2011). Flat-bottomed 96 well microtitration plates (Nunc Immunoplate, Life Technologies, USA) were coated with a buffer containing 1 $\mu l \cdot ml^{-1}$ rabbit anti-human IgA secretary component capture antibody (Dako, Australia) in 1 ml^{-1} of carbonate/bicarbonate solution (pH 9.6), covered, and incubated overnight at 4°C. Plates were then washed four times (300 $\mu l \cdot well^{-1}$) with a wash buffer (PBS, 0.03 $mmol \cdot L^{-1}$,0.1%Tween) and blocked with 2% bovine serum albumin in PBS (100 $\mu l \cdot well^{-1}$) (Fraction V, Sigma-Aldrich, St. Louis, Missouri, USA), covered and incubated at room temperature (22°C) for 60 minutes.

Thawed saliva samples were spun for two minutes at 13,400 rpm and the supernatant was diluted by 1:1000 with PBS. Purified secretory IgA from bovine colostrum was used as a standard (Sigma-Aldrich, St. Louis, Missouri, USA). Working standards were prepared as follows: the top working standard of 1.0 $\mu g \cdot ml^{-1}$ IgA was prepared in PBS and diluted serially with PBS to give working standards of 1 $\mu g \cdot ml^{-1}$, 0.5 $\mu g \cdot ml^{-1}$, 0.25 $\mu g \cdot ml^{-1}$, 0.125 $\mu g \cdot ml^{-1}$, 0.0625 $\mu g \cdot ml^{-1}$, 0.03125 $\mu g \cdot ml^{-1}$, 0.015625 $\mu g \cdot ml^{-1}$ and 0 $\mu g \cdot ml^{-1}$

(PBS only). Following plate washing, 50 μl of standards and diluted saliva samples were loaded in duplicate, plates were sealed, and incubated at 4°C overnight.

After washing the plates, detection antibody (1:2000 dilution of Polyclonal Rabbit Anti-Human IgA/HRP (Dako, Australia) in PBS) was loaded and plates were incubated at room temperature (22°C) for 90 minutes. Following the final plate washing, 100 $\mu l \cdot well^{-1}$ of a chromogenic substance (TMB + Substrate-Chromogen, Dako, Australia) was applied and plates were incubated in the dark at room temperature (22°C) for 8 minutes; 100 μl of a stopping solution (1 $mmol \cdot L^{-1}$ H_2SO_4) was then added to each well and the absorbance of individual samples were immediately determined spectrophotometrically at 490 nm on an automated absorbance plate reader (Multiskan Go, Thermo Fisher Scientific, USA). A graph was then plotted using prism (version 6) with the mean optical density readings of the standards on the γ axis and SIgA concentrations of the standards plotted on the χ axis. A polynomial standard curve was fitted and the SIgA concentration of the samples calculated. The absolute SIgA concentration of samples were calculated by multiplying the sample concentration by the dilution factor (1000) to give a final concentration ($\mu g \cdot ml^{-1}$).

Salivary cortisol analysis

Thawed and centrifuged saliva samples were analysed for cortisol concentration using an electrochemiluminescence immunoassay (Cobas Modular P170 Analyser, Roche Diagnostics, New Zealand). According to the manufacturers' protocol, measurement range was between 1.5 and 1750 $nmol \cdot L^{-1}$.

4.2 Blood sampling and analysis

Blood collection

All blood samples in this thesis were collected by venepuncture from an antecubital forearm vein with the athlete in an upright seated position. Blood was collected into serum and dipotassium ethylenediamine tetra-acetic acid (K_2EDTA) vacutainers (Becton Dickinson and Co, USA).

Haematological analysis

Blood in K_2EDTA vacutainers were used for haematological analysis to determine total circulating leukocyte and differential white blood cell (WBC) counts: neutrophils, lymphocytes, monocytes and eosinophils (XT-2000i, Sysmex Corporation of America, Long Grove, Illinois, USA). $CD4^+$ and $CD8^+$ subsets of lymphocytes were assessed with a Muse Cell Analyser (Merck Millipore, Abacus Dx, New Zealand). The Muse Cell Analyser features miniaturised fluorescence detection and microcapillary technology for rapid, accurate and reliable quantitative cell analysis. Cell concentrations for $CD4^+$ and $CD8^+$ were calculated by multiplying the percentage of these cells with the total circulating lymphocyte cell number.

CMV antibody analysis

Thawed and centrifuged serum samples were analysed for detection of CMV antibodies, immunoglobulin (Ig)M and IgG (Cobas Modular P170 Analyser, Roche Diagnostics, New Zealand), in accordance with the manufacturers' instructions. The presence of CMV IgM antibodies is suggestive of an acute, recent or reactive infection and the presence of IgG antibodies indicates prior infection (Griffiths et al., 2015).

Multi-antigen stimulated cytokine production by whole blood culture

Stimulated whole blood cultures were set up as follows: in non-treated, sterile tissue culture plates (Guangzhou Jet Bio-Filtration Co. Ltd, China), 3600 μL of blood in K_2EDTA

vacutainers was added to 400 μL of RPMI medium (Thermo Fischer Scientific, USA) with an added stimulant at a dilution of 1:100, giving a final stimulant dilution of 1:1000. The stimulant was a commercially available multi-antigen vaccine and the dilution was chosen based on a separate experiment, which established the dose-response curves for the measured cytokines over the dilution range of 1:100–1:20,000. Our selected stimulant dilutions are similar to those previously used (He, Fraser, et al., 2014; Svendsen et al., 2014). Whole blood cultures were incubated for 24 hours at 37°C and 5% CO_2 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Following incubation, stimulated whole blood samples were transferred into Eppendorf tubes and centrifuged for 4 minutes at 12,000 g, and separated supernatant was collected and aliquoted into Eppendorf tubes to be stored at -80°C for later analysis. A multiplex kit (HCYTOMAG-60K, MILLIPLEX) was used to analyse thawed stimulated supernatant for IFN-γ, IL-10 and IL-4 concentrations. Results were obtained with a MAGPIX system (Luminex MAGPIX, Austin, Texas) with Luminex xPONENT software for MAGPIX (version 4.2).

4.3 Self-reported data

Self-reported URTS data

Self-reported URTS data were collected using a modified Jackson URTS questionnaire (Jackson et al., 1958), as previously reported (Gleeson et al., 2012). The URTS listed on the questionnaire included repetitive sneezing, runny and/or blocked nose, sore throat, catarrh in throat, cough, fever, persistent muscle soreness, joint aches and pains, weakness, and loss of sleep. Athletes provided a severity rating for each symptom of light, moderate or severe (scored as 1, 2 or 3, respectively). A weekly total symptom score was calculated by multiplying the total number of days that the symptom was present by the numerical rating (1-3). In any given week, a total symptom score ≥12 indicated the presence of an URTS episode (Gleeson et al., 2012). A single URTS episode was defined as a period where the weekly total symptom score was ≥12 and

separated by at least one week from another week with a total symptom score ≥12 (Gleeson et al., 2012).

Self-reported training and competition load data

Duration and session ratings of perceived exertion (sRPE) were recorded for all training sessions and matches to calculate an overall weekly physical load. Athletes provided a global intensity rating of the entire training session or match on a scale using rating ranges from 0 (rest) to 10 (maximal) (CR-10 RPE scale), as presented in Table 4.1 (Foster et al., 2001). Internal training load was calculated by multiplying the sRPE by the session duration (min) (Foster et al., 2001).

Table 4.1 The CR-10 RPE scale used by athletes to classify training and match intensity

Rating	Descriptor
0	Rest
1	Very, Very Easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	-
7	Very Hard
8	-
9	-
10	Maximal

CHAPTER 5 PREDICTORS OF UPPER RESPIRATORY TRACT ILLNESS RISK: DIFFERENCES BETWEEN ELITE RUGBY UNION AND LEAGUE PLAYERS.

Prelude

Chapter 2 explored the potential for biomarkers and self-reported data to predict team-sport athletes' risk for URTS episodes. However, discrepant findings for URTS predictors were revealed in the literature, and this may be explained by two factors: 1) methodological differences between studies; and 2) possible sport and/or cohort specific differences. To eliminate methodological differences, this study used the same methodology to compare the efficacy of biomarker and self-reported data in predicting URTS risk in two different elite sporting teams; namely, elite rugby union and league.

5.1 Abstract

Purpose: This prospective cohort study examined possible predictors of URTS risk in elite rugby union and league players (n=51) over an intensive pre-season training period.

Methods: Baseline saliva and blood samples were collected in the first week of pre-season training for analysis of SIgA, S-cortisol, WBC counts and CMV. Thereafter, SIgA, S-cortisol, URTS, internal training load and self-reported wellness data including stress, mood, motivation, fatigue, muscle soreness and sleep quality were repeatedly measured throughout a 10-week pre-season training period.

Results: Rugby union and league players experienced a similar number of URTS episodes; however, predictors of URTS episodes differed between the codes. No biomarkers or self-reported measures significantly predicted URTS risk in rugby union players, while reductions in self-reported total wellness and sleep quality predicted increased URTS risk in rugby league players. In both rugby codes, internal training load was positively associated with S-cortisol, suggesting it may be a useful marker to assess players' physiological responses to training and risk for maladaptive responses.

Conclusions: The findings from this study highlight that factors influencing URTS risk are perhaps sport specific and this may be attributed to different sporting demands and/or different management of players by team-practitioners.

5.2 Introduction

Pre-season is a critical phase of training for elite rugby union and league players to prepare for the physical demands of competition. Preventing illness—caused by opportunistic infection or reactivation of latent viruses—is paramount to optimise athletes' training availability and, ultimately, performance (Raysmith & Drew, 2016). However, intensified training, a typical characteristic of pre-season training in elite rugby union and league (Argus et al., 2010; Killen et al., 2010), can increase illness risk by suppressing various aspects of immune function (Gleeson & Pyne, 2016). Indeed, despite the relatively short duration of pre-season training (8-11 weeks), ~20-50% of elite rugby union and league players experience at least one illness; most commonly URTS (e.g., sore throat, headache, runny nose and coughing) (Cunniffe et al., 2011; Thornton et al., 2016; Tiernan et al., 2020).

To maintain rugby union and league players' health during pre-season training, identifying valid biomarkers and/or self-reported measures with the potential to predict URTS risk is critical. SIgA is a biomarker that contributes to the bodies first line of immune defence against infectious pathogens and antigens presented at the mucosa (Bishop & Gleeson, 2009). In athletes, low or declining levels of SIgA have been associated with an increased risk of URTS (Gleeson & Pyne, 2016; Gleeson et al., 2017). However, discrepant findings have been reported in elite Northern Hemisphere rugby union players, with a significant inverse association (Tiernan et al., 2020) or no association (Cunniffe et al., 2011) found between SIgA concentration and URTS. To date, the relationship between SIgA and URTS remains unknown in elite Southern hemisphere rugby union and league players who differ from their Northern Hemisphere counterparts in training regimes, competition schedules, climate exposure and player ethnicities (Jones et al., 2017; Schwellnus et al., 2012).

CMV is another biomarker that has shown promise in predicting athletes' 'potential' URTS risk (Gleeson & Pyne, 2016; Simpson et al., 2016). CMV is a herpes virus that

persists in the human body for life following primary infection (Griffiths et al., 2015). Reactivation of latent CMV can occur when the cellular immune system is compromised (Glaser & Kiecolt-Glaser, 1994; Simpson et al., 2016). In athletes, reactivation of latent herpes viruses have been found to precede the appearance of URTS (Gleeson et al., 2002; Yamauchi et al., 2011b), and are suggested to account for ~25-55% of all URTS (Gleeson & Pyne, 2016). However, the relationship between CMV and URTS risk remains unknown in elite rugby union and league players. CMV is a particularly interesting marker to assess in rugby union and league players given close physical contact and trauma (i.e., abrasions and lacerations of skin) can facilitate the transmission of herpes viral infections (White & Grant-Kels, 1984).

In addition to SIgA and CMV, simple and cost-effective self-reported measures of internal training load and wellness may predict athletes' URTS risk. To date, URTS in team-sport athletes have been linked to changes in internal training load and/or wellness measures in some (Fitzgerald et al., 2019; Thornton et al., 2016; Watson et al., 2016), but not all studies (Ahmun et al., 2019; Martin Buchheit et al., 2013). Previous team-sport studies are limited in that they have tended to examine either self-reported measures (Drew et al., 2017; Thornton et al., 2016) or biomarkers (Cunniffe et al., 2011; Tiernan et al., 2020) as predictors of URTS. Further research simultaneously evaluating both measures is required to ascertain whether one approach or the combination of approaches is most effective in predicting URTS risk.

Methodological heterogeneity may account for the conflicting evidence in the team-sport literature evaluating predictors of URTS. For example, specimen collection and analysis (Papacosta & Nassis, 2011), and self-reported questionnaires (Taylor et al., 2012) vary considerably between previous studies. However, it is possible that predictors of URTS are sport specific. To eliminate methodological differences, research directly comparing different sports is needed. Rugby union and rugby league are closely aligned sports that contain similarities yet fundamental differences; rules of play vary between the codes,

and generally, rugby union is a slower paced more tactical game, while rugby league is more free flowing with more ball-running and tackling (Hogarth et al., 2016). Due to the different sporting demands associated with rugby union and league, it is possible that predictors of URTS may differ. It is therefore of interest to determine if there are any biomarkers and/or self-reported measures strong enough to predict URTS risk in both rugby union and league players.

Accordingly, given the lack of research directly comparing biomarkers, self-reported measures and URTS episodes between different elite sporting teams, the aim of this study was to identify possible predictors of URTS risk in elite rugby union and league players during an intensive pre-season training period.

5.3 Methods

Participants

Twenty-eight male rugby union players and twenty-three male rugby league players participated in the study. All rugby union and league players were contracted to elite teams that competed in the 2017 Southern hemisphere premier rugby union competition (Super Rugby) and Australian premier rugby league competition (National Rugby League), respectively. Supplement use was permitted during the study. Rugby union players were provided oral and gastrointestinal probiotics, berry (polyphenol) concentrate and a multi-vitamin by the team dietitian; rugby league players were provided zinc, glutamine, vitamin D3 and vitamin C by the team trainer. The rugby union team dietitian and rugby league team trainer reported good compliance with supplement use; however, precise data on each player's supplement intake was not provided to the researchers. Players were informed of the rationale, aims and requirements of the study before providing written consent (Appendices A and B). The study was approved by the Auckland University of Technology Ethics Committee (Auckland, New Zealand). A summary of participant characteristics is presented in Table 5.1.

Table 5.1 Participant characteristics

	Rugby union (n=28)	**Rugby league (n=23)**	**P value**
Age (yr)	25.1 ± 3.4	23.8 ± 4.2	0.204
Body mass (kg)	101.1 ± 10.8	100.2 ± 9.9	0.766
Height (cm)	186.9 ± 7.1	185.1 ± 4.9	0.317
Positions (backs, forwards)	13, 15	12, 11	NC

NC: Not calculable

Study design

A prospective cohort repeated-measures study design was used to collect URTS, biomarker, internal training load and self-reported wellness data during a pre-season training period in elite rugby union and league players. In both codes, pre-season training primarily consisted of a high-volume, high-intensity training regime incorporating conditioning, gym/resistance, and field-based rugby training. Rugby union and league

teams competed in pre-season tournaments; the 'Brisbane Tens' and the 'Auckland NRL Nines' in weeks 7 and 9, respectively. Rugby union and league games were modified for the tournaments: ten players per side with ten minutes per half and nine players per side with nine minutes per half were the competition formats for the 'Brisbane Tens' and 'Auckland NRL Nines' tournaments, respectively. The knockout tournaments ran over two days, and both teams played three games each. All data were collected from the teams' training facilities during Southern hemisphere summer.

Baseline blood and saliva collection

Baseline blood and saliva samples were collected in the first week of pre-season training. Players arrived at their team training facility between 08:00 and 09:00 hours following an overnight fast. They were requested to avoid caffeine and alcohol 24 hours prior to arrival. Players sat quietly for 10 minutes and completed a health screen questionnaire (Appendix C). Subsequently, resting blood and saliva samples were collected, as described in Chapter 4. For all players, blood was collected into a 1 x 8 ml serum vacutainer and a 1x 6 ml K_2EDTA vacutainer. Blood was kept at room temperature and saliva was stored on ice for 1-1.5 hours before being transported back to the laboratory for sample preparation and analysis. Blood in the serum vacutainer was centrifuged at 1500 *g* for 10 minutes at 4˚C and separated serum was aliquoted into Eppendorf tubes and stored at -80˚C until CMV analysis. K_2EDTA blood was used for haematological analysis. Saliva was transferred into Eppendorf tubes to be stored at -80˚C until SIgA and S-cortisol analysis.

Saliva collection

During the pre-season period, saliva samples were collected weekly (excluding the Christmas break) between 07:00 and 8:00 hours, following an overnight fast. A total of 10 saliva samples were collected mid to late week (i.e., Wednesday, Thursday or Friday) during the pre-season training period.

URTS data

Self-reported URTS data were collected on a weekly basis using a modified URTS questionnaire (see Chapter 4).

Training and competition load

Duration (minutes) and sRPE using the CR-10 sRPE scale were recorded for all training sessions and matches to calculate internal training load (see Chapter 4).

Self-reported wellness

Rugby union and league players completed an online wellness questionnaire (4±1 days·week^{-1}). The teams existing wellness questionnaires were used becuase team practitioners were not prepared to change these for the purpose of this study; therefore, questionnaires differed with regards to the questions asked and scales used. Rugby union players numerically rated sleep quality (1 = 'terrible' to 5 = 'good'), mood (1 = 'highly annoyed, irritable &/or down' to 5 = 'great'), muscle soreness, stress levels and fatigue (1 = 'very high' to 5 = 'none'). Rugby league players numerically rated sleep quality (1 = 'terrible' to 10 = 'good'), motivation to train (1 = 'none' to 10 = 'very high'), stress, upper body soreness and lower body soreness (1 = 'very high' to 10 = 'none'). For both teams, the summation of the five individual ratings provided a total wellness score (Buchheit et al., 2013). Rugby union and league players also reported sleep quantity (hours).

Laboratory analysis

SIgA analysis

SIgA concentration was determined using an in-house sandwich ELISA method, as described in Chapter 4. All saliva samples from each player were analysed in duplicate on one plate. The between-run coefficient of variation (CV) was 5.8 ± 4.7%.

S-cortisol analysis

Saliva samples were analysed for cortisol at specified time-points: baseline (week 0), midway through pre-season training (week 5), the week prior to pre-season tournaments (rugby union: week 7; rugby league: week 9), the week following pre-season tournaments (rugby union: week 8; rugby league: week 10) and at the end of the pre-season monitoring period (week 10). Thawed and centrifuged saliva samples were analysed for S-cortisol concentration (see Chapter 4).

Baseline blood cell count analysis

Blood in K_2EDTA vacutainers were used for haematological analysis to determine total circulating leukocyte and differential WBC counts: neutrophils, lymphocytes, monocytes and eosinophils (see Chapter 4).

Baseline CMV antibody analysis

Thawed and centrifuged serum samples were analysed for detection of CMV antibodies, IgM and IgG (see Chapter 4). The presence of CMV IgM antibodies is suggestive of an acute, recent or reactive infection and the presence of IgG antibodies indicates prior infection (Griffiths et al., 2015).

Data analysis

Prior to analysis, the data were appraised for normality using the Shapiro-Wilk test. The data are presented as mean ± standard deviation (unless otherwise specified). The variability of SIgA concentration within- and between- players is presented as the CV. Internal training load, SIgA concentration, S-cortisol concentration and baseline immune data were compared between rugby union and league players using unpaired t-tests or Mann Whitney U tests. Associations between SIgA and S-cortisol, and training load and S-cortisol, were examined using a linear regression for rugby union and league players collectively.

To determine predictors of URTS risk, survival analysis was performed for each rugby code. Frailty model analysis was selected as it enables analysis where subjects can experience an episode (i.e., URTS episode) more than once over the study period. Univariate frailty model analysis for URTS risk was run for the following independent predictor variables: SIgA concentration, internal training load, total wellness, sleep quantity, sleep quality and stress. The significance threshold for the current study was $p<0.05$. Frailty model analysis was performed using Stata (version 15) and all other statistical analysis was conducted in IBM SPSS (version 25).

5.4 Results

URTS incidence, severity, and duration

A total of 31 URTS episodes occurred over the pre-season period; 13 (42%) in rugby union and 18 (58%) in rugby league players. The proportion of players that experienced ≥1 URTS episodes was 0.36 (i.e., 36% of players) in rugby union and 0.52 in rugby league ($p = 0.242$). Thirty players (rugby union: 18; rugby league: 12) did not experience a single URTS episode, fourteen players (rugby union: 7; rugby league: 7) experienced one URTS episode and seven players (rugby union: 3; rugby league: 4) experienced ≥2 URTS episodes. When a URTS episode was present, there was no difference in the total weekly symptom severity score (rugby union 20.9 ± 8.5, rugby league 29.2 ± 15.2; $p = 0.117$) or duration of symptoms (rugby union 3.4 ± 0.8, rugby league 3.6 ± 1.82; $p = 0.867$) between rugby union and league players. A cluster of URTS episodes occurred during the final week of monitoring (week 10) in rugby league players (Figure 5.1).

Internal training load and pre-season tournaments

Rugby union and league players' pre-season internal training load distributions are presented in Figure 5.1. Internal training load was significantly higher in rugby union players (6017 ± 1353 arbitrary units (AU)) than rugby league players (3610 ± 910 AU) ($p < 0.001$). Of the players that participated in the study, 43% and 57% of rugby union and league players, respectively, competed in pre-season tournaments.

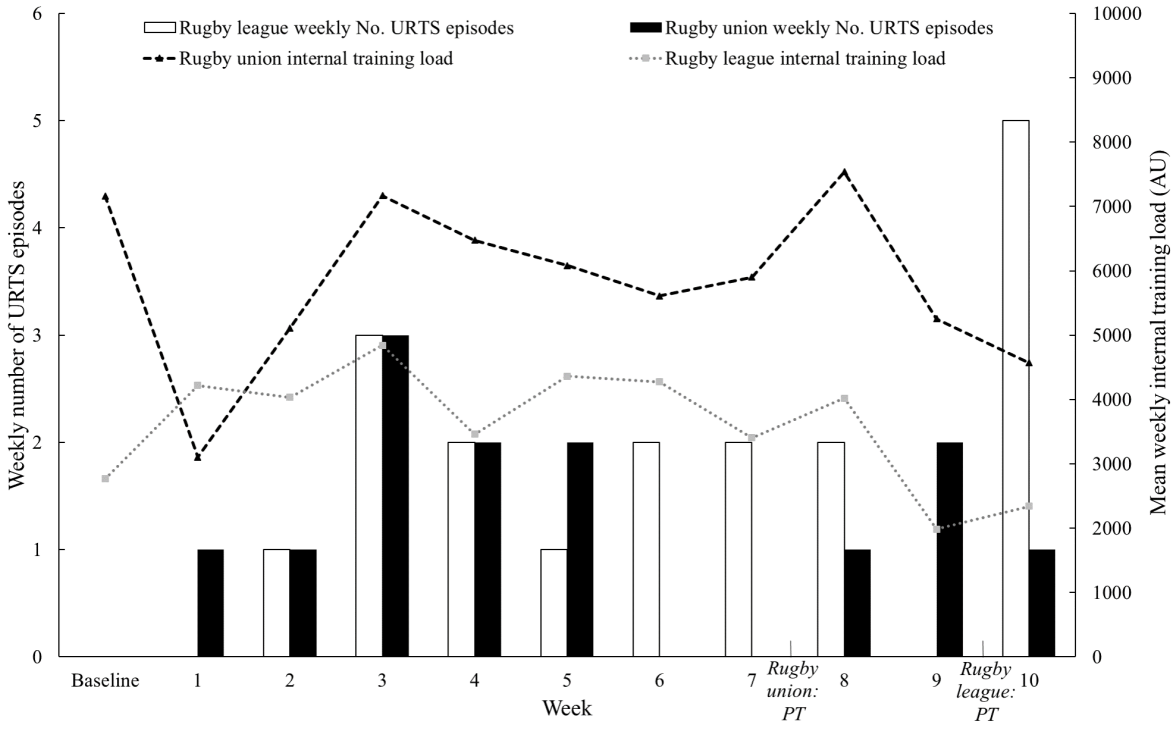

Figure 5.1 Rugby union and league players' weekly number of URTS episodes and internal training loads over the pre-season. The pre-season tournaments (PT) are demonstrated on χ the axis; the rugby union 'Brisbane Tens' and rugby league 'Auckland NRL Nines' occurred at the end of weeks seven and nine, respectively. Standard error bars were omitted for clarity.

SIgA and S-Cortisol

Rugby union and league players' SIgA and S-cortisol concentrations over the pre-season period are presented in Figure 5.2. Five-hundred samples were analysed for SIgA (rugby union: 276 samples; rugby league: 224 samples). SIgA concentration was significantly higher in rugby union players (345 ± 195 $\mu g \cdot mL^{-1}$) compared to rugby league players (273 ± 175 $\mu g \cdot mL^{-1}$) ($p < 0.001$). The CVs for SIgA concentration were 35% and 48% (within-player) and 56% and 61% (between-player) in rugby union and league players, respectively. Two-hundred and fifteen samples were analysed for S-cortisol concentration (rugby union: 125; rugby league: 90). S-cortisol concentration was significantly higher in rugby union players (12.7 ± 5.7 $nmol \cdot L^{-1}$) compared to rugby league

players (8.8 ± 4.6 nmol·L^{-1}) ($p < 0.001$). No association was found between SIgA concentration and S-cortisol concentration ($p = 0.121$). However, S-cortisol was positively associated with internal training load ($r = 0.501$, $p = 0.033$).

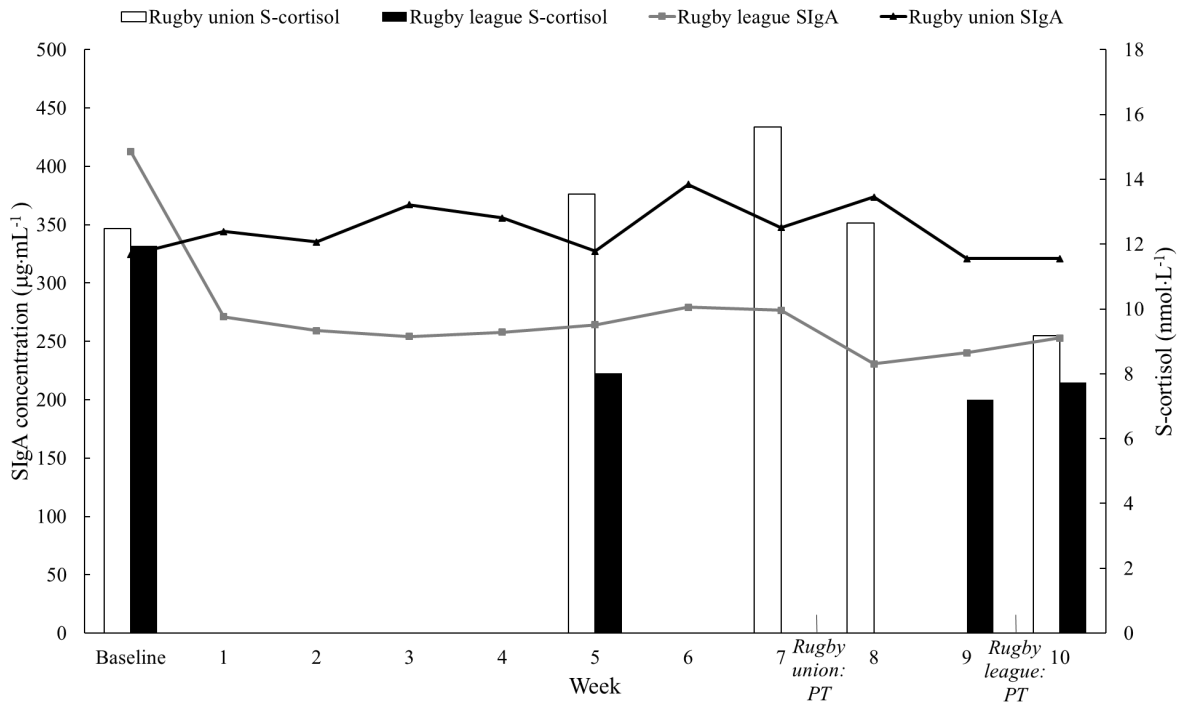

Figure 5.2 Rugby union and league players SIgA and S-cortisol concentrations over the pre-season period. The pre-season tournaments (PT) are demonstrated on the χ axis; the rugby union 'Brisbane Tens' and rugby league 'Auckland NRL Nines' occurred at the end of weeks seven and nine, respectively. Where S-cortisol concentration equals 0 nmol·L-1, saliva samples were not analysed for cortisol. Standard error bars were omitted for clarity.

Baseline data

There were no significant differences in baseline biomarkers between rugby league and union players (Table 5.2).

Table 5.2 Baseline data in rugby union and rugby league players

	Rugby union (28)	Rugby league (23)	P value
History of asthma (%)	18	26	NC
History of allergies (%)	18	26	NC
History of recurrent URTS (%)	36	30	NC
SIgA concentration ($\mu g \cdot mL^{-1}$)	324 ± 179	413 ± 197	0.121
S-cortisol concentration ($nmol \cdot L^{-1}$)	13.3 ± 7.9	12.0 ± 5.1	0.984
Leukocyte count (x 10^9 $cells \cdot L^{-1}$)	6.3 ± 1.2	6.2 ±1.2	0.439
Neutrophil count (x 10^9 $cells \cdot L^{-1}$)	3.2 ± 1.0	2.8 ±0.8	0.200
Monocyte count (x 10^9 $cells \cdot L^{-1}$)	0.6 ± 0.2	0.6 ± 0.2	0.765
Eosinophil count (x 10^9 $cells \cdot L^{-1}$)	0.2 ± 0.1	0.3 ± 0.2	0.346
Total lymphocyte count (x 10^9 $cells \cdot L^{-1}$)	2.2 ± 0.5	2.5 ± 0.6	0.809
CMV seropositive (%)	73	96	NC
CMV IgG antibody titer ($IU \cdot mL^{-1}$)	120 ± 143	173 ± 171	0.145

NC: Not calculable

SIgA concentration and URTS risk

SIgA concentration was inversely associated with URTS incidence in rugby union players (Table 5.3); however, the increase in URTS risk was small (~1%) and nonsignificant (p = 0.094). No association between SIgA concentration and URTS risk was demonstrated in rugby league players (p = 0.593) (Table 5.4).

Self-reported data and URTS risk

Total wellness and sleep quality were significant predictors of URTS risk in rugby league players, for which players were ~2-3% more at risk for URTS when total wellness (p = 0.004) and sleep quality (p = 0.001) were reduced (Table 5.4). However, these self-reported measures did not predict URTS risk in rugby union players (Table 5.3). Internal training load, sleep quantity and stress did not predict URTS risk in rugby union (Table 5.3) or league players (Table 5.4).

Table 5.3 Rugby union players (n=28) hazard ratios for URTS episodes

	Hazard ratio	Standard error	Z value	P value	95% confidence interval
SIgA concentration	0.997	0.002	-1.67	0.094	0.993-1.000
Internal training load	1.000	0.000	0.96	0.339	0.999-1.000
Total wellness	0.955	0.121	-0.37	0.714	0.744-1.22
Sleep quantity	0.542	0.217	-1.53	0.126	0.276-1.393
Sleep quality	0.621	0.256	-1.16	0.248	0.276-1.393
Stress	0.711	0.314	-0.77	0.441	0.299-1.691

Table 5.4 Rugby league players (n=23) hazard ratios for URTS episodes

	Hazard ratio	Standard error	Z value	P value	95% confidence interval
SIgA concentration	1.001	0.001	0.53	0.593	0.998-1.003
Internal training load	1.000	0.000	0.38	0.703	0.999-1.000
Total wellness	0.731	0.080	-2.84	0.004	0.590-0.907
Sleep quantity	0.758	0.190	-1.10	0.269	0.464-1.239
Sleep quality	0.345	0.114	-3.20	0.001	0.180-0.662
Stress	0.559	0.248	-1.31	0.189	0.235-1.333

5.5 Discussion

The main findings of the current study were; 1) URTS incidence did not significantly differ between elite rugby union and league players; 2) predictors of URTS risk appeared to differ between the codes; and 3) internal training load was positively associated with S-cortisol. These findings may assist team-sport practitioners in effectively monitoring and managing players to potentially reduce URTS risk.

The URTS incidence in rugby union and league players in the current study was consistent with previous studies (Cunniffe et al., 2011; Thornton et al., 2016; Tiernan et al., 2020) which collectively suggest that ~20-50% of rugby union and league players experience a least one URTS episode during pre-season training. Congested competition has been identified as a period of increased risk for URTS in athletes (Gleeson & Pyne, 2016), and in support this study found a spike in URTS incidence in rugby league players following the 'NRL Nines' pre-season tournament. However, given only two out of five rugby league players experiencing an URTS episode competed in the pre-season tournament, accumulation of pre-season training loads rather than the pre-season tournament itself may explain the increased URTS incidence. Indeed, a systematic review concluded that accumulation in training load is a key risk factor for illness (Jones et al., 2016). It is interesting that this period of increased risk for URTS in rugby league players was not observed in rugby union players. Nevertheless, the rugby union and league teams were managed differently (e.g., training periodisation and supplementation) which may explain why URTS distribution differed between the rugby codes.

Previous studies conducted in team-sport athletes have often reported either biomarker (Cunniffe et al., 2011; Tiernan et al., 2020) or self-reported (Drew et al., 2017; Thornton et al., 2016) measures when it comes to predicting URTS risk. To gain a more comprehensive understanding and to determine whether one is a better predictor than the other, we captured and evaluated these measures together. Interestingly, predictors

differed between rugby codes; no biomarkers or self-reported measures predicted URTS risk in rugby union players, while self-reported total wellness and sleep quality predicted increased URTS risk in rugby league players. Although rugby codes are similar, physical and physiological demands do differ (Hogarth et al., 2016), as evidenced by the significantly higher pre-season internal training loads observed in rugby union players compared to rugby league players. As such, our findings suggest that predictors of URTS risk may, in fact, be sport and/or cohort specific. Furthermore, it is possible that predictors of URTS risk are macrocycle specific, given players are exposed to different stressors in each macrocycle. For example, pre-season training involves intensive training regimes for which training loads can be 2-4 times greater than the competition period (Argus et al., 2010); whereas during competition, players are exposed to stressors of air travel and intensive competition (Walsh, Gleeson, Pyne, et al., 2011). Therefore, team-sport practitioners should be mindful that predictors of URTS risk during the pre-season may not necessarily be applicable during competition due to the different demands associated with each macrocycle.

SIgA concentration did not predict URTS risk in rugby union or league players. Similarly, previous research by Cunniffe et al. (2011) found no relationships between URTS and SIgA in elite rugby union players during an 11-month season. In contrast, Tiernan et al. (2019) demonstrated a significant inverse association between SIgA concentration and URTS in elite rugby union players during pre-season training. The discrepant findings presented by Tiernan et al. (2019) may be explained by their use of biweekly saliva sampling. Saliva sampling was only performed weekly in the current study and monthly by Cunnife et al. (2011). As such, the usefulness of measuring SIgA to predict URTS risk may depend on the saliva sampling frequency, with more frequent sampling increasing the likelihood of detecting a reduction in SIgA (Tiernan et al., 2020). However, frequent SIgA measurement may be impractical in an elite team-sport setting due to the constraints of SIgA monitoring such as analysis costs and the pre-sample standardisation required to control for factors known to influence SIgA (e.g., nutrition,

oral health, caffeine, diurnal and seasonal variation) (Bishop & Gleeson, 2009; Dumortier et al., 2020; Pritchard et al., 2017). Therefore, a more feasible approach may be to only monitor SIgA concentration in at risk rugby union and league players (i.e., those who experience recurrent URTS) (Simpson et al., 2020).

S-cortisol appears to be a mechanism underlying alterations in SIgA, with an inverse association between S-cortisol and SIgA reported in team-sport athletes (Cunniffe et al., 2011; He et al., 2010). In contrast, we found no association between S-cortisol and SIgA; a finding also supported in soccer players (Moreira et al., 2014; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012). However, we did find a significant positive association between S-cortisol and training load which aligns with previous team-sport research (Moreira et al., 2012; Rowell et al., 2018). Pre-season training is characterised by high training loads which may increase players risk for maladaptive responses including non-functional overreaching (NFO) and overtraining syndrome (OTS) (Cadegiani & Kater, 2017). Hormonal and immunological disturbances contribute to the development of NFO and OTS, and increased URTS incidence is a common symptom of these conditions (Cadegiani & Kater, 2017). Therefore, our findings suggest S-cortisol is a useful marker to monitor rugby players' physiological response to training; it could also serve as a potential indicator for NFO and OTS, and associated recurrent illness.

In the current study, the percentage of CMV seropositive rugby union players (73%) and league players (96%) were considerably higher than reported rates in endurance athletes (23-25%) (Gleeson et al., 2016; He, Handzlik, Muhamad, et al., 2013). The skin-to-skin contact and trauma (i.e., abrasions and lacerations of skin) inherent in playing rugby can increase risk for viral infection transmission (Stacey & Atkins, 2000), which likely explains the high CMV seropositivity observed in rugby union and league players. CMV seropositive athletes may be at an increased risk for URTS because latent CMV can reactivate and elicit URTS (Simpson et al., 2016). Transient reactivation of latent

CMV may account for the relatively short duration (2–4 days) of URTS experienced by athletes rather than a primary infection per se (Gleeson & Pyne, 2016). Indeed, in the current study the duration of URTS episodes in both rugby codes were relatively short (rugby union: 3.4 ± 0.8 days; rugby league: 3.6 ± 1.82 days); however, the relationship between CMV reactivation and URTS risk could not be assessed as CMV antibody titers were only measured at baseline. Given the high CMV seropositive results observed in elite rugby players, future studies incorporating more frequent measurement of CMV reactivation are needed to determine the link between viral control and URTS incidence.

Self-reported measures of total wellness and sleep quality were identified as predictors of URTS risk in rugby league players. In support, previous studies in elite rugby codes have demonstrated increased URTS susceptibility with reductions in overall wellbeing (Thornton et al., 2016) and sleep quality/quantity (Fitzgerald et al., 2019). However, in the current study, no relationship between these measures and URTS risk was found in rugby union players; a finding also reported in soccer (Buchheit et al., 2013) and cricket players (Ahmun et al., 2019). A limitation of the current study is that wellness questionnaires differed between rugby union and league teams, which may explain the discrepant results in this study and with the previous literature. A 10-point scale was used in the rugby league team and previous research supporting an association between self-reported wellness measures and URTS (Thornton et al., 2016); whereas, in the rugby union team and studies reporting no relationship (Ahmun et al., 2019; Buchheit et al., 2013), a 5-point scale was used. A greater number of points on a scale has been found to increase measurement sensitivity and reliability (Preston & Colman, 2000). Therefore, team practitioners should consider using a 10-point scale for wellness measures, given it appears to be more effective in predicting URTS risk.

Internal training load did not predict URTS risk in rugby union or league players. Our data contradicts the positive association found between internal training load and illness incidence in amateur team-sport athletes (Putlur et al., 2004; Watson et al., 2016).

However, the elite status of our participants may explain why no relationship was observed. Both rugby union and league teams had sport scientists who managed players training loads, thus minimising risk for URTS. Furthermore, our findings support the S-shape curve hypothesis which suggests that high training loads are associated with increased illness risk in recreational and sub-elite athletes, but not in elite athletes (Malm, 2006). Malm (2006) proposed that elite athletes are at a lower risk for illness, as to reach and maintain an elite status they must intrinsically possess or develop robust immune systems capable of withstanding infections during even severe physiological and psychological stress. Therefore, the observed lack of association between internal training load and URTS incidence may be attributed to the team-sport practitioners' management of players training loads and/or players robust immune systems.

5.6 Conclusion

In conclusion, predictors of URTS risk appeared to differ between rugby codes. No biomarker or self-reported measures predicted URTS risk in rugby union players, while reductions in self-reported wellness and sleep quality were predictive of increased URTS risk in rugby league players. The discrepant findings indicate that factors influencing URTS risk are perhaps sport and/or cohort specific, and this in part may be attributed to different sporting demands and/or different management of players by team-practitioners.

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CHAPTER 6 SELF-REPORTED LIFESTYLE AND BEHAVIOURAL FACTORS AS AN ALTERNATIVE TO BIOMARKER PREDICTORS OF RESPIRATORY ILLNESS RISK IN ATHLETES.

Prelude

Chapter 5 examined predictors of URTS risk in elite rugby union and league players during an 11-week preseason training period. However, as mentioned in Chapter 5, URTS predictors found during the pre-season may not necessarily be applicable during competition, because the competition period presents different demands and stressors that may influence URTS risk. Given the rugby codes followed differing programs and had different URTS predictors, it was decided that only one code would be followed throughout an entire season. As such, the study reported in this chapter described factors influencing URTS risk in elite rugby union players during an 8-month sporting season.

6.1 Abstract

Purpose: To identify periods of increased risk for URTS, and examine whether biomarkers and/or self-reported lifestyle and behavioural data have the potential to predict URTS risk in elite rugby union players.

Methods: A prospective longitudinal study was conducted in elite Southern hemisphere rugby union players (n=28). Baseline saliva and blood samples were collected for analysis of SIgA, S-cortisol, WBC counts, CMV and multi-antigen stimulated cytokine production. Thereafter, SIgA, S-cortisol, URTS, household illness, internal training load and self-reported wellness measures including stress, mood, fatigue, muscle soreness and sleep quality were repeatedly measured throughout an entire season. Univariate frailty model analysis, which included 495 observations over 30-weeks, was used to determine predictors of URTS risk.

Results: Surprisingly, rest weeks were associated with an increased risk for URTS; whereas URTS risk was reduced during weeks involving international travel (HR: 0.438, $p < 0.001$). Illness in players' households and SIgA concentration predicted URTS risk. Household illness was the strongest predictor; players were almost three-fold more at risk for URTS episodes when illness in the household was present (HR: 2.902, $p = 0.002$). A non-significant, but potentially important trend for an inverse association between SIgA concentration and URTS incidence was observed (HR: 0.99, $p = 0.070$), and a significant reduction (25%, $p = 0.008$) in relative SIgA concentration occurred in players experiencing URTS episodes.

Conclusion: Rest weeks were identified as periods of increased risk for URTS; while international travel did not appear to increase players risk for URTS. Incidence of household illness and reductions in SIgA concentration independently predicted increased URTS risk, with household illness being the strongest predictor. Household illness monitoring is a simple and inexpensive tool that practitioners can use in surrogate of SIgA to identify athletes' risk for URTS.

6.2 Introduction

Recurrent illness in athletes detracts from training availability, performance and success (Gleeson & Pyne, 2016). Elite rugby union players are invariably exposed to sport-related stressors that may suppress immunity and increase illness risk, including high training loads, competition and long-haul travel (Cunniffe et al., 2011; Schwellnus et al., 2012). Illness risk is compounded by the contact nature of rugby (tackling, scrums, rucks, mauls and collisions) and typical team-sport practices, such as bottle sharing and shaking hands, which augment pathogen transmission (Mela & Whitworth, 2014; White & Grant-Kels, 1984). Given the multi-stressor exposure associated with elite rugby union participation, it is unsurprising that ~70-90% of players experience at least one illness during training and competition periods; most commonly URTS episodes (Cunniffe et al., 2011; Schwellnus et al., 2012).

To monitor players' susceptibility to illness, identifying valid and relevant biomarkers to predict URTS risk is paramount. Blood biomarkers including CMV and multi-antigen stimulated cytokine production have been found to predict endurance athletes' 'potential' URTS risk (Gleeson et al., 2012; He, Handzlik, Muhamad, et al., 2013), although possible associations between these biomarkers and URTS susceptibility remains unknown in rugby players. It is acknowledged, however, that measurement of blood biomarkers may be limited in elite rugby settings due to the expense and invasiveness of these measures. SIgA is a more accessible biomarker that may predict athletes URTS risk, with low or declining levels of SIgA associated with increased incidence of URTS (Gleeson & Pyne, 2016).

Nevertheless, the use of SIgA as a biomarker to determine URTS risk in athletes remains uncertain (Simpson et al., 2020). Indeed, discrepant findings have been reported in elite Northern hemisphere rugby union players, with an inverse association (Tiernan et al., 2020) or no association (Cunniffe et al., 2011) found between SIgA concentration and URTS incidence. Whereas, the relationship between SIgA and URTS risk is currently

unknown in elite Southern hemisphere rugby union players who differ from their Northern hemisphere counterparts in competition formats, climate exposure and player ethnicities. Another key difference between Southern and Northern hemisphere elite rugby union is the travel schedule; Southern hemisphere competitions often span across countries that are greater distances apart (i.e., New Zealand, Australia, South Africa, Argentina and Japan) than Northern hemisphere rugby competitions which take place in neighbouring countries (i.e., England, Scotland, Wales, Ireland, France and Italy).

Emerging evidence suggests that long-haul travel is a risk factor for URTS in athletes (Walsh, 2018). Indeed, increased URTS incidence and severity has been reported in elite Southern hemisphere rugby players following long-haul travel (Fowler et al., 2016; Schwellnus et al., 2012). Heightened URTS risk following long-haul travel may be attributed to the direct stressors associated with aircraft travel including the confined space, close proximity to others, limited ventilation and recirculating dry air which can increase risk for infection transmission (Pipe, 2011). However, jet lag also occurs following travel across multiple time-zones, with disruptions of the circadian system and desynchronisation of immune modulating hormones including cortisol occurring (Doane et al., 2010; Waterhouse et al., 2002). Elevated cortisol concentrations can have immunosuppressive effects (Gleeson, 2007), which may explain athletes heightened URTS risk following long-haul travel. However, the current limitation to previous rugby studies (Fowler et al., 2016; Schwellnus et al., 2012), is that hormones and immune makers were not measured alongside illness reports. Therefore, possible immunoendocrine mechanisms underpinning increased URTS risk with long-haul travel remain unknown in elite rugby union players.

Another method to assess players risk for URTS relies on non-biological self-reported data, including lifestyle and behavioural factors such as wellness indicators and household illness incidence. Indeed, self-reported wellbeing (Thornton et al., 2016), stress (Watson et al., 2016) and sleep quantity (Fitzgerald et al., 2019) have been linked

to the incidence of URTS in team-sport athletes. However, in Chapter 5, self-reported wellness measures were not predictive of URTS risk in elite rugby union players during an intensive pre-season training period. Self-reported household illness may be a worthwhile measure for predicting URTS risk given close-contact between household members facilitates respiratory virus transmission (Tsang et al., 2016); however, this is yet to be investigated in athletic populations. Given self-reported lifestyle and behavioural data offer a more convenient, instantaneous and cost-effective approach than biomarker monitoring, further research is required to determine their effectiveness in predicting elite rugby union players' URTS risk.

The uniqueness of the elite Southern hemisphere rugby season provides a model to understand how different stressors influence URTS risk. If factors influencing URTS risk are identified, illness prevention strategies can be targeted to reduce players' risk for URTS. Therefore, the aims of the current study were to identify periods of increased risk for URTS and examine whether biomarkers and/or more accessible self-reported lifestyle and behavioural data can predict URTS risk in elite rugby union players.

6.3 Methods

Participants

Twenty-eight elite male rugby union players were included in the study (age 25.1 ± 3.4 y, body mass 101.1 ± 10.8 kg, height 1.88 ± 0.07 m). Positions included: forwards (n=15) and backs (n=13). Supplement use was permitted during the study; the team dietitian prescribed supplements to support immune function, including oral and gastrointestinal probiotics, berry (polyphenol) concentrate and a multi-vitamin. The team dietitian reported good compliance with supplement use; however, precise supplement intake was not measured as this was difficult to validate, and supplement intake varied between players. All players were informed of the rationale, aims and requirements of the study, before providing informed written consent (Appendices A and B). The study was approved by the Auckland University of Technology Ethics Committee (Auckland, New Zealand).

Study design

A prospective, longitudinal, repeated-measures study design was used to collect URTS, biomarker, internal training load, and self-reported lifestyle and behavioural data during an 8-month Super Rugby season (December 2016 to July 2017). Pre-season and competition phases are briefly summarised in Table 6.1. The first seven weeks of pre-season training involved a high-volume, high-intensity training regime incorporating conditioning, gym/resistance, and field-based rugby training, with no matches. The final three weeks of pre-season training included two domestic pre-season matches and a pre-season tournament—the ‘Brisbane Tens’. From late-February to mid-July, the team competed in the Southern hemisphere premier 2017 rugby union competition, ‘Super Rugby’; including 15 matches: 6 at home, 3 requiring domestic travel, and 6 requiring international travel. All analysis was completed after the season; therefore, no data were provided to players, team-practitioners, or coaches during the study period.

Table 6.1 Overview of the pre-season and competition phases

	Training week	Description of training week
Pre-season (Dec-Feb)	1	Start of pre-season, after 4-week rugby union offseason
	2-7	Pre-season training: High volume training, no matches
	8-10	Pre-season training and two matches and a tournament
Competition (Late Feb-Jul)	11-17, 19-25, 30	In-season training and 2017 Super Rugby competition
	18, 26	Bye weeks: No scheduled trainings or matches
	27, 28, 29	International test window: Training but no matches

Baseline blood and saliva collection

Baseline blood and saliva samples were collected in the first week of pre-season training; after a 4-week off-season. Players arrived at the team training facility between 08:00 and 09:00 hours following an overnight fast. They were instructed to avoid caffeine and alcohol 24 hours prior to arrival. Players then sat quietly for 10 minutes and completed a health screen questionnaire (Appendix C). Subsequently, resting blood and saliva samples were collected (see Chapter 4). For all players, approximately 20 ml of blood was collected into 1 x 8 ml serum and 2 x 6 ml K_2EDTA vacutainers. Blood was kept at room temperature and saliva was stored on ice for 1-1.5 hours before being transported back to the laboratory for sample preparation and analysis. Blood in the serum vacutainer was centrifuged at 1500 *g* for 10 minutes at 4˚C and separated serum was aliquoted into Eppendorf tubes and stored at -80˚C until CMV analysis. K_2EDTA blood was used for haematological analysis and whole blood cultures. Saliva was transferred into Eppendorf tubes to be stored at -80˚C until SIgA and S-cortisol analysis.

In-season saliva collection

Saliva samples were collected weekly, between 07:00 and 07:30 hours, following an overnight fast; excluding the Christmas break, bye weeks (i.e., no scheduled trainings or matches) and travel periods where the team was not in New Zealand. Ten saliva samples

were collected during the pre-season period; saliva collection took place on Thursday or Friday to capture the effects of weekly loading. During the competition phase, saliva samples were collected following 10 of the 15 matches played, including home matches (n=3), domestic travel matches (n=2) and international travel matches (n=5). Matches were played on Fridays, Saturdays or Sundays, and saliva samples were always collected 2- or 3- days post-match day for two reasons: 1) to avoid capturing acute perturbations in SIgA and S-cortisol levels associated with match-play; and 2) to provide time for the team to return to New Zealand following overseas matches. Of the 20 saliva samples collected during the season, six were collected following overseas matches; once during the pre-season (i.e., week 9) and five times during the competition phase (i.e., weeks 11, 21, 23, 25 and 30). Following overseas matches, the team travelled back to New Zealand ~1-day post-match, thus samples were typically provided 1- to 2- days post-international travel.

Player URTS monitoring

All players visited the team doctor on a weekly basis and those experiencing illness were required to complete a modified URTS questionnaire (see Chapter 4). The impact of URTS on training availability was determined by the team-practitioners through daily classification of players as: (i) unable to train; (ii) able to partake in modified training; or (iii) fit to train.

Training and competition load monitoring

Duration and sRPE using the CR-10 sRPE scale were recorded for all training sessions and matches to calculate internal training load (Foster et al., 2001) (see Chapter 4).

Self-reported lifestyle and behavioural measures

Players completed an online wellness questionnaire (3 ± 1 days·week^{-1}) to numerically rate their sleep quality (1 = ‘terrible’ to 5 = ‘good’), mood (1 = ‘highly annoyed, irritable &/or down’ to 5 = ‘great’), muscle soreness, stress levels and fatigue (1 = ‘very high’ to

5 = ‘none’). A total wellness score (ranging from 5-25) was calculated by adding the five individual numerical ratings (Buchheit et al., 2013). Players were also asked to provide a ‘yes’ or ‘no’ response to the question: *“Is anyone in your household ill?”*

Laboratory analysis

SIgA analysis

SIgA concentration was determined using an in-house sandwich ELISA method (see Chapter 4). All saliva samples from each player were analysed in duplicate on one plate. The between-run CV was 5.7 ± 4.7%. SIgA secretion rate ($\mu l \cdot min^{-1}$) was calculated by multiplying SIgA concentration ($\mu g \cdot mL^{-1}$) with saliva flow rate ($ml \cdot min^{-1}$) (Gleeson et al., 2012).

Salivary cortisol analysis

Thawed and centrifuged saliva samples were analysed for cortisol concentration (see Chapter 4).

Baseline blood cell count analysis

Samples in K_2EDTA vacutainers were used for haematological analysis to determine total circulating leukocyte and differential WBC counts: neutrophils, lymphocytes, monocytes and eosinophils (see Chapter 4). $CD4^+$ and $CD8^+$ subsets of lymphocytes were assessed with a Muse Cell Analyser (see Chapter 4).

Baseline CMV antibody analysis

Thawed and centrifuged serum samples were analysed for detection of CMV antibodies, IgM and IgG (see Chapter 4). The presence of CMV IgM antibodies is suggestive of an acute, recent or reactive infection and the presence of IgG antibodies indicates prior infection (Griffiths et al., 2015).

Baseline stimulated whole blood cytokine analysis

Samples in K_2EDTA vacutainers were used for determination of multi-antigen stimulated cytokine (IFN-γ, IL-10 and IL-4) concentrations by whole blood culture (see Chapter 4).

Data analysis

Data were appraised for normality using the Shapiro-Wilk test and reported using mean and standard deviation (unless otherwise specified). The variability of SIgA concentration and secretion rate within- and between- players was reported as the CV. Baseline demographic and biomarker data were compared between URTS-free players (i.e., no URTS), players who experienced 1-2 URTS episodes and URTS-prone players (i.e., players who experienced three or more URTS episodes) using one-way analysis of variance (ANOVA) tests or Kruskal–Wallis tests. Paired sample t-tests or Wilcoxon Signed Rank tests were performed to compare pooled mean SIgA concentration across time points from international travel weeks (n = 6 weeks) and non-travel weeks (n = 14 weeks). Additionally, the mean SIgA concentration from each of the individual six international travel weeks (i.e., week 9, 11, 16, 17, 19 and 2) were compared to the pooled mean SIgA concentration from all fourteen non-travel weeks using one-way analysis of variance (ANOVA) tests with Bonferroni post-hoc tests, or Kruskal-Wallis tests with pairwise Mann-Whitney U tests. T-tests or Mann Whitney U tests were used to compare mean internal training load, household illness and total wellness between pre-season and competition phases.

To examine the relationship between SIgA concentration and URTS risk, pooled mean SIgA concentration for each player was calculated when in an URTS-free state (i.e., SIgA values were excluded from the mean during weeks players were experiencing an URTS episode), and individual relative SIgA concentrations were calculated as a percentage of this mean value. Relative SIgA concentrations before, during and after URTS episodes were analysed using one-way ANOVA tests with a Bonferroni post-hoc test or Kruskal–Wallis tests.

To determine predictors of URTS risk, survival analysis was performed; specifically, Frailty model analysis was selected as it enables analysis where subjects can experience an event (i.e., URTS episode) more than once over the study period. Univariate frailty model analysis for URTS risk was run for the following independent predictor variables: SIgA concentration and secretion rate, household illness, internal training load, total wellness and international travel weeks. The significance for the current study was set at $p<0.05$. Frailty model analysis was performed using Stata (version 15) and all other statistical analysis was conducted in IBM SPSS (version 25).

6.4 Results

URTS incidence, severity, and duration

A total of 35 incidences of URTS episodes were reported; 13 (37%) and 22 (63%) URTS episodes occurred in the pre-season and competition phases, respectively (Figure 6.1). The highest URTS incidence followed rest weeks, specifically the Christmas break (week 4) and bye weeks (weeks 19 and 27) (Figure 6.1). When a URTS episode was present, total weekly symptom severity score and duration of symptoms were 27 ± 20 and 3.3 ± 0.9 days, respectively. Nine players (32%) did not experience a single URTS episode, fourteen players (50%) experienced 1-2 URTS episodes and five players (18%) experienced 3 or more URTS episodes. URTS episodes did not affect players training or match availability (i.e., no trainings were modified, and no trainings or matches were missed due to URTS).

Relationships between baseline data and URTS risk

Five players met the definition for URTS-prone (≥3 URTS episodes); therefore, limiting the power to detect differences between URTS-prone and UTRS-free athletes. Baseline data did not differ between URTS-free players, players who experienced 1-2 URTS episodes and URTS-prone players (Table 6.2).

SIgA and S-cortisol

Four-hundred and ninety-five saliva samples were analysed for SIgA. Season SIgA concentration and secretion rate were 335 ± 196 $\mu g \cdot mL^{-1}$ and 190 ± 106 $\mu l \cdot min^{-1}$, respectively. The within- and between- player CV for SIgA concentration were 37% and 58%, respectively. CVs for SIgA secretion rate were 45% (within-player) and 55% (between-player). S-cortisol was analysed for 200 samples; the season S-cortisol concentration was 11.0 ± 5.6 $nmol \cdot L^{-1}$. No association was found between SIgA and S-cortisol ($p = 0.69$).

SIgA and international travel

Pooled mean SIgA concentration did not differ between international travel weeks (324 ± 207 $\mu g \cdot mL^{-1}$) and non-travel weeks (342 ± 192 $\mu g \cdot mL^{-1}$) ($p = 0.222$). Additionally, SIgA concentration following international travel from Australia (weeks 9, 11 and 21), Samoa (week 25) and Japan (week 30) to New Zealand did not significantly differ to the pooled mean SIgA concentration from non-travel weeks ($p \geq 0.05$) (Figure 6.2). However, SIgA concentration was significantly lower following travel from South Africa to New Zealand (week 23: 224 ± 146 $\mu g \cdot mL^{-1}$) than the pooled mean SIgA concentration from non-travel weeks (342 ± 192 $\mu g \cdot mL^{-1}$) ($p = 0.001$) (Figure 6.2).

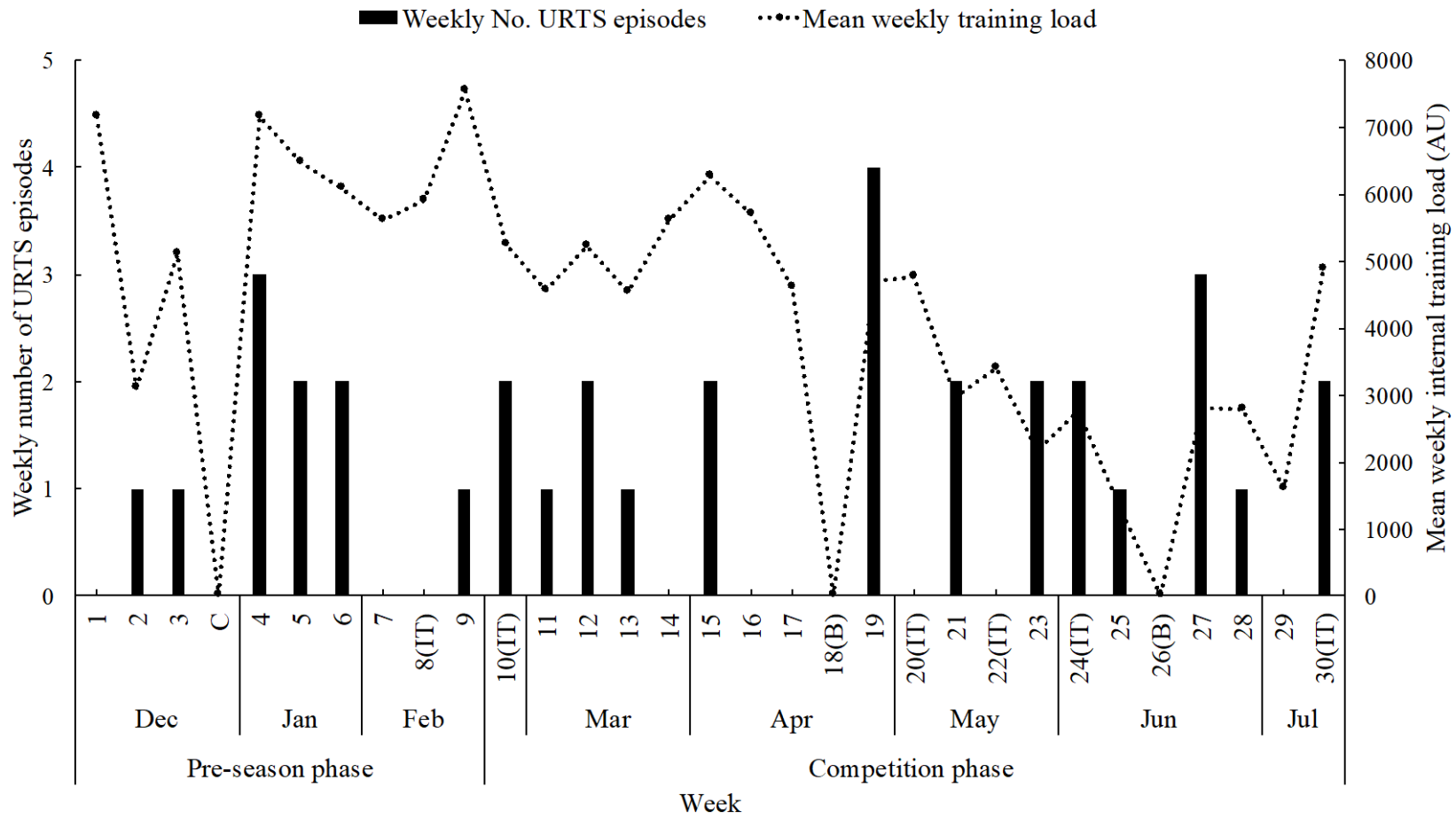

Figure 6.1 Weekly number of URTS and mean weekly internal training load (arbitrary units (AU)) over the season. The Christmas break (C), international travel (IT) and bye weeks (B) demonstrated in the χ axis.

Table 6.2 Baseline measures between URTS-free players, players who experienced 1-2 URTS episodes and URTS-prone players (≥3 URTS episodes)

	URTS-free players (9)	1-2 URTS players (14)	URTS prone players (5)	P value
URTS episodes	0 ± 0	1.3 ± 0.5	3.4 ± 0.5	NC
Age (yrs)	24.6 ± 3.2	24.7 ± 3.9	27.4 ± 3.9	0.261
Body mass (kg)	99 ± 11.3	102 ± 10.6	104 ± 12.2	0.668
Height (m)	184 ± 5.0	187 ± 8.4	191 ± 5.0	0.265
Forwards (F), Backs (B)	4, 5	7, 7	2, 3	NC
History of asthma (#)	1	3	1	NC
History of allergies (#)	2	2	1	NC
History of recurrent URTS (#)	0	8	2	NC
Saliva flow rate ($mL \cdot min^{-1}$)	0.4 ± 0.1	0.5 ± 0.2	0.6 ± 0.3	0.477
SIgA concentration ($\mu g \cdot mL^{-1}$)	325 ± 192	365 ± 181	208 ± 119	0.187
SIgA secretion rate ($\mu l \cdot min^{-1}$)	131 ± 56	168 ± 71	112 ± 56	0.188
S-cortisol concentration ($nmol \cdot L^{-1}$)	10.1 ± 5.6	15.5 ± 9.3	13.0 ± 6.0	0.316
WBC count (x 10^9 cells $\cdot L^{-1}$)	6.1 ± 1.1	6.4 ± 1.1	6.7 ± 1.3	0.657
Neutrophil count (x 10^9 cells $\cdot L^{-1}$)	3.1 ± 0.9	3.4 ± 1.2	3.2 ± 1.1	0.846
Lymphocyte count (x 10^9 cells $\cdot L^{-1}$)	2.1 ± 0.4	2.2 ± 0.5	2.4 ± 0.9	0.571
Monocyte count (x 10^9 cells $\cdot L^{-1}$)	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	0.885
Eosinophil count (x 10^9 cells $\cdot L^{-1}$)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.429
$CD4^+$ cell count (x 10^9 cells $\cdot L^{-1}$)	0.9 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	0.297
$CD8^+$ cell count (x 10^9 cells $\cdot L^{-1}$)	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.4	0.666
CMV seropositive (%)	78	78	100	NC
CMV IgG antibody titre ($IU \cdot mL^{-1}$)	75 ± 97	132 ± 158	193 ± 193	0.360
IFN-γ production ($pg \cdot mL^{-1}$)	18.0 ± 13.2	7.1 ± 3.5	8.6 ± 4.1	0.151
IL-4 production ($pg \cdot mL^{-1}$)	23.9 ± 8.5	15.8 ± 5.5	23.9 ± 10.2	0.078
IL-10 production ($pg \cdot ml^{-1}$)	613 ± 256	710 ± 312	794 ± 465	0.627

NC: Not calculable

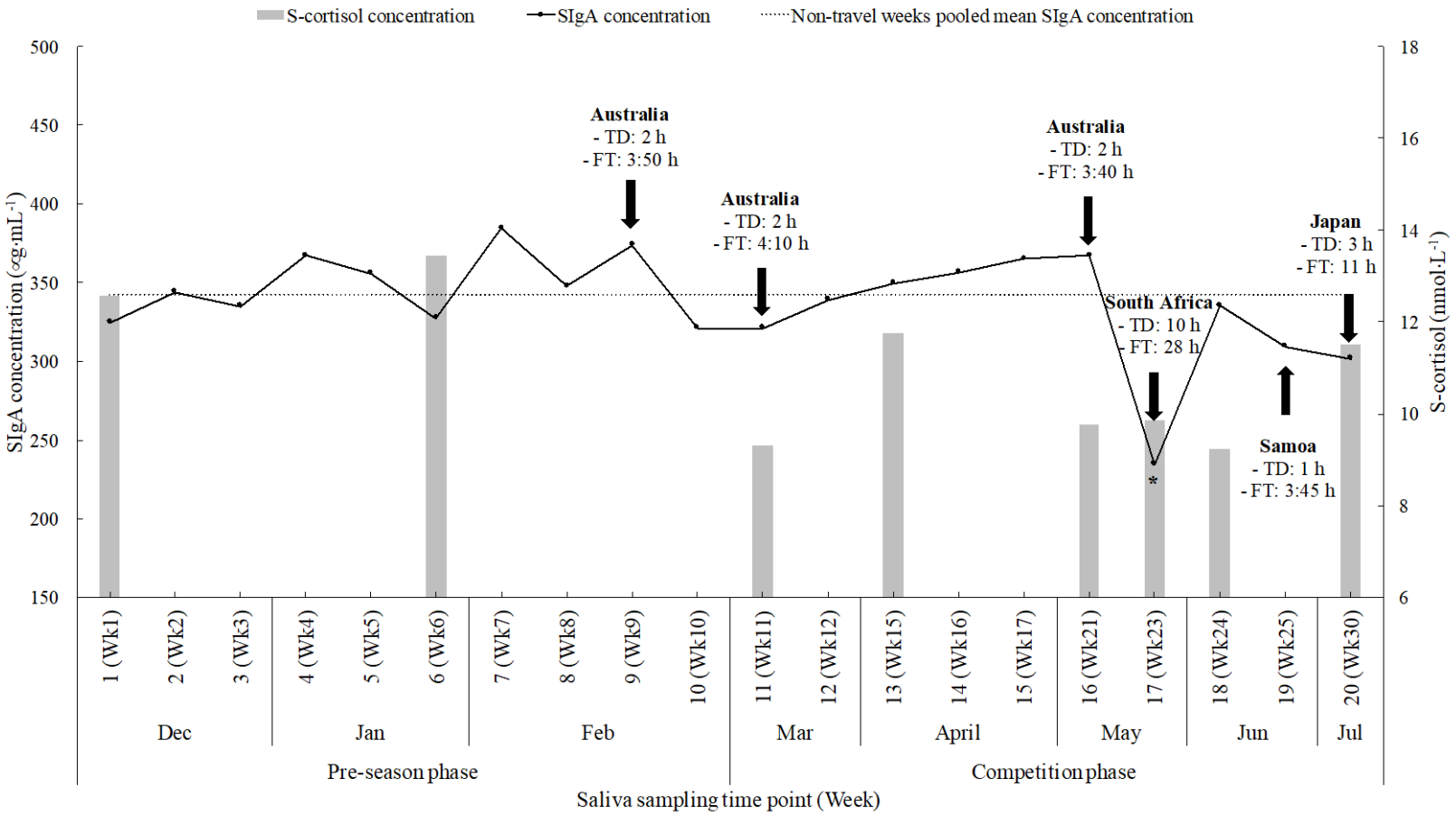

Figure 6.2 SIgA and S-cortisol concentrations over saliva sampling time points. Six saliva samples were collected following matches requiring international travel; the country, time zone differences (TD) and flight duration (FD) are indicated on the figure. Where S-cortisol concentration equals 0 nmol·L-1, saliva samples were not analysed for cortisol. *SIgA concentration at saliva sampling time-point 17/wk (week) 23 was significantly lower than the pooled mean SIgA concentration measured for all other non-travel time-points combined ($p = 0.001$).

SIgA and URTS risk

A non-significant, but potentially important trend for an inverse association between SIgA concentration and URTS incidence was observed (HR: 0.998, p = 0.070) (Table 6.3). When SIgA values were normalised to each players' mean healthy concentration, relative SIgA concentration (75 ± 31%) was 25% lower during an URTS episode (p = 0.008) (Figure 6.3).

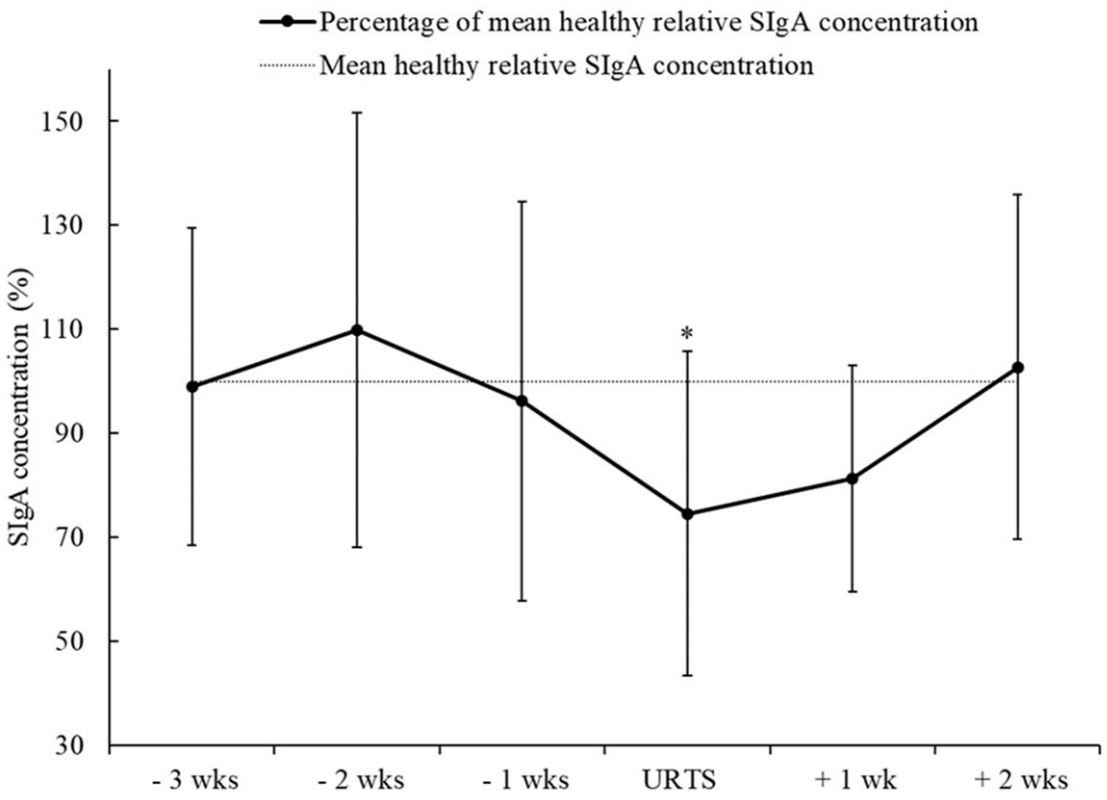

Figure 6.3 Players relative SIgA concentration (i.e., percentage of SIgA when no URTS episode present) for each week (wk) pre-, during- and post- URTS episodes. *SIgA concentration during an URTS episode was significantly lower than -2 weeks pre- the UTRS episode (p = 0.008).

Internal training load and URTS risk

Weekly internal training load distribution over the season is presented in Figure 6.1. Internal training load was significantly higher during pre-season training (6017 ± 1354 AU) compared to the competition phase (3996 ± 1465 AU) (p = 0.002). However, internal training load did not predict players URTS risk (Table 6.3).

Self-reported lifestyle and behavioural measures, travel and URTS risk

A total of 137 household illness incidences were reported over the season, with no difference in weekly household illness incidence observed between pre-season (5.5 ± 3.1 AU) and competition phases (3.9 ± 3.2 AU) (p = 0.159). Household illness was identified to be a significant predictor for URTS risk (p = 0.002), whereby players were almost three-fold more at risk for an URTS episode when illness in the household was present (Table 6.3). Self-reported total wellness (i.e., summation of sleep quality, mood, muscle soreness, stress levels and fatigue) was significantly lower during the pre-season (18.3 ± 0.9 AU) compared with the competition phase (19.1 ± 0.7 AU) (p = 0.014). However, total wellness score did not predict URTS risk (Table 6.3). International travel reduced players risk for URTS compared with non-travel weeks and this difference was statistically significant (Table 1; p = <0.001) (Table 6.3).

Table 6.3 Hazard ratios for risk of URTS episodes

	Hazard ratio	**Standard error**	**Z value**	**P value**	**95% confidence interval**
SIgA concentration	0.998	0.001	-1.81	0.070	0.995-1.000
SIgA secretion rate	0.996	0.002	-1.71	0.087	0.993-1.000
Household illness	2.902	1.005	3.08	0.002	1.472-5.720
Internal training load	1.000	1.13	-1.19	0.235	0.999-1.001
Total wellness	0.926	0.076	-0.48	0.631	0.824-1.120
International travel	0.438	0.096	-3.78	0.000	0.285-0.672

6.5 Discussion

The main findings of the current study include; 1) players' household illness and SIgA concentration predicted URTS risk, with household illness being the strongest predictor; 2) clusters of URTS episodes occurred after rest weeks (i.e., the Christmas break and bye weeks); and 3) URTS risk was reduced during weeks involving international travel.

A novel finding of this study was the relationship between players' household illness and URTS incidence. Specifically, players were almost three times more likely to experience an URTS episode when a member in their household was ill. This outcome aligns with general population research, as households seem to play a significant role in the spread of infection because of the frequency and intensity of contacts between household members (Petrie et al., 2013). Our finding has major implications beyond rugby players as household illness monitoring is an accessible tool for all sports given it is cheap, convenient and does not require a specialist to administer or analyse. Sport practitioners can easily monitor household illness through simply adding the 'yes' or 'no' question '*Is anyone in your household ill?'* within existing wellness questionnaires. If players report household illness, illness prevention strategies can be targeted to maintain player health and minimise disruptions to training and competition preparation.

Other accessible non-biological self-reported measures of internal training load and self-reported total wellness were not predictive of URTS risk in rugby players; corroborating previous findings in soccer (Buchheit et al., 2013), basketball (Anderson et al., 2003) and cricket players (Ahmun et al., 2019). In the last decade, several exercise immunology reviews have been published outlining risk factors for URTS (e.g., heavy training, psychological stress, poor sleep, etc.) and strategies for maintaining athletes' health (Schwellnus et al., 2016; Walsh, 2018; Walsh, Gleeson, Pyne, et al., 2011; Walsh, Gleeson, Shephard, et al., 2011). As such, the current and previous study findings of no association between URTS risk and internal training load or wellness could be explained by team-sport practitioners' increased education and awareness of URTS risk factors,

and better implementation of illness prevention strategies. However, an association between these self-reported measures and URTS incidence has been reported in elite Australian rugby league and football rules players (Fitzgerald et al., 2019; Thornton et al., 2016). The discrepant findings support Chapter 5 and suggest that predictors of URTS risk could well be sport and/or cohort specific. Indeed, sporting teams are exposed to different stressors (e.g., competition and travel schedules), and managed differently with respect to training periodisation, supplementation, wellness questionnaires (e.g., 5-point- versus 10-point- Likert scale), and illness prevention strategies. Therefore, practitioners should be mindful that predictors of URTS risk in one sporting team may not necessarily be applicable to another.

The current study found that the highest incidence of URTS occurred after rest weeks, specifically the Christmas break and bye weeks during the pre-season and competition phases, respectively. This was surprising given clusters of illness have generally been found to occur during and/or directly following stressful periods (e.g., strenuous training, congested match schedules and long-haul travel) (Walsh, 2018). Speculatively, our finding may be explained by the 'let-down effect'; a pattern in which people experience an illness not during a concentrated period of stress but after it dissipates (Lipton et al., 2014). The 'let-down effect' has not been well researched, although immunoendocrine alterations may be responsible for increased illness susceptibility following periods of increased stress (Lipton et al., 2014). During training weeks, players were frequently exposed to the short-term stressor of exercise (i.e., training and matches). Previous research has shown that exposure to short term stress can enhance immune activation and responses (Dhabhar, 2014). The absence of the potential immune priming effect elicited by acute trainings and matches may explain why players appeared to be more at risk for URTS following rest weeks. However, saliva samples were not collected during rest weeks; therefore, possible immunoendocrine mechanisms underlying increased URTS risk could not be determined. The change in environment and players' behaviour during rest weeks (e.g., reduced access to medical support, self-regulated nutritional

practices and increased social contact) may have also contributed to increased URTS incidence. Therefore, to maintain player health, illness prevention strategies should be targeted around rest weeks.

An unanticipated finding from the current study was that international travel appeared to protect against URTS incidence. The team doctor and dietitian applied illness prevention strategies during periods of travel (i.e., face masks on planes, hygiene practices and supplements) which might explain why player health was well-maintained. Indeed, a recent study found that the application of a team illness prevention strategy, including international travel guidelines, reduced elite rugby union players' URTS incidence by 59% during the Southern hemisphere Super Rugby Competition (Schwellnus et al., 2019). However, our finding contradicts previous evidence in elite rugby players showing increased illness risk following international travel to a foreign country across >5 hour time zones (Schwellnus et al., 2012) and 11 hour times zones (Fowler et al., 2016). In the current study, the team travelled internationally six times; although, five of the travel destinations only had minor time zone changes (2-3 hours) and no reduction in SIgA concentration occurred after these flights, which may explain why URTS risk did not increase. Nevertheless, it was unexpected that URTS rates were not increased following travel from South Africa to New Zealand, given it had the greatest time zone change (-10 hours), flight time duration (28 hours) and a significant reduction in SIgA concentration occurred. The observed decline in SIgA concentration following the flight from South Africa may not reflect immunosuppression; rather, it could be attributed to circadian rhythm misalignment of immune-modulating hormones, including cortisol and melatonin which can modulate SIgA (Park & Tokura, 1999; Piérard et al., 2001). Collectively these findings suggest that if players are well-managed and illness prevention strategies are applied, international travel may not be a prominent risk factor for URTS; however, further research is required.

The current study found a trend for increased URTS risk when players SIgA concentration decreased. This finding differs to evidence presented in elite Northern hemisphere players, for which no relationship between players SIgA concentration and the total number of URTS episodes was observed during an 11-month season (Cunniffe et al., 2011). Cunniffe et al. (2011) only collected saliva samples monthly; therefore, infrequent sampling may explain the lack of association in their study. In the current study, saliva samples were collected 20 times over an 8-month season, revealing similar findings to a previous study in elite rugby union players involving bi-weekly saliva sampling (Tiernan et al., 2020). As such, the usefulness of measuring SIgA concentration to predict elite rugby players' URTS risk may depend on saliva sampling frequency.

However, the measurement of absolute SIgA concentration to determine players' URTS risk is `limited by the variability of this marker; for example, our study and others have shown high within-individual CVs (41-54%) (Cunniffe et al., 2011; Leicht et al., 2012; Neville et al., 2008). As such, it has been proposed that it is more appropriate to express SIgA concentration relative to an athlete's mean healthy SIgA concentration when trying to predict URTS risk (Neville et al., 2008). Therefore, we examined players' relative SIgA concentration in the 3-weeks pre-, during- and 2-weeks post- URTS episodes. Relative SIgA concentrations were not significantly reduced in the weeks preceding an URTS episode, and this contradicts previous research demonstrating decreased SIgA concentration in the 2-3 weeks before an URTS episode (Neville et al., 2008; Tiernan et al., 2020). It is possible that weekly saliva sampling may have prevented us from detecting the pre-URTS drop in SIgA as significant reductions have been shown to occur just days before URTS onset in team-sport athletes (Nakamura et al., 2006; Yamauchi et al., 2011a). Nonetheless, in the current study, relative SIgA concentration was found to be almost 25% lower during an URTS episode than when no URTS episode was present. This is comparable to the 15% and 28% reduction in relative SIgA reported in

elite rugby union players (Cunniffe et al., 2011) and sailors (Neville et al., 2008), respectively, when experiencing an URTS episode compared with when URTS-free.

The mechanisms underlying reductions in SIgA concentration are unclear; although, cortisol may play a mediating role through decreasing the expression of pIgR mRNA and subsequently reducing SIgA translocation into saliva (Rosato et al., 1995). Indeed, an inverse association between SIgA and S-cortisol has been demonstrated in elite team-sport athletes (Cunniffe et al., 2011; He et al., 2010). In contrast, no relationship between S-cortisol and SIgA was observed in this study; a finding reported elsewhere (Moreira et al., 2014; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012). Therefore, other mechanisms, including altered sympathetic innervation of the salivary glands, IgA synthesis, and IgA transportation via availability of the pIgR, may elicit greater influence on SIgA compared to S-cortisol (Bishop & Gleeson, 2009). Nevertheless, the current studies failure to detect an association between SIgA and S-cortisol may also be explained by infrequent S-cortisol analysis; due to financial constraints S-cortisol was only analysed for eight saliva sampling time-points.

Associations between baseline biomarkers and URTS risk have been demonstrated in previous studies, with SIgA (Gleeson et al., 2012), CMV serostaus (He, Handzlik, Muhamad, et al., 2013) and whole blood stimulated cytokine production (IL-4 and IL-10) (Gleeson et al., 2012) found to differ between illness-prone and illness-free athletes. In contrast, no significant difference in baseline biomarkers was found between URTS-free players, players who experienced 1-2 URTS episodes and URTS-prone players (≥3 URTS episodes). Our discrepant results may be explained by our small sample size and study population; the current study was conducted in 28 elite male rugby players, whereas previous studies examined large samples (n=80-236) of recreational male and female endurance-based athletes (Gleeson et al., 2012; He, Handzlik, Muhamad, et al., 2013). Given our non-significant findings and the invasiveness and expense of measuring these preconceived predictors, screening of baseline biomarkers does not

appear to be a worthwhile or accessible tool for practitioners to use to predict risk for URTS in elite team-sport athletes.

One potentially confounding factor of the current study is that players were prescribed supplements including probiotics, polyphenols and a multi-vitamin. These supplements have been proposed to alter specific aspects of the immune system and potentially reduce athletes' URTS risk (Maughan et al., 2018). Despite this, predictors of URTS risk were still evident in the current study. Given 40-100% of elite athletes use supplements (Garthe & Maughan, 2018), there is a need for practitioners to know best URTS predictors to monitor even when athletes' are using supplements.

6.6 Conclusions

In summary, the current study found that URTS risk in elite rugby union players varies during a season with players being at greatest risk during rest (i.e., Christmas break) and non-competition (i.e., bye weeks) periods. Identification of players' risk for URTS episodes were best revealed by a self-reported household illness survey and measurement of SIgA concentration. Therefore, to maintain athlete health, focussed illness prevention strategies should be used around rest-weeks, the routine monitoring of household illness should be incorporated and, where resources permit, the measurement of SIgA concentration should be conducted.

CHAPTER 7 A MULTI-FACTORIAL ASSESSMENT OF ELITE FIELD HOCKEY PLAYERS RESPONSES TO AN INTERNATIONAL TOUR.

Prelude

Chapters 5 and 6 identified factors influencing URTS risk in elite rugby union and league players. However, there are several factors that may limit the applicability of these study findings to other team-sports, such as elite field hockey. Firstly, while elite rugby union and league players are professional, elite field hockey players are often amateur (i.e., not paid to play) or semi-professional. Secondly, rugby union and league are contact sports, whereas field hockey is a non-contact sport. Finally, the competition format differs markedly between these sports, with a schedule of weekly competition in elite rugby codes compared to multiple matches within an intense congested tournament format in elite field hockey. Therefore, this chapter described the impact of a multi-stressor overseas tour, involving 8 international matches in 21 days, on mucosal immunity, self-reported wellness, hydration status and URTS risk in elite field hockey players.

7.1 Abstract

Purpose: To examine the impact of a multi-stressor overseas tour on URTS incidence and mucosal immunoendocrine, self-reported wellness and hydration measures in elite field hockey players; and to determine whether any of these measures can predict URTS risk.

Methods: A prospective cohort study was conducted in elite national male field hockey players (n=8) during a real-life 3-week overseas tour involving long-haul travel, a congested competition schedule and extreme environmental conditions. Repeated-measurements of SIgA, S-cortisol, self-reported wellness indicators (i.e., stress, mood, fatigue, muscle soreness and sleep quality) and urine specific gravity (USG) were taken throughout the tour. Self-reported URTS were monitored one month before and during the tour.

Results: Despite players being exposed to several stressors during the tour, mucosal immunoendocrine, self-reported wellness and hydration measures remained stable. Additionally, URTS incidence was unexpectedly low, with only one player experiencing an URTS episode.

Conclusion: The low URTS incidence during the 3-week tour may be explained by the unique traits of elite athletes' immune systems, such as enhanced immunotolerance and/or the management of tour stressors by team-practitioners to prevent URTS.

7.2 Introduction

Field hockey is a high-intensity, intermittent team-sport that requires physical, technical and tactical attributes to perform (Lidor & Ziv, 2015). Elite field hockey teams often compete in tournaments (e.g., World Cup, Olympic Games), involving several stressors such as congested competition schedules (e.g., ~5 matches in 7–8 days) (Vescovi & Watson, 2019), long-haul travel to unfamiliar environments (i.e., heat) and time-zones, dehydration, psychological stress and inadequate sleep (Walsh, 2018). These stressors have the potential to increase URTS risk (Walsh, 2018). URTS can pose a serious problem for elite field hockey players during competition, because severe URTS episodes can impair performance and even render players ineligible for match selection (Gleeson & Pyne, 2016). To understand the mechanisms underlying URTS incidence in athletes, previous studies have examined mucosal immunoendocrine (Morgans et al., 2014; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012; Stevens et al., 2018; Vardiman et al., 2011), self-reported wellness (Fitzgerald et al., 2019; Thornton et al., 2016) and hydration measures (Killer et al., 2015; Penkman et al., 2008). Characterising these measures in association with URTS may inform management/treatment strategies to minimise URTS risk and associated disruptions to competition performance in elite field hockey players.

SIgA is a mucosal immune marker that has been found to predict athletes' risk for URTS, with low or declining levels of SIgA associated with increased URTS susceptibility (Gleeson & Pyne, 2016). However, in Chapters 5 and 6, no significant association between absolute SIgA concentration and URTS incidence was found in elite rugby union and league players. SIgA secretion is influenced by neuroendocrine responses, including the stress hormone cortisol (Bishop & Gleeson, 2009). In previous team-sport studies, reductions in SIgA secretion have been linked to elevations in S-cortisol concentration (Cunniffe et al., 2011; He et al., 2010); although findings from Chapters 5 and 6 did not support this inverse association. Despite limited research examining mucosal immunoendocrine measures in elite field hockey players, an inverse association

between SIgA and URTS incidence has been demonstrated (Mackinnon et al., 1993), suggesting measurement of SIgA and S-cortisol may be worthwhile in this particular sport. However, the sensitivity of these measures to field hockey competition stressors (e.g., congested competition, long-haul travel, dehydration etc.) is uncertain. Measurement of SIgA and S-cortisol in elite field hockey has recently become more accessible with the emergence of point-of-care devices (Dunbar et al., 2011). Yet, regular monitoring of these measures may be limited due the cost (USD ~$200 to test a team of 16 players) and rigorous pre-sample standardisation required to get valid results (Bishop & Gleeson, 2009).

Inexpensive and simple self-reported wellness and hydration measures tend to be more routinely monitored in elite field hockey players during competition. These measures appear to be sensitive to competition stressors, with changes in self-reported wellness indicators (e.g., mood, sleep quality, muscle soreness and total wellness) (Ihsan et al., 2017; McGuinness et al., 2018) and hydration status (MacLeod & Sunderland, 2009; Vescovi & Watson, 2019) reported during real-life tournaments in elite field hockey players. However, the clinical relevance of these changes remains unknown as URTS were not reported (Ihsan et al., 2017; MacLeod & Sunderland, 2009; McGuinness et al., 2018; Vescovi & Watson, 2019).

Given the multiple and cumulative stressors imposed on elite field hockey players during an overseas tour, they represent a unique athlete population to investigate. The aims of this study were: (1) to examine how a real-life tour influences mucosal immunoendocrine responses, self-reported wellness measures, hydration status and URTS incidence in elite national field hockey players; and (2) to determine whether mucosal immunoendocrine, self-reported wellness and/or hydration measures are reliable indicators of URTS incidence.

7.3 Methods

Participants

Ten elite male field hockey players (age 23.0 ± 2.2 y, body mass 82.0 ± 7.0 kg, height 1.8 ± 0.07 m) belonging to the New Zealand national field hockey team (ranked 8th in the world) participated in this study. Positions included: goalkeepers (n=2), defenders (n=3), midfielders (n=2) and strikers (n=3). Supplements that have been proposed to alter specific aspects of the immune system and reduce URTS risk (e.g., probiotics, echinacea, bovine colostrum, flavonoids, multivitamins and multi-minerals) (Maughan et al., 2018) were prohibited during the study period. All players were informed of the rationale, aims and requirements of the study, before providing a written consent (Appendices D and E). This study was approved by the Auckland University of Technology Ethics Committee (Auckland, New Zealand) and endorsed by Hockey New Zealand and the High Performance Sport New Zealand Research Committee (Appendices F and G).

Study design

This prospective cohort study repeatedly measured SIgA, S-cortisol, self-reported wellness indicators, USG, URTS, performance outcomes and environmental data over a 3-week multi-stressor tour (hereinafter 'the tour'). URTS data were also collected during the month leading up to the tour which consisted of 2-weeks normal training and 2-weeks of supervised heat acclimation (8x30 minute post-exercise sauna sessions in 70-75˚C and 10-15% RH; and 3x90 minute heated gym sessions in 28-32˚C and 30-45% RH). At the start of July 2018, the team travelled from the New Zealand winter to the Northern hemisphere summer to compete in eight international matches: four matches versus Japan, one match versus Germany and three matches versus India. The first 10 days of the tour were in Japan and consisted of four training sessions and five matches. The team then travelled to India for the final week of the tour which involved three training sessions and three matches. Figure 7.1 is a schematic representation of the tour timeline in which players completed 38.75 hours of international, economy class travel, as

detailed in Table 7.1. A High Performance Sport New Zealand Prime Ministers Scholarship provided some funding for this study (Appendices H, I and J).

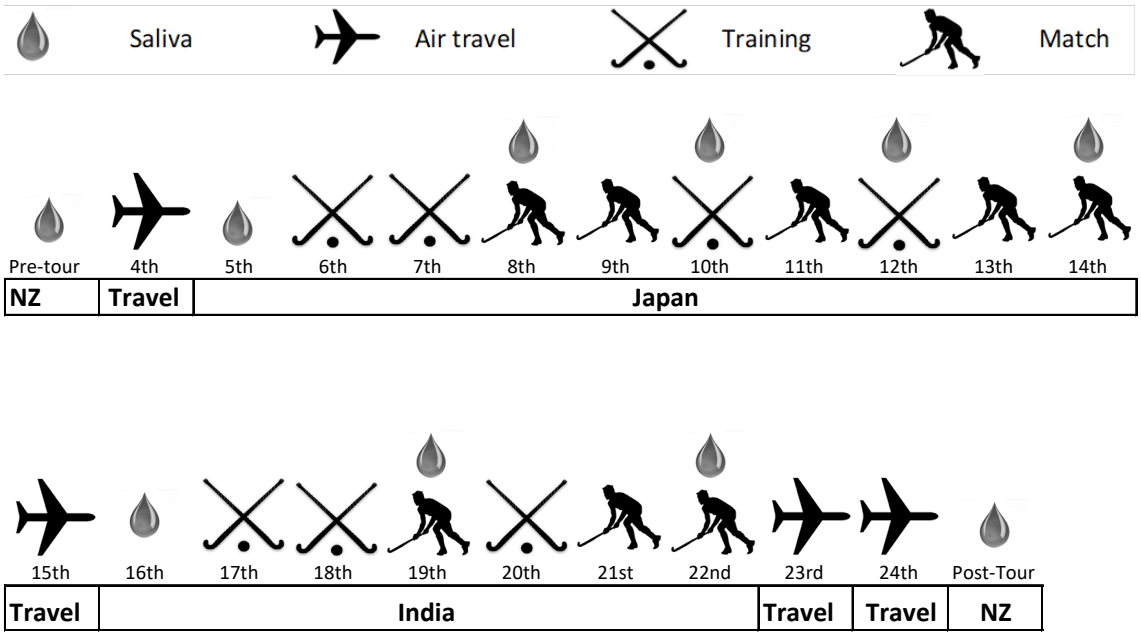

Figure 7.1 A schematic representation of the tour timeline and saliva sampling time-points.

Table 7.1 Players international travel schedule during the 3-week tour

	Total flight duration (h:min)	**Time zone difference from NZ (h:min)**	**Flights and stopover details**	**Flight and stopover durations (h:min)**
NZ to Japan	13:35	3:00	Auckland to Sydney flight	3:40
			Sydney stopover	1:45
			Sydney to Osaka flight	9:55
Japan to India	11:00	6.30	Osaka to Singapore flight	6:45
			Singapore stopover	3:25
			Singapore to Bengaluru flight	4:15
India to NZ	14:10		Bengaluru to Kuala Lumpur flight	4:15
			Kuala Lumpur stopover	4:40
			Kuala Lumpur to Auckland flight	9:55

Note. h=hours, min=minutes

Measures

Mucosal immunoendocrine responses

SIgA and S-cortisol concentrations were determined at 10 time-points during the study period (Figure 7.1): once before the tour (3.3 ± 1.5 days), eight times during the tour, and once following the tour (8.2 ± 1.8 days). Unstimulated whole saliva samples were collected in the morning (07:00-08:00 hours), ~30 minutes after waking and before breakfast using an IPRO oral fluid collector (OFC) (IPRO Interactive, Wallingford, UK). Samples were analysed using an IPRO lateral flow device (LFD) and LFD reader (IPRO Interactive, Wallingford, UK). Players placed the OFC in their mouth until the volume indicator changed colour to signify 0.5 mL of saliva had been collected. The OFC swab was then placed into a buffer containing extraction agents to draw SIgA and S-cortisol. Two drops of the OFC/buffer mix were then added to the sample window of the SIgA/s-cortisol LFD. After a 15-minute incubation period, SIgA and S-cortisol concentrations were determined by the LFD reader. This method has previously been validated against ELISA analysis (r = 0.90 and coefficient of variation = 7.2%) (Dunbar et al., 2015).

Self-reported wellness and URTS data

Players completed an online wellness questionnaire (3.0 ± 1.2 days·week^{-1}) to numerically rate their sleep quality (1 = 'terrible' to 5 = 'good'), mood (1 = 'highly annoyed, irritable &/or down' to 5 = 'great'), muscle soreness, stress levels and fatigue (1 = 'very high' to 5 = 'none'). A total wellness score (ranging from 5-25) was calculated by adding the five individual ratings (Buchheit et al., 2013). Players were also asked to provide a 'yes' or 'no' response to the question: *"Are you ill?"*. Players who responded 'yes' completed a modified URTS questionnaire (see Chapter 4).

Match-day hydration, performance, and environmental data

USG, performance outcomes and environmental data were collected on match days during the tour. Players collected their first-morning, mid-stream urine samples into a plastic container. USG was measured within 10-15 minutes of collection using a

handheld digital refractometer. Match outcomes (i.e., win, draw or loss) and score margins were recorded for each match. The wet bulb globe temperature (WBGT) was measured based on a weighted sum of natural wet bulb temperature (Nwetbulb), globe temperature (Tglobe) and dry bulb temperature (Tdrybulb) (i.e., WBGT(°C) = 0.7·Nwetbulb + 0.2·Tglobe + 0.1.Tdrybulb). WBGT data were collected before, during (quarters and half times) and at the end of each match with a weather meter (Kestrel 5400, Nielsen Kellerman, Philadelphia, USA).

Data analysis

Data were assessed for normality using the Shapiro-Wilk test and reported using descriptive statistics including mean and standard deviation (unless otherwise specified). A percentage variation (Δ%) in SIgA concentration was calculated through the relative change of samples collected during and following the tour (i.e., sample time-points 2-10) to the sample collected at baseline (i.e., sample time-point 1). Unpaired t-tests or Mann Whitney U tests were performed to compare the Δ% of SIgA concentration between players who experienced no URTS episodes (URTS-free players) and those with at least one URTS episode (≥1 URTS) in the month prior to the tour. One-way within-measures ANOVA tests (parametric) or Friedman's tests (nonparametric) were used to assess changes in absolute SIgA concentration, the Δ% of SIgA concentration, S-cortisol concentration and USG over time. Assumptions of homogeneity and sphericity were checked using Levene's test and, where appropriate, adjustments to the degrees of freedom was made using Greenhouse-Geisser correction method. The Bonferroni post-hoc test was used to evaluate pairwise comparisons of time-points. Self-reported wellness scores during weeks one, two and three of the tour were compared using a one-way ANOVA test with a Bonferroni post-hoc test. A linear regression was used to examine the relationship between SIgA, S-cortisol and USG from match days where all measures were collected (i.e., matches 1, 5, 6 and 8) (Figure 7.1). The significance for the current study was set at $p<0.05$ and all statistical analyses were conducted in IBM SPSS (version 25).

7.4 Results

Performance outcomes and environmental conditions

Two players suffered serious injuries and were unable to play in the final three matches versus India; as such, their data were excluded from analyses. The match outcomes, score margins and WBGT are presented in Table 7.2. During the tour, the team had six losses, one win and one draw, with the score margin between -6 and 1. All matches were played in hot and humid environmental conditions.

Table 7.2 Performance outcomes and WBGT from each match

	Opposition (world ranking)	Match outcome	Score	WBGT (°C)
Match 1	Japan (16th)	Draw	3-3	28.8 ± 1.8
Match 2	Japan (16th)	Loss	1-7	28.2 ± 2.5
Match 3	Japan (16th)	Win	2-3	26.5 ± 0.3
Match 4	Japan (16th)	Loss	0-1	28.4 ± 1.4
Match 5	Germany (6th)	Loss	4-7	29.5 ± 2.4
Match 6	India (5th)	Loss	2-4	25.2 ± 1.6
Match 7	India (5th)	Loss	1-1	26.2 ± 1.0
Match 8	India (5th)	Loss	0-4	27.2 ± 1.4

URTS incidence, severity and duration

Only one player experienced an URTS episode during the first two weeks of the tour. The player's total symptom severity score was 40 ('runny nose' = 13; 'sore throat' = 12; 'cough' = 8; 'headache' = 3; 'fever' = 2; 'weakness' = 1; 'loss of sleep' = 1) and the total duration of the symptoms was 12 days. Due to the low URTS incidence among the players, there was insufficient power to determine whether mucosal immunoendocrine, self-reported wellness and USG measures were reliable indicators of URTS incidence.

SIgA and S-cortisol

Eighty saliva samples were analysed for SIgA and S-cortisol. The within- and between-player CVs for SIgA concentration was 42% and 50%, respectively. CVs for S-cortisol concentration were 57% (within-player) and 60% (between-player). SIgA and S-cortisol concentrations over the study period are presented in Figure 7.2. There were no

significant differences in SIgA concentration, S-cortisol concentration or Δ% of SIgA concentration between time-point 1 (baseline) and the following time-points (2-10) ($p \geq 0.05$). The Δ% of SIgA concentration in URTS-free players (n=3) was higher than that in ≥1 URTS players (n=5) at all time-points (2-10), but particularly at time-point five ($p = 0.038$) (Figure 7.3).

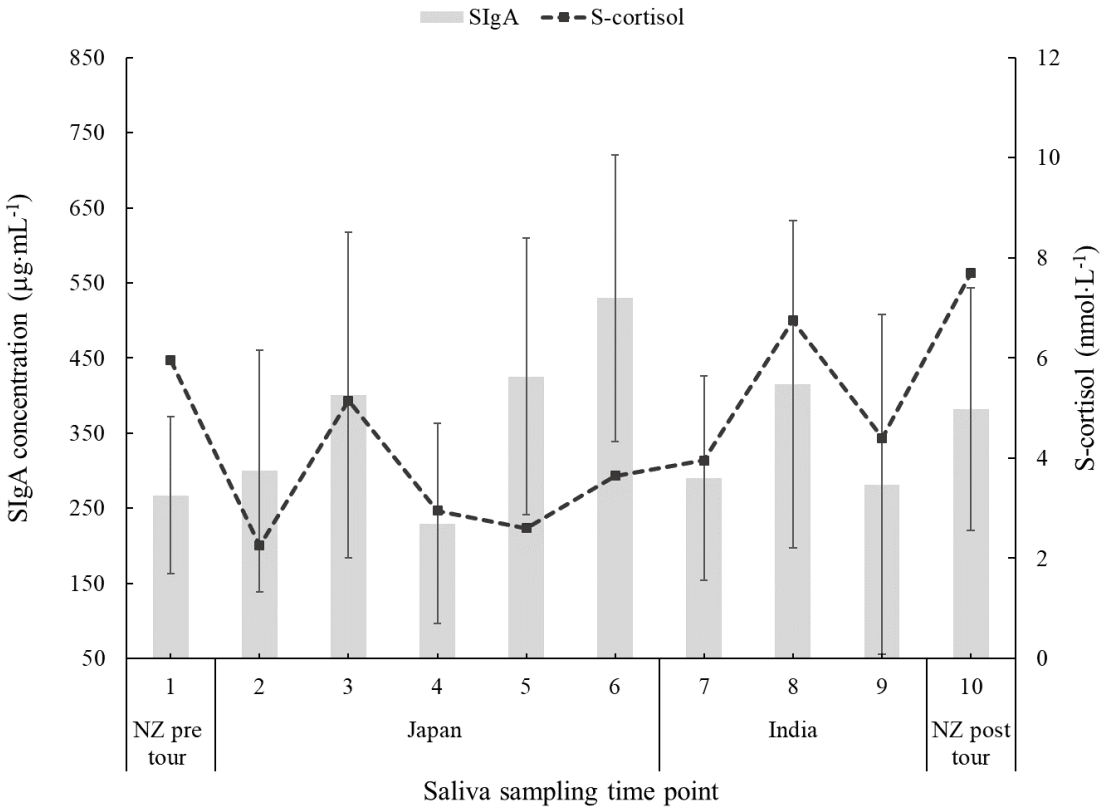

Figure 7.2 SIgA and S-cortisol concentrations before (New Zealand (NZ) pre-tour), during (Japan and India) and following (NZ post-tour) the tour (n=8). For clarity, the standard deviation is only illustrated for SIgA concentration.

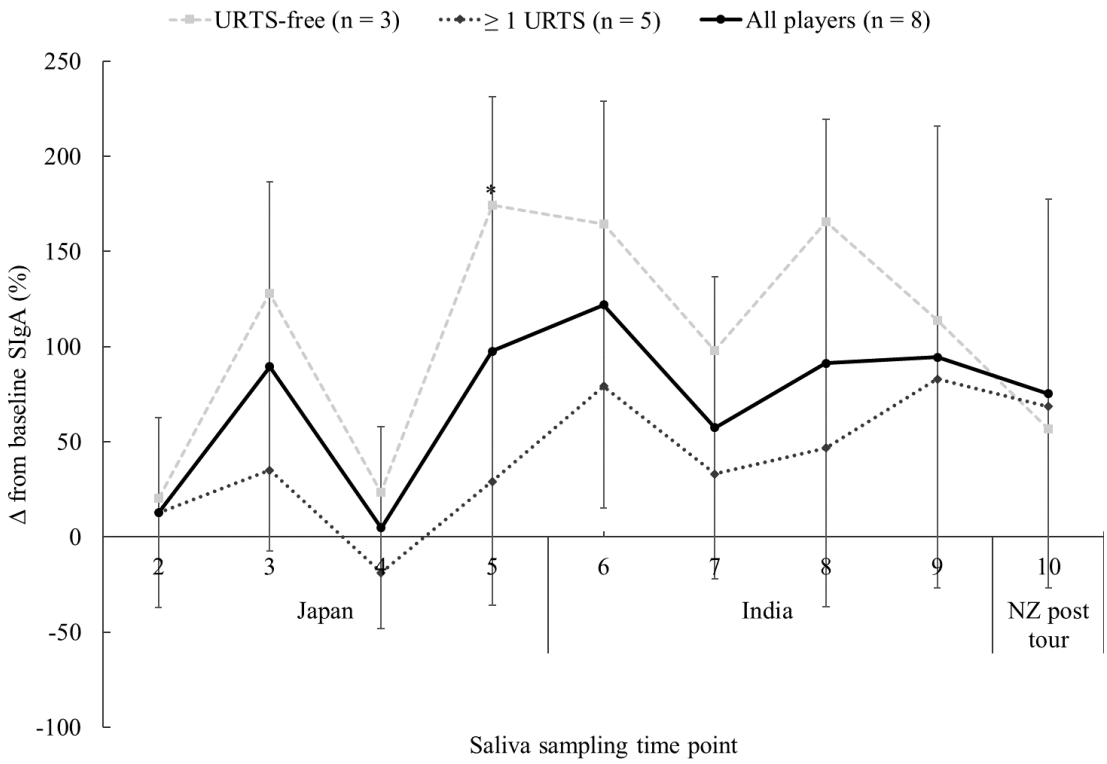

Figure 7.3 The Δ% of SIgA concentration from baseline for all players (n=8), players who had not experienced an URTS in the month prior to the tour (URTS-free players) (n=3) and players who had experienced at least one URTS episode in the month prior to the tour (≥1 URTS) (n=5). *A significant difference ($p < 0.05$) between URTS-free and ≥1 UTRS players. For clarity, the standard deviation is only illustrated Δ% of SIgA concentration for all players.

Self-reported wellness and match-day hydration

No significant changes across weeks were evident for any self-reported wellness indicator ($p \geq 0.05$) (Table 7.3). Players' morning USG was 1.021 ± 0.007 $g \cdot mL^{-1}$; there were no significant changes in players' USG values over the tour ($p \geq 0.005$). In addition, no association was found between USG and SIgA concentration ($p = 0.328$) or S-cortisol concentration ($p = 0.146$).

Table 7.3 Self-reported wellness measures during weeks one, two and three of the tour

	Week 1	Week 2	Week 3	P value
Total wellness (AU)	18.8 ± 3.6	17.5 ± 2.7	18.6 ± 2.9	0.507
Fatigue (AU)	3.9 ± 0.5	3.5 ± 0.6	3.6 ± 0.4	0.237
Muscle soreness (AU)	3.9 ± 0.6	3.4 ± 0.6	3.9 ± 0.7	0.188
Stress (AU)	3.9 ± 1.0	3.7 ± 0.5	3.9 ± 0.7	0.632
Mood (AU)	3.8 ± 1.1	3.5 ± 0.8	3.6 ± 0.8	0.341
Sleep quality (AU)	3.6 ± 0.4	3.5 ± 0.6	3.7 ± 0.6	0.581

AU = Arbitrary unit

7.5 Discussion

This study provided a multi-factorial assessment of elite field hockey players' responses to a real-life international tour. During the tour, players were exposed to several stressors including long-haul travel, a congested competition schedule, hot and humid environmental conditions and hypohydration. Despite the multi-stressor exposure, there was only one URTS incidence, and mucosal immunoendocrine, self-reported wellness and hydration measures remained stable throughout the tour.

Increased URTS incidence has been demonstrated in team-sport athletes with exposure to sport-related stressors, including congested competition schedules (Cunniffe et al., 2011; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012) and long-haul travel (Fowler et al., 2016; Schwellnus et al., 2012). Although elite field hockey players in this study were exposed to such stressors, URTS incidence was unexpectedly low, with only one player experiencing an URTS episode. Comparably low URTS incidence has been reported in elite basketball and football players during intensified training and competition periods, respectively (Moreira et al., 2008; Morgans et al., 2014). It is assumed that these low UTRS rates can be explained by the S-shape curve hypothesis which suggests that elite athletes possess or develop robust immune systems able to withstand infections even during severe psychophysiological stress (Malm, 2006). Indeed, in the current study, no impairment in SIgA concentration was observed in players during the tour. However, it is acknowledged that the low URTS incidence could also be explained by the fact that players were well managed; their team doctor and sport scientists applied several illness prevention strategies (e.g., face masks on planes, hygiene practices, diet/hydration strategies) during travel, training and competition environments to maintain players' health. Therefore, the low URTS incidence observed in this study may be attributed to the players' immunotolerance and/or the team-practitioners' management of tour stressors to prevent URTS.

Higher URTS incidence was reported in the month leading up to the tour, with five (63%) players experiencing at least one URTS episode. Previous research in endurance athletes showed an association between underlying or recent infection and increased risk for illness (Ekblom et al., 2006; Hellard et al., 2015). It was suggested that resuming intensive training or competition too soon after illness might allow for reactivation of the virus responsible for the athletes' primary illness episode (Ekblom et al., 2006; Hellard et al., 2015). Indeed, viral reactivation is thought to account for 25-55% of all URTS episodes in athletes (Gleeson & Pyne, 2016). In the current study, the one player who experienced URTS during the tour had reported URTS episodes in the month leading up to the tour. On the other hand, four of the players who also reported URTS prior to the tour remained URTS-free throughout the tour. URTS episodes do not always have an infectious aetiology, they can also be caused by non-infectious inflammatory stimuli (e.g., allergies, asthma and trauma to respiratory epithelial membranes) (Gleeson & Pyne, 2016), which may explain why some players with recent URTS did not experience recurrent URTS during the tour. The aetiology of URTS is outside the scope of this study; however, future research should seek to clinically diagnose (i.e., pathology analyses) upper respiratory tract infection in team-sport athletes to better elucidate the relationship between pre-competition health status and URTS risk.

A reduction in SIgA concentration is thought to reflect a decrease in immune function, and has been associated with increased risk of URTS in athletes (Gleeson & Pyne, 2016; Keaney et al., 2018). In the current study, SIgA concentration remained stable throughout the tour which may in part explain the observed low URTS incidence. SIgA has also been found to be a sensitive marker to exercise stress, with significant reductions in athletes' SIgA levels demonstrated during congested competition periods (Coad et al., 2015; Cunniffe et al., 2011; Morgans et al., 2014; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & José Filho, 2012). However, the current study showed that players' SIgA and S-cortisol concentrations were unaltered even with multi-stressor exposure (e.g., congested competition schedule, hot and humid environmental playing

conditions and long-haul travel). In support, previous studies have also observed no change in athletes' SIgA and/or S-cortisol with a congested competition schedule (Moreira et al., 2016), heat stress (Sari-Sarraf et al., 2011) and long-haul travel (Stevens et al., 2018). The observed lack of mucosal immunoendocrine disturbance in the current study may be explained by a number of factors. Firstly, players were accustomed to congested competition schedules, with international field hockey tournaments typically consisting of 3-6 matches played within 4-9 days (McGuinness et al., 2018). Secondly, players prepared for hot and humid conditions by completing 2-weeks of heat acclimation prior to the tour. Finally, team-practitioners closely monitored and managed field hockey players throughout the tour, particularly during long-haul travel.

Absolute SIgA concentration is known to be highly variable, with the current and previous studies reporting within-individual CVs of ~40-50% (Cunniffe et al., 2011; Neville et al., 2008). Therefore, it is arguably more appropriate to express SIgA concentration as a Δ% from players' baseline levels (Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & José Filho, 2012; Owen et al., 2014). Throughout the tour, the Δ% in SIgA from baseline was non-significantly increased. In line with our finding, increases in SIgA concentration have been demonstrated with stressors of competition and intensified training in elite taekwondo athletes (Tsai et al., 2011) and elite race walkers (McKay et al., 2019), respectively. Such findings challenge the concept of stressor-induced mucosal immunosuppression, and support the notion that stressor exposure could in fact benefit/prime the mucosal immune system (Born et al., 2017). In the current study, players who had not experienced an URTS episode in the month prior to the tour (i.e., URTS-free) had a higher Δ% in SIgA from baseline throughout the tour, compared to players who had experienced URTS in the month before the tour (i.e., ≥1 URTS players). Due to our small sample size, limited conclusions can be drawn; however, it is possible URTS-free players' mucosal immune systems are more resilient and/or responsive to stress, which may explain why they did not experience any URTS throughout the study period.

Field hockey players' self-reported wellness scores remained stable throughout the tour. Similar findings have been reported in elite Australian Rules footballers, with no change in wellness scores observed during a two-week training camp involving several sport-related stressors (i.e., hot environmental conditions, altitude, intensified training period) (Bucheit et al., 2013). In contrast, changes in self-reported mood, sleep quality and muscle soreness have been observed in elite field hockey players competing in international tournaments (Ihsan et al., 2017; McGuinness et al., 2018). The discrepant findings may be explained by methodological differences; a 5-point scale was used in this study and the study on Australian Rules footballers (Buchheit et al., 2013); whereas a 10-point scale was used in previous elite field hockey studies (Ihsan et al., 2017; McGuinness et al., 2018). Due to the low URTS incidence in the current study, the relationship between self-reported wellness and URTS was not examined. Nevertheless, further research is warranted given self-reported wellness measures have been found to predict URTS risk in other team-sport athletes (Fitzgerald et al., 2019; Thornton et al., 2016), including elite rugby league players, as demonstrated in Chapter 5.

Players' hydration status was assessed via USG and a hypohydration threshold greater than 1.020 $g.mL^{-1}$ (Vescovi & Watson, 2019). During the tour, mean morning USG was 1.021 $g.mL^{-1}$, indicating most players awoke in a hypohydrated state. This may, in part, be due to the tour game times; all games occurred in the late afternoon or evening, meaning players had limited window of time to rehydrate before going to sleep. Despite players' morning hypohydrated status, no significant association was found between USG and SIgA concertation or S-cortisol concentration. In support, no change in resting SIgA concentration (Killer et al., 2015) and cortisol concentration (Killer et al., 2015; Svendsen et al., 2014) has been found with mild hypohydration in endurance athletes. Therefore, hydration status does not appear to influence mucosal immunoendocrine responses in athletes; however, its effect on URTS risk is yet to be elucidated.

Given the logistical constraints of conducting research in applied settings, such as during an overseas tour, the frequency of data collection in the current study may be considered a limitation, because mucosal immunoendocrine, self-reported wellness and hydration measures were not collected on a daily basis. A further limitation of this study was the small sample size and lack of statistical power, which may have prevented us from detecting any significant changes in mucosal immunoendocrine, self-reported wellness and hydration measures during tour. Despite these limitations, our findings have real-world applied significance for elite team-sport athletes who often compete in multi-stressor congested competition schedules.

7.6 Conclusions

This study provided a multi-factorial assessment of elite field hockey players' responses to a real-life tour. Despite multi-stressor exposure during the 3-week tour, field hockey players' mucosal immunoendocrine, self-reported wellness and hydration measures remained stable. Additionally, URTS incidence was unexpectedly low, which may be due to the unique traits of elite athletes' immune systems, such as enhanced immunotolerance and/or the management of tour stressors by team-practitioners to prevent URTS.

CHAPTER 8 UPPER RESPIRATORY TRACT SYMPTOM RISK IN ELITE FIELD HOCKEY PLAYERS DURING A DRY RUN FOR THE 2020 OLYMPIC GAMES.

Prelude

Chapter 7 found no changes in mucosal immunoendocrine, self-reported wellness and hydration measures during a 3-week overseas tour in elite field hockey players. Additionally, URTS rates were unexpectedly low, with only one player experiencing an URTS episode. The study, however, was limited in terms of a small sample size (n=8) and/or short study period (3-week overseas tour), which may explain the lack of significant changes and low incidence of URTS. Therefore, in Chapter 8, factors influencing URTS risk were examined in a larger cohort of elite field hockey players (n=19) during an 8-week multi-stressor training and competition period that simulated the expected preparatory and competition phases of the 2020 Tokyo Olympic Games.

8.1 Abstract

Purpose: The aims of this study were to: (1) examine if biomarkers and/or self-reported lifestyle and behavioural data can predict URTS risk in elite field hockey players; and (2) investigate the effect of the additional stressor 'repeated heat exposure' on measures of thermoregulation and immunity in this population.

Methods: A prospective cohort repeated measures study design was used to collect URTS, household illness, self-reported wellness, biomarker and thermoregulatory data from elite male field hockey players (n=19), during an 8-week training and competition period that simulated the preparatory and competition phases of the 2020 Tokyo Olympics Games. Heat response testing (HRT) was performed at the beginning of the study period, following heat acclimation (HA) and following an intensified competition period (ICP) played in hot and humid conditions.

Results: Univariate frailty analysis demonstrated that illness in players' households (HR: 4.90; $p < 0.001$) and increased self-reported stress (HR: 0.63; $p = 0.043$) predicted players' risk for URTS. Additionally, low baseline resting SIgA concentration predicted players' 'potential' URTS risk ($p = 0.021$). Repeated heat exposure facilitated thermoregulatory adaptation without attenuating resting immune functions.

Conclusions: Lifestyle and behavioural factors (i.e., household illness and stress) influenced players risk for URTS more than sport-related stressors. Furthermore, the additional sport-related stressor 'repeated heat exposure' did not appear to compromise players resting immunity. To assess elite athletes' risk for URTS during the preparatory and competition phases of pinnacle events, baseline screening of SIgA concentration and regular monitoring of self-reported lifestyle and behavioural data are recommended.

8.2 Introduction

URTS episodes are the most common non-injury related presentation in sports medicine, accounting for 35-65% of illnesses in athletes (Fricker, 1997). The susceptibility of URTS in athletes is suggested be multi-factorial, with stressors of intensified training and competition periods, long-haul travel, high levels of psychological stress and sleep deprivation causing or contributing to increased URTS risk (Simpson et al., 2020; Walsh, 2018). Although elite field hockey players are invariably exposed to such stressors, particularly during the preparatory and competition phases of pinnacle events such as the Olympic Games, factors influencing URTS risk are yet to be defined in this sport.

In comparison to other professional team-sport athletes (e.g., football, rugby), elite field hockey players in New Zealand are unique as they are often amateur (i.e., not paid to play) or semi-professional, and may need to manage additional stressors, such as work and/or study commitments alongside international hockey commitments. As such, elite field hockey players may experience high levels of life-related stress. Previous research in both general (Cohen et al., 1991) and athletic populations (Brink et al., 2010; Drew et al., 2017; Hamlin et al., 2019) have demonstrated a positive association between perceived stress levels and risk of URTS. Therefore, it is possible that elite amateur or semi-professional field hockey players may be more susceptible to URTS than other elite professional team-sport athletes.

Hot environmental conditions are a sport-related stressor that elite field hockey players often encounter during training and competition periods. In previous studies, athletes' responses to thermal stress were predominately assessed using thermoregulatory outcomes (Racinais et al., 2015), as opposed to immune markers. Nevertheless, no exacerbation of exercise-induced immune perturbations has been found with acute exercise in hot conditions compared to thermoneutral conditions (Walsh & Oliver, 2015). Previous studies, however, examined only a one-off bout of exercise in the heat, as opposed to multiple heat exposures which elite field hockey players more often

experience (e.g., during HA and tournaments held in hot locations). Of the limited work available, no change in immunity has been demonstrated in athletes following HA; although, URTS were not examined (Guy et al., 2016; Willmott et al., 2016). Many elite athletes will train and compete in hot conditions during the preparatory and competition phases of the 2020 Tokyo Olympic Games, which are expected to be the hottest ever (Gerrett et al., 2019). Thus, the examination of combined measures of thermoregulatory, biomarker and URTS data is required to obtain a more comprehensive understanding of elite athletes' responses to repeated heat exposures.

To assess players' URTS susceptibility, identifying biomarker and/or self-reported data with the potential to predict URTS risk is paramount. Previous studies have shown that SIgA (Gleeson et al., 2012), multi-antigen stimulated cytokine responses (Gleeson et al., 2012) and CMV (He, Handzlik, Muhamad, et al., 2013) can predict URTS risk in athletes. In contrast, no significant associations between the above biomarkers and URTS incidence were found in Chapters 5-7. More recently, an association between non-biological self-reported lifestyle and behavioural data and risk for URTS has been demonstrated in the team-sport literature (Fitzgerald et al., 2019; Thornton et al., 2016; Watson et al., 2016). In support, in Chapters 5 and 6, self-reported household illness and sleep quality were found to predict URTS risk in elite rugby union and league players', respectively. However, research examining possible predictors of URTS risk in elite field hockey players is limited, with only one study reporting an inverse association between SIgA and URTS incidence (Mackinnon et al., 1993). Therefore, further research examining associations between biomarkers, self-reported data (lifestyle and behavioural) and URTS in elite field hockey players is warranted.

The current study was conducted in elite male field hockey players during an 8-week multi-stressor training and competition period that acted as a dry run for the preparatory and competition phases of the 2020 Tokyo Olympics. The aims of this study were to: (1) examine if any biomarker and/or self-reported lifestyle and behavioural data can predict

URTS risk in elite field hockey players; and (2) investigate the effect of repeated heat exposure on measures of thermoregulation and immunity in this population.

8.3 Methods

Participants

Nineteen elite male field hockey players (age 24.1 ± 3.1 y, body mass 81.4 ± 7.3 kg, height 183.3 ± 6.8 cm) belonging to the New Zealand national squad (ranked 8th in the world) participated in this study. Positions included: strikers (n=4), midfielders (n=6), defenders (n=6) and goalkeepers (n=3). Supplements that have been proposed to alter specific aspects of the immune system and reduce athletes' URTS risk (e.g., probiotics, echinacea, bovine colostrum, flavonoids, multivitamins and multi-minerals) (Maughan et al., 2018) were prohibited during the study period. All players were informed of the rationale, aims and requirements of the study, before providing written consent (Appendices D and E). The study was approved by the Auckland University of Technology Ethics Committee (Auckland, New Zealand) and endorsed by Hockey New Zealand and the High Performance Sport New Zealand Research Committee (Appendices F and G).

Study design

Self-reported URTS and lifestyle and behavioural data were repeatedly-measured over the 8-week study period, consisting of two-weeks of normal training (NT), two-weeks of HA, one-week of NT and three-weeks of an ICP played in hot and humid conditions. The study period was designed to simulate the teams preparatory (weeks 1-5) and competition (weeks 6-8) phases of the 2020 Tokyo Olympics Games. NT and HA occurred domestically during the Southern hemisphere winter (May-June 2018); whereas the ICP occurred in Japan and India during the Northern hemisphere summer (July 2018). During the three-week ICP, players competed in eight international matches and completed 38:75 hours of international economy class air travel (see Chapter 7 for details). HRT was performed at the beginning of the study during NT (HRT1), following HA (HRT2) and following the ICP (HRT3). Thermoregulatory and biomarker data were collected for each HRT. A schematic of the study timeline and a description of NT, HA and the ICP is illustrated in Figure 8.1.

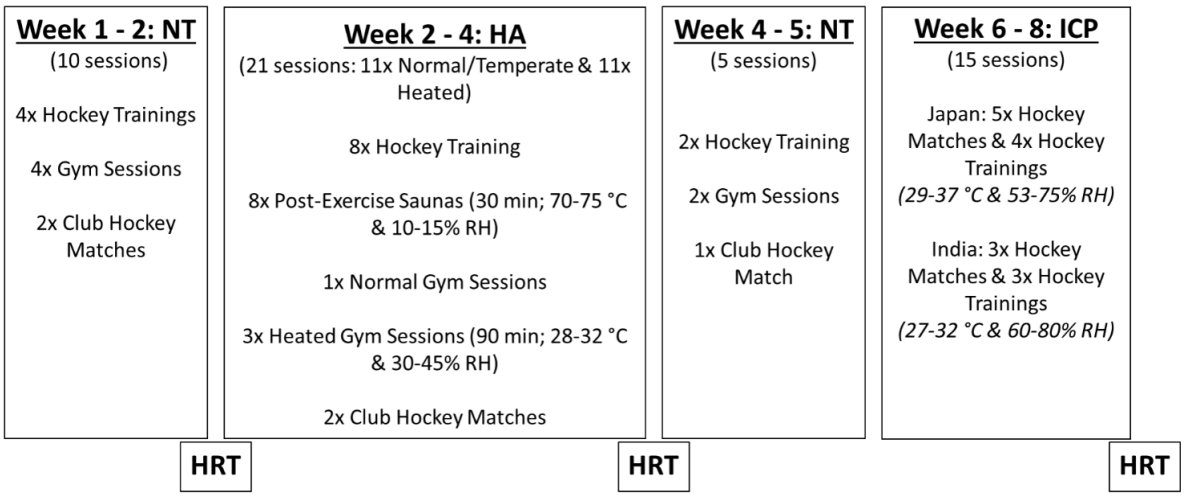

Figure 8.1 A schematic of the 8-week study period involving normal training (NT), heat acclimation (HA), an intensified competition period (ICP) and heat response testing (HRT).

Self-reported URTS and lifestyle and behavioural measures

Players completed an online questionnaire two days per week requiring a 'yes' or 'no' response to the question: "*Are you ill?*". Players who responded 'yes' completed a modified URTS questionnaire (see Chapter 4). Players also provided a 'yes' or 'no' response to the question: "*Is anyone in your household ill?*". Finally, players reported their sleep quantity (in hour) and numerically rated their sleep quality (1 = 'terrible' to 5 = 'good'), mood (1 = 'highly annoyed, irritable and/or down' to 5 = 'great'), muscle soreness, stress levels and fatigue (1 = 'very high' to 5 = 'none'). A total wellness score (ranging from 5-25) was obtained by adding the five individual numerical wellness ratings (Buchheit et al., 2013).

Heat response testing

Twelve players visited the laboratory to perform HRT, on three separate occasions (between 07:00 and 09:00 hours): at the start of the study period during NT (i.e., baseline measurement), following HA (3.6 ± 1.8 days) and following the ICP (8.2 ± 1.8 days) (Figure 8.1). Players were requested to refrain from consuming food for at least 2 hours

and alcohol, caffeine and strenuous exercise for 24 hours prior to their HRT. HRT was 34 minutes and involved 30 minutes of constant-pace cycling (2 Watts/kg and 75-85 reps per minute) followed by 4 minutes of repeated cycling sprint efforts (10 x 4 second supramaximal sprints, 20 second recovery) on a cycle ergometer (Wattbike, Pro/trainer, UK). All HRT was performed in an environmental chamber (Design Environmental, Wales, UK) in which temperature and RH were set at 35°C and 80% RH, respectively. Players were removed from the environmental chamber if core temperate exceeded the predetermined value of 39.5°C or if symptoms of exertional heat illness (e.g., dizziness, nauseous, headache etc.) were reported by players or observed by trained researchers.

Sample collection and preparation

Upon arrival to the laboratory, the players rested in an upright position for 10 minutes and completed a health screen questionnaire (Appendix C), subsequently resting saliva and blood samples were collected (see Chapter 4). Saliva was immediately transferred into Eppendorf tubes and stored at -80°C for SIgA analyses. Approximately 20 ml of blood was collected from each player into 1 x 8 ml serum and 2 x 6 ml K_2EDTA vacutainers. Blood in the serum vacutainer was left to clot for 30 minutes prior to centrifugation at 1500 *g* for 10 minutes at 4°C and separated serum was aliquoted into Eppendorf tubes and stored at -80°C for CMV analysis. K_2EDTA blood was immediately used for haematological analysis and whole blood cultures.

Thermoregulatory responses

Body weight (with shorts only) and drink bottle weight were measured (HW-200KGL/KGV, Weightec Ltd, Albany, NZ) before and after HRT for calculation of sweat rate. Players inserted a rectal thermometer (Monatherm Thermistor, 400 Series, Mallinckrodt Medical, St Louis, MO) ~12cm beyond the anal sphincter for continuous determination of rectal temperature (T_{re}). Wireless telemetric sensors (iButtons, Maxim DS1921G, San Jose, California) were applied to the skin of the left-side chest, upper arm, thigh and calf for measurement of skin temperature (T_{sk}) at 1 minute intervals. Heart

rate was recorded continuously throughout HRT (RS800, Polar Electro Oy, Kempele, Finland). Perceptual ratings including thermal comfort (1 = 'comfortable' to 10 = 'extremely uncomfortable' = 10), thermal sensation (1 = 'unbearably cold' to 14 = 'unbearably hot') and rate of perceived exertion (RPE; 6 = 'no exertion' to 20 = 'maximal exertion') were collected upon entry into the environmental chamber, at 5 minute intervals during constant paced cycling and at the end of HRT.

Laboratory biomarker analysis

SIgA analysis

SIgA concentration was determined using an in-house sandwich ELISA method, (see Chapter 4). The between-run coefficient of variation was 6.5 ± 5.5%. SIgA secretion rate ($\mu l \cdot min^{-1}$) was calculated by multiplying SIgA concentration ($\mu g \cdot mL^{-1}$) with saliva flow rate ($ml \cdot min^{-1}$) (Gleeson et al., 2012).

Serum cortisol analysis

Thawed and centrifuged serum samples were analysed for cortisol concentration using an electrochemoluminescence immunoassay (Cobas Modular P170 Analyser, Roche Diagnostics, New Zealand). According to the manufacturers' protocol, measurement range was between 1.5 and 1750 $nmol \cdot L^{-1}$.

Blood cell count and plasma volume analysis

Samples in K_2EDTA vacutainers were used for haematological analysis to determine total circulating leukocyte and differential WBC counts: neutrophils, lymphocytes, monocytes and eosinophils (see Chapter 4). $CD4^+$ and $CD8^+$ subsets of lymphocytes were measured for HRT1 only with a Muse Cell Analyser (see Chapter 4). Haematocrit and haemoglobin concentrations were also determined using haematological analysis, and resting plasma volume change (%) from HRT1 to HRT2 and HRT1 to HRT3 was calculated using the Dill and Costill (1974) method.

Cytomegalovirus antibody analysis

Thawed and centrifuged serum samples were analysed for detection of CMV antibodies, IgM and IgG (see Chapter 4). The presence of CMV IgM antibodies is suggestive of an acute, recent or reactive infection and the presence of IgG antibodies indicates prior infection (Griffiths et al., 2015).

Stimulated whole blood cytokine analysis

Samples in K_2EDTA vacutainers were used for determination of multi-antigen stimulated cytokine (IFN-γ, IL-10 and IL-4) concentrations by whole blood culture (see Chapter 4).

Data analysis

Data were assessed for normality using the Shapiro-Wilk test and reported using mean and standard deviation (unless otherwise specified). Resting baseline biomarker data from HRT1 was compared between players who experienced no URTS (URTS-free) and players who experienced at least one URTS episode (≥1 URTS) using unpaired t-tests and Mann Whitney U tests. Resting biomarkers and thermoregulatory measures between HRT1, HRT2 and HRT3 were analysed using one-way ANOVA tests with Bonferroni post-hoc tests or Kruskal-Wallis tests, with follow up Mann-Whitney U tests. Changes in total wellness, sleep quantity, sleep quality, mood, muscle soreness, stress and fatigue over time were assessed using a one-way within-measures ANOVA tests or Friedmans tests. Assumptions of homogeneity and sphericity were checked using Levene's test and, where appropriate, adjustments to the degrees of freedom were made using Greenhouse-Geisser correction method. The Bonferroni post-hoc test was used to evaluate pairwise comparisons of time points.

Survival analysis was performed to determine predictors of URTS risk. Frailty model analysis was selected as it enables analysis where subjects can experience an event (i.e., URTS episode) more than once over the study period. Univariate frailty model analysis for URTS risk was run for the following independent predictor variables:

household illness, total wellness, sleep quantity, sleep quality, mood, muscle soreness, stress and fatigue. The significance for the current study was set at $p<0.05$. Frailty model analysis was performed using Stata (version 15) and all other statistical analysis was conducted in IBM SPSS (version 25).

8.4 Results

URTS incidence, severity, and duration

A total of 17 URTS episodes were reported during the 8-week study period: nine players (48%) did not experience a single URTS episode, four players (21%) experienced one URTS episode, five players (26%) experienced two URTS episodes, and one player (5%) experienced three URTS episodes. When a URTS episode was present, the total weekly symptom severity score and duration of symptoms were 30.6 ± 15.0 and 5.8 ± 3.0 days, respectively. The number of players reporting URTS were 15 (60%), 8 (32%) and 2 (8%) during NT, HA and the ICP, respectively (Figure 8.2). Three players missed one training session each during HA due to URTS severity. No training sessions were modified because of URTS.

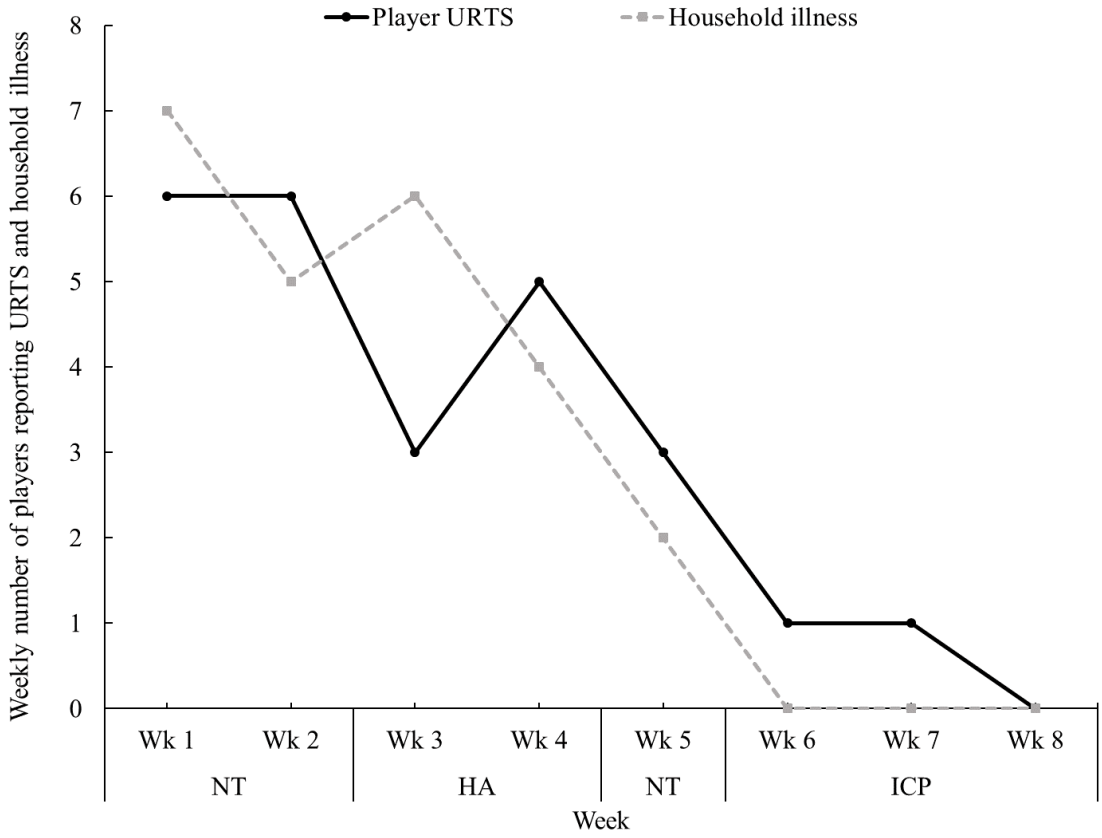

Figure 8.2 Weekly number of players reporting URTS and household illness over the 8-week monitoring period. Normal training (NT), heat acclimation (HA) and the intensified competition period (ICP) are displayed on the χ axis.

Resting biomarkers and URTS risk

Of the 12 players who provided blood and saliva samples, five players (42%) did not experience a single URTS episode (i.e., URTS-free) and seven players (58%) experienced at least one URTS episode (i.e., ≥1 URTS). Baseline resting SIgA concentration was significantly lower in ≥1 URTS players compared with URTS-free players ($p = 0.021$) (Table 8.1). However, no other biomarkers significantly differed between URTS-free and ≥ 1 URTS players (Table 8.1).

Table 8.1 Baseline measures between URTS-free players and players who experienced at least one URTS episode (≥ 1 URTS)

	URTS-free (5)	**≥ 1URTS (7)**	**P value**
URTS episodes	0 ± 0	1.9 ± 0.7	NC
History of asthma (%)	20	0	NC
History of allergies (%)	40	0	NC
History of recurrent URTS (%)	0	30	NC
Saliva flow rate ($mL \cdot min^{-1}$)	0.5 ± 0.2	0.5 ± 0.2	0.350
SIgA concentration ($\mu g \cdot mL^{-1}$)	382 ± 34	280 ± 127	0.021
SIgA secretion rate ($\mu l \cdot min^{-1}$)	178 ± 60	145 ± 91	0.299
WBC count (x 10^9 cells $\cdot L^{-1}$)	5.6 ± 1.2	6.3 ± 2.0	0.101
Neutrophil count (x 10^9 cells $\cdot L^{-1}$)	2.8 ± 1.0	3.5 ± 1.6	0.175
Lymphocyte count (x 10^9 cells $\cdot L^{-1}$)	2.1 ± 0.4	2.0 ± 0.3	0.181
Monocyte count (x 10^9 cells $\cdot L^{-1}$)	0.5 ± 0.1	0.6 ± 0.2	0.074
Eosinophil count (x 10^9 cells $\cdot L^{-1}$)	0.2 ± 0.1	0.2 ± 0.1	0.894
$CD4^+$ cell count (x 10^9 cells $\cdot L^{-1}$)	1.0 ± 0.2	0.8 ± 0.2	0.475
$CD8^+$ cell count (x 10^9 cells $\cdot L^{-1}$)	0.6 ± 0.1	0.6 ± 0.2	0.141
CMV seropositive (#)	0	3	NC
CMV IgG antibody titre ($IU \cdot mL^{-1}$)	0.2 ± 0.0	37.6 ± 74.4	0.268
Serum cortisol ($nmol \cdot L^{-1}$)	411 ± 86	329 ± 104	0.646
IFN-γ production ($pg \cdot mL^{-1}$)	5.4 ± 4.3	4.1 ± 3.1	0.432
IL-4 production ($pg \cdot mL^{-1}$)	7.2 ± 4.4	9.0 ± 3.5	0.784
IL-10 production ($pg \cdot ml^{-1}$)	6.4 ± 2.0	7.5 ± 2.3	0.565

Self-reported lifestyle and behavioural measures, and URTS risk

A total of 24 household illness incidences were reported over the 8-week monitoring period; 14 (58%), 10 (42%) and 0 (0%) household illness incidences occurred during NT, HA and the ICP, respectively (Figure 8.2). Household illness was a significant predictor for URTS risk ($p < 0.001$), whereby players were almost five-fold more at risk for an URTS episode when illness in the household was present (Table 8.2). Stress was also a significant predictor for URTS, for which players were ~2% more at risk for URTS episodes when self-reported stress levels were increased ($p = 0.043$) (Table 8.2). In contrast, total wellness, sleep quantity, sleep quality, mood, muscle soreness and fatigue did not predict URTS risk (Table 8.2). Sleep quantity was significantly ($p < 0.001$) higher during the ICP (8.1 ± 1.0 h) compared with NT (7.3 ± 1.0) and HA (7.2 ± 0.9). However, total wellness, sleep quality, mood, muscle soreness, stress and fatigue scores were unaltered across the study period ($p \geq 0.05$) (Figure 8.3).

Table 8.2 Field hockey players (n=19) hazard ratios for URTS episodes

	Hazard ratio	**Standard error**	**Z value**	**P value**	**95% confidence interval**
Household illness	4.924	2.234	3.51	0.000	2.020-11.980
Total wellness	0.897	0.062	-1.56	0.118	0.783-1.028
Sleep quantity	0.851	0.173	-0.79	0.427	0.571-1.268
Sleep quality	0.961	0.181	-0.21	0.833	0.664-1.390
Mood	0.883	0.254	-0.43	0.665	0.502-1.553
Muscle soreness	1.150	0.331	0.48	0.628	0.654-2.022
Stress	0.627	0.145	-2.02	0.043	0.398-0.986
Fatigue	0.688	0.196	-1.31	0.190	0.394-1.204

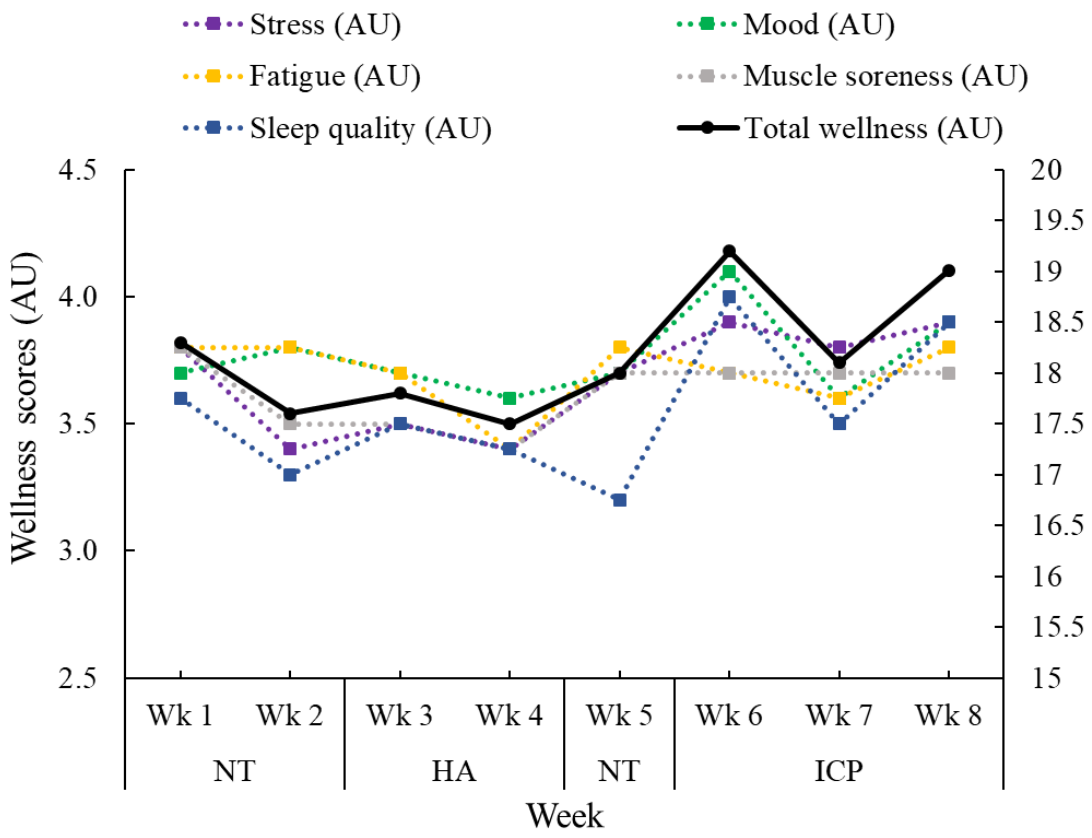

Figure 8.3 Mean weekly total wellness score individual wellness indicators across the 8-week study period. Normal training (NT), heat acclimation (HA) and the intensified competition period (ICP) are displayed on the χ axis.

Biomarker responses to repeated heat exposure

No significant changes in resting biomarkers were evident between HRT1, HRT2 and HRT3 (Table 8.3).

Table 8.3 Field hockey players (n=12) resting biomarkers at baseline (HRT1), following HA (HRT2) and following the ICP (HRT3)

	HRT1	HRT2	HRT3	P value
Saliva flow rate ($mL \cdot min^{-1}$)	0.5 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.906
SIgA concentration ($\mu g \cdot mL^{-1}$)	323 ± 109	267 ± 71	278 ± 95	0.339
SIgA secretion rate ($\mu l \cdot min^{-1}$)	159 ± 79	142 ± 82	158 ± 78	0.845
WBC count (x 10^9 cells $\cdot L^{-1}$)	6.0 ± 1.7	5.5 ± 1.7	5.5 ± 1.3	0.630
Neutrophil count (x 10^9 cells $\cdot L^{-1}$)	3.2 ± 1.4	3.0 ± 1.3	2.7 ± 0.8	0.795
Lymphocyte count (x 10^9 cells $\cdot L^{-1}$)	2.1 ± 0.3	1.8 ± 0.3	2.1 ± 0.6	0.537
Monocyte count (x 10^9 cells $\cdot L^{-1}$)	0.6 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.125
Eosinophil count (x 10^9 cells $\cdot L^{-1}$)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.698
CMV seropositive (#)	3	3	3	NC
CMV IgG antibody titre ($IU \cdot mL^{-1}$)	22 ± 58	23 ± 59	32 ± 63	0.974
Serum cortisol ($nmol \cdot L^{-1}$)	364 ± 102	380 ± 113	459 ± 119	0.110
IFN-γ production ($pg \cdot mL^{-1}$)	4.6 ± 3.5	4.2 ± 2.8	4.4 ± 3.0	0.997
IL-4 production ($pg \cdot mL^{-1}$)	8.2 ± 3.8	7.5 ± 3.6	7.4 ± 4.2	0.358
IL-10 production ($pg \cdot ml^{-1}$)	7.0 ± 2.2	7.5 ± 3.6	7.4 ± 4.2	0.975

Thermoregulatory responses to repeated heat exposure

Peak T_{re} was lower in HRT2 (p = 0.002) and HRT3 (p = 0.001), compared with HRT1 (Table 8.4). Similarly, mean thermal sensation was significantly reduced in HRT2 (p = 0.013) and HRT3 (p = 0.004), compared with HRT1 (Table 8.4). A trend for lower mean RPE was observed in HRT 2 (p = 0.087) and HRT3 (p = 0.061), compared with HRT1 (Table 8.4). A trend also occurred for increased resting plasma volume in HRT3 (p = 0.053), compared with HRT1. However, no significant differences were observed for sweat rate, resting T_{re}, peak T_{sk}, mean heart rate and thermal comfort, between HRT1, HR2 and HRT3 (Table 8.4).

Table 8.4 Field hockey players (n=12) thermoregulatory responses at baseline (HRT1), following HA (HRT2) and following the ICP (HRT3)

	HRT1	HRT2	HRT3	P value
Resting T_{re} (°C)	36.7 ± 0.5	36.7 ± 0.3	36.7 ± 0.4	0.798
Peak T_{re} (°C)	38.4 ± 0.2	38.1 ± 0.3	37.9 ± 0.3	0.001
Peak T_{sk} (°C)	37.1 ± 0.4	36.7 ± 0.5	36.8 ± 0.4	0.085
Heart rate (bpm)	161 ± 5.7	156 ± 8.5	155 ± 11.0	0.241
Sweat rate ($L \cdot h^{-1}$)	1.4 ± 0.2	1.6 ± 0.4	1.4 ± 0.3	0.439
Δ resting plasma volume (%)	-	4.4 ± 6.6	4.8 ± 4.8	0.031
Thermal comfort (AU)	5.8 ± 1.3	4.7 ± 1.2	5.1 ± 1.2	0.107
Thermal sensation (AU)	11.2 ± 0.9	10.1 ± 0.6	10.0 ± 0.7	0.002
RPE (AU)	15.0 ± 1.3	13.6 ± 1.6	13.5 ± 1.4	0.031

8.5 Discussion

This study provides a comprehensive assessment of elite field hockey players' responses to a real-life multi-stressor training and competition period that acted as a dry run for the 2020 Tokyo Olympics. The main findings of the current study were; 1) URTS risk was predicted by illness in players' households and self-reported stress; 2) low baseline SIgA concentration predicted players 'potential' URTS risk; and 3) repeated heat exposures facilitated partial thermoregulatory adaptation without altering resting immunity.

Household illness was found to be the strongest predictor of URTS risk, with players almost five times more at risk for an URTS episode when a member in their household was ill. This finding corroborates research in the general population (Petrie et al., 2013; Tsang et al., 2016) and supports the evidence presented in elite rugby union players (Chapter 6). The timeframe between household illness and players actual onset of URTS could not be ascertained in the current study, as self-reported household illness and player URTS data were only collected twice a week. However, in the general population, infection transmission from one household member to another typically occurs within three days (Tsang et al., 2016). To reduce risk for household infection transmission, general population research suggests that illness prevention strategies (e.g., antiviral drugs, face masks and intensified hand hygiene) should be applied to all household occupants within 36 hours of the household members' symptoms onset (Tsang et al., 2016). Early identification of household illness and intervention may maintain field hockey player health and minimise disruptions to training and competition performance.

Higher self-reported stress was found to predict increased URTS risk in elite field hockey players. In support, a positive association between perceived stress levels and illness incidence has been demonstrated in Australian Olympic athletes (Drew et al., 2017), elite youth soccer players (Brink et al., 2010) and university athletes (Hamlin et al., 2019). In this study, despite playing for an elite national field hockey team, players were not paid

to play; and thus, had to balance work and/or study alongside sporting commitments. High levels of life-stress (e.g., work/study, relationships, finance, home-life etc.), particularly during domestic NT and HA periods, may explain why stress was identified as a significant predictor of URTS risk. Indeed, in the general population it is well established that long lasting high levels of psychological stress impair immune functions (Dhabhar, 2014) and increase URTS susceptibility (Cohen et al., 1991). Recently, stress has also been shown to effect immune responses to exercise (Edwards et al., 2018), suggesting athletes' stress levels may be important for determining whether a given exercise regimen is salubrious or harmful. Therefore, team-sport practitioners should regularly monitor athletes' stress levels and consider stress management strategies (e.g., mindfulness, relaxation techniques, consultation with psychologists, etc.), particularly during domestic training periods where players may be more exposed to multiple life-stressors.

Resting biomarkers have been shown to predict athletes' 'potential' URTS risk (Gleeson & Bishop, 2013). In support, the current study found that baseline SIgA concentration was significantly lower in players who experienced at least one URTS episode compared to URTS-free players. However, no difference in multi-antigen stimulated cytokine production and CMV IgG antibody titers was observed between ≥1 URTS and URTS-free players. Thus, SIgA appeared to be a worthwhile measure for predicting players 'potential' URTS risk, whereas the utility of screening blood biomarkers was uncertain. A limitation of this study is that SIgA was only measured at three time-points. Previous studies have found that ~50% of illnesses can be explained by a preceding decrease in SIgA, when more frequent saliva sampling (e.g., weekly, bi-weekly) is employed (Neville et al., 2008; Tiernan et al., 2020). Therefore, team-sport practitioners should consider assessing SIgA at baseline and, if feasible, regularly monitoring SIgA, particularly in players who exhibit low resting baseline SIgA concentration.

The current study found that self-reported total wellness, sleep quantity and quality, mood, muscle soreness and fatigue did not predict URTS risk, which corroborates previous findings in football (Watson et al., 2016) and cricket players (Ahmun et al., 2019). Players were exposed to several stressors during the study period; yet, most wellness indicators remained stable, possibly explaining why these measures were not associated with URTS risk. Sleep quantity was the only wellness indicator to change, for which athletes slept an additional one hour per night during the ICP compared with NT and HA. During NT and HA, training sessions typically started in the early hours of the morning (~06:00-06:30 hours) to accommodate for players work and/or study commitments, whereas, during the ICP players woke at ~08:00 hours. Thus, later wake times may explain the increased sleep quantity during the ICP. Sleep duration has been shown to influence illness risk, as Cohen and colleagues (2009) demonstrated that individuals sleeping less than 7 hours per night prior to inoculation with an active cold virus were three times more likely to develop a cold than those sleeping 8 hours or more (Cohen et al., 2009). In support, a significant inverse association between sleep quantity and illness incidence was recently demonstrated in elite Australian footballers (Fitzgerald et al., 2019). In the current study URTS incidence was lower during the ICP, and this in part may be explained by players increased sleep quantity. However, frailty model analysis revealed sleep quantity was in fact not a significant predictor of URTS episodes. Research in this area is still in its infancy; therefore, more work is needed to clarify whether monitoring of sleep parameters can assist practitioners in predicting UTRS risk in elite team-sport athletes.

Exercise immunology research suggests that elite athletes have a similar distribution of URTS episodes to the general population (Gleeson & Pyne, 2016). However, rather than following seasonal trends, URTS tend to cluster around sport-related stressors, namely intensified training periods, congested competition schedules and long-haul travel (Gleeson & Pyne, 2016). Our findings challenge this notion, as a higher URTS incidence was observed during winter training weeks (i.e., NT and HA) than during the overseas

ICP which involved several sport-related stressors yet occurred during the summer. Similar findings have been reported in elite cross country skiers and swimmers, with athletes 2-3 times more at risk for URTS episodes during winter- versus summer- training and competition periods (Hellard et al., 2015; Svendsen et al., 2016). The reasons for higher URTS incidence in winter remain controversial; and several causal factors, such as changes in immune function, vitamin D deficiency, dehydration of mucus in the nose and respiratory tract and increased contact due to more times spent indoors, have been suggested (Bloom-Feshbach et al., 2013). Thus, winter months may in fact be a stronger risk factor for URTS incidence than sport-related stressors. However, causes of URTS episodes are multi-factorial; therefore, in the current study, the combination of winter months, HA, household illness incidence, stress and other lifestyle and behavioural factors (e.g., sleep) likely increased URTS incidence during the domestic training period, compared with the overseas ICP.

Elite field hockey players are often exposed to extreme environmental conditions during training and competition. Indeed, athletes will encounter multiple heat exposures during the preparatory and competition phases of the 2020 Tokyo Olympics which are expected to be extremely hot and humid (Gerrett et al., 2019). This is the first study to simultaneously examine elite athletes thermoregulatory and biomarker responses to repeated heat exposures, specifically HA and an ICP in hot and humid conditions. Following HA and the ICP, improvements in peak T_{re}, mean thermal sensation, RPE and plasma volume occurred, while sweat rate, resting T_{re}, peak T_{sk}, mean heart rate and thermal comfort were unaltered. These results indicate that repeated heat exposure was a significant stressor as players partially adapted. However, no change in resting biomarkers occurred following HA and the ICP. Therefore, repeated heat exposure appears to facilitate thermoregulatory adaption without compromising resting immune function. A limitation of the current study is that saliva and blood samples were collected ~8 days after the ICP (i.e., HRT3), thus it is possible changes in biomarkers were not captured due to this sampling delay. Nevertheless, our results corroborate evidence in

ultra-endurance athletes (Willmott et al., 2016) and recreationally active males (Guy et al., 2016) demonstrating no change in resting immune markers following repeated heat exposure.

8.6 Conclusions

Understanding the factors that meaningfully impact upon elite athletes' risk for URTS is crucial for informing management and treatment strategies to maintain their health. The results of this study suggest factors outside of the team-environment (i.e., household illness, stress and winter months) influence elite field hockey players risk for URTS more than sport-related stressors (i.e., congested competition schedule, long-haul travel). Furthermore, the addition of heat exposure as an additional sport-related stressor did not appear to compromise players' resting immunity during training and subsequent competition. To assess elite athletes' risk for URTS during the preparatory and competition phases of pinnacle events, baseline screening of SIgA concentration and regular monitoring of self-reported lifestyle and behavioural data (i.e., household illness and stress) are recommended.

CHAPTER 9 GENERAL DISCUSSION

9.1 Overview

Given recurrent or severe URTS can impair athletes training availability and performance (Gleeson & Pyne, 2016), keeping athletes healthy is a primary objective in elite team-sport. Despite this, research in elite team-sport athletes is lacking, with most exercise immunology studies focusing on endurance athletes. Considering the vast differences between team and endurance sports, it is unrealistic to extrapolate results found using endurance athletes. To address this gap, this thesis involved four studies that have extended current knowledge on factors influencing URTS risk in elite team-sport athletes. Specifically, a multifaceted/holistic approach was used in this thesis, whereby several stressors and measures were examined in a range of settings (i.e., different team-sports and periods of the season), to better understand the complex and often interrelating factors that influence elite team-sport athletes risk for URTS. The findings of this thesis have revealed important implications for team-sport practitioners in informing athlete management to maintain athlete health, and potentially minimise disruptions to training and competition performance. In this chapter, the studies comprising this thesis (Chapters 5-8) will be discussed as a cohesive whole under four key themes: 1) URTS incidence in elite team-sport athletes; 2) periods of increased risk/risk factors for URTS; 3) predictors of URTS risk; and 4) strategies to minimise team-sport athletes risk for URTS. The chapter will then describe research limitations, directions for future research and practical recommendations.

9.2 Theme 1: URTS incidence in elite team-sport athletes

Based on the findings from this thesis, URTS incidence varies between sporting teams, with 36-68% of rugby union, rugby league and field hockey players experiencing at least one URTS episode during training and competition periods (Chapters 5, 6 and 8). The between-sport differences in URTS incidence may be explained by differences in sport-

related stressors (e.g., training, competition and travel schedules). However, they could also reflect methodological differences: for example, 30-week (Chapter 6) and 8-week (Chapter 8) monitoring periods were used with elite rugby union and field hockey players, respectively. In Chapter 5**,** when the same monitoring period (i.e., 11 weeks) and macrocycle (i.e., pre-season training) were examined, no difference in URTS incidence was observed between elite rugby union and league players. It is important to note that the present studies (Chapters 5, 6 and 8) observed lower total URTS incidence than previous team-sport studies (Bury et al., 1998; Cunniffe et al., 2011; Schwellnus et al., 2012). For example, in previous studies 72-92% of elite rugby union and football players have been found to experience URTS episodes during 16-52-week training and competition periods (Bury et al., 1998; Cunniffe et al., 2011; Schwellnus et al., 2012). These longitudinal studies were conducted in 1995-1996 (Bury et al., 1998), 2005-2006 (Cunniffe et al., 2011) and 2010 (Schwellnus et al., 2012), whereas data in the present thesis was collected from 2016-2018. As such, URTS rates appear to be declining in elite team-sport, and this concurs with research conducted on Olympic athletes, whereby lower illness incidence was demonstrated at the 2016 Olympics compared with the 2008 and 2012 Olympics (Soligard et al., 2017). In the past decade, numerous exercise immunology studies and reviews have been published outlining recommended guidelines and strategies to keep athletes healthy (Schwellnus et al., 2016; Walsh, 2018; Walsh, Gleeson, Pyne, et al., 2011; Walsh, Gleeson, Shephard, et al., 2011). Therefore, declining UTRS rates in elite sport may, in part, be explained by a greater awareness of athletes, coaches and practitioners with respect to factors influencing URTS risk and implementation of illness prevention strategies.

This thesis demonstrated high inter-individual variability in URTS incidence among a homogenous group of elite team-sport athletes. Specifically, rugby league players experienced 0-5 URTS episodes (Chapter 5), rugby union players experienced 0-4 URTS episodes (Chapter 6) and field hockey players experienced 0-3 URTS episodes (Chapter 8) during 8-30-week training and competition periods. It was assumed that

athletes from the same sporting team were exposed to similar sport-related stressors (e.g., training load, travel and competition schedule). Thus, the high inter-individual variability may be explained by differences in individuals' genetics and/or lifestyle and behaviours. Previous research suggests cytokines may account for differences in URTS susceptibility by influencing immune cell functions (Gleeson & Bishop, 2013). Indeed, lower unstimulated IFN-γ production (Clancy et al., 2006) and IL-2 low-expression genotype (Cox et al., 2010) as well as higher multi-antigen stimulated IL-4 and IL-10 production (Gleeson et al., 2012) have been found in URTS-prone athletes compared with URTS-free athletes. In contrast, in Chapter 6, no difference in resting multi-antigen stimulated cytokine production (i.e., IFN-γ, IL-4 and IL-10) was found between URTS-prone and URTS-free rugby union players. Similarly, these measures did not differ between URTS-free elite field hockey players and those who experienced one or more URTS episodes (Chapter 8). As such, the inter-individual variability in URTS incidence observed in this thesis may instead be due to athletes' lifestyle and behavioural factors. Indeed, household illness incidence (Chapters 6 and 8), decreased sleep quality (Chapter 5) and increased stress (Chapter 8) were identified as risk factors for URTS. In support, recent team-sport studies have also demonstrated a relationship between URTS risk and lifestyle and behavioural factors including overall well-being (Thornton et al., 2016), stress (Hamlin et al., 2019) and sleep quantity (Fitzgerald et al., 2019).

In comparison to recreational and sub-elite athletes, fewer studies have examined URTS incidence in elite athletes (Gleeson & Pyne, 2016). Nevertheless, emerging evidence suggests that performance level is associated with URTS risk, with elite athletes found to be less URTS-prone than sub-elite athletes (Hellard et al., 2015; Raysmith & Drew, 2016). These research findings support the S-shape curve hypothesis which suggests that athletes at an elite level possess robust immune systems capable of resisting infection even during severe psychophysiological stress (Malm, 2006). In accordance with the S-shape curve, a 'survival' effect was more recently proposed, whereby elite athletes' immune systems can be trained to adapt or attenuate responses to stressors

(Simpson et al., 2020). However, lower URTS incidence may also reflect better management and illness prevention strategies in elite settings (Williams et al., 2018). To date, research comparing URTS incidence between elite and sub-elite athletes has been conducted in endurance-sport athletes (Hellard et al., 2015; Raysmith & Drew, 2016). In this thesis, the influence of performance level on team-sport athletes' URTS incidence could not be determined as all studies were conducted on elites. However, sporting teams differed in that elite rugby union and league players were professional, while elite field hockey players were amateur or semi-professional. It is possible that URTS incidence differs between professional and amateur or semi-professional elite team-sport athletes; however, this could not be examined in the present thesis as monitoring periods differed between the studies. Therefore, to better understand the incidence of URTS in team-sport athletes, further research is warranted comparing URTS incidence in elite versus sub-elite athletes and elite professional versus elite amateur or semi-professional athletes.

9.3 Theme 2: Periods of increased risk/risk factors for URTS

Sport-related stressors

Intensified training and competition periods have been identified as periods of increased risk for URTS in team-sport athletes (Gleeson & Pyne, 2016; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & Jose Filho, 2012; Putlur et al., 2004; Thornton et al., 2016; Watson et al., 2016). In contrast, in the present thesis, URTS risk was not influenced by internal training load in elite rugby union and league players (Chapters 5 and 6). Moreover, in elite field hockey players, URTS incidence was not increased during a congested competition schedule, as only one player experienced an URTS episode during an overseas tour involving eight matches in 21 days (Chapter 7). In support, previous studies have also found no relationship between athletes' training/competition load and illness incidence (Anderson et al., 2003; Fitzgerald et al., 2019; Hamlin et al., 2019; Hellard et al., 2015; Svendsen et al., 2016). In the present thesis, all sporting teams had full time sport-scientists who managed athletes' training/competition loads,

and consequently training/competition stress, which may explain the lack of association with URTS risk. Therefore, if elite athletes are managed correctly, intensified training and competition periods may not be as prominent of risk factors for URTS incidence, as previously thought (Nieman, 2000) .

Previous research suggests that long-haul air travel is a key risk factor for URTS in athletes (Fowler et al., 2016; Schwellnus et al., 2012; Svendsen et al., 2016). Increased URTS incidence following long-haul travel has been attributed to a number of factors including the impact of long-haul travel on circadian variations (e.g., immune cells, stress hormones) and sleep, increased exposure to pathogens in the aircraft cabin, and crowded airports (Walsh, 2018). However, in the present thesis, long haul travel did not increase risk for URTS in elite rugby union (Chapter 6) or field hockey players' (Chapter 8). In fact, long-haul travel appeared to protect against URTS incidence in elite rugby union players (Chapter 6). It was noted that rugby union and field hockey team-practitioners targeted illness prevention strategies during periods of international travel (i.e., face masks on planes, hygiene practices, supplementation, etc.), which may explain why athlete health was well-maintained. Indeed, a recent study found that the application of a team illness prevention strategy, including international travel guidelines (i.e., good hygiene practices, prophylactic local antimicrobial spray, probiotics and antibiotic prophylaxis) reduced elite rugby union players' URTS incidence by 59% during the Super Rugby competition which involved periods of long-haul travel (Schwellnus et al., 2019). Therefore, these findings indicate the importance of applying illness prevention strategies during travel periods to maintain athlete health.

It is well established that exercising in hot conditions imposes significant physiological strain (Hargreaves, 2008), because exercise in the heat elicits a more substantial increase in core temperature, cardiovascular drift and circulating stress hormone concentrations than exercise in thermoneutral conditions (Walsh & Whitham, 2006). However, exercise in hot environments does not appear to cause further

immunosuppression than exercise in a thermoneutral environment (Walsh, 2018; Walsh & Oliver, 2015). Furthermore, no change in immunity has been demonstrated following HA (Guy et al., 2016; Willmott et al., 2016). In support, in Chapters 7 and 8, immune markers were unaltered during and following real-life repeated heat exposures in elite field hockey players. However, the direct impact of thermal stress on URTS risk could not be ascertained as elite field hockey players were simultaneously exposed to heat and other sport-related stressors (e.g., training, competition). Therefore, current evidence suggests that exercise in hot environments does not provide an additional threat to athletes' immunity; however, more work is needed to determine its impact on URTS risk.

Stressors outside the team environment

Rest weeks (i.e., Christmas break and bye weeks (i.e., no scheduled trainings or matches)), a period in which athletes were not exposed to sport-related stressors, were identified as a period of increased risk for URTS incidence in elite rugby union players (Chapter 6). This finding may be explained by several factors including the 'let-down effect' a pattern in which people experience an illness not during a concentrated period of stress but after it dissipates (Lipton et al., 2014), or changes in environment and athletes' behaviour during rest weeks. Another interesting finding in this thesis was that elite rugby union and field hockey players appeared to be more at risk for URTS episodes during domestic training weeks compared to the weeks that the teams were competing overseas (Chapters 6 and 8). The higher URTS incidence during domestic weeks may be explained by athletes' lifestyle and behavioural factors (e.g., household illness incidence, high life stress and inadequate sleep). These factors may be less of an issue during weeks in which the teams competed overseas, given athletes, practitioners and coaches all stayed together, allowing athletes to be more closely monitored and managed.

Summary

In the exercise immunology literature, strenuous training, congested competition schedules and long-haul travel have been identified as key periods of increased risk for URTS in athletes (Gleeson & Pyne, 2016). In the present thesis, however, these sport-related stressors were not associated with URTS incidence in elite rugby league (Chapter 5), rugby union (Chapter 6) and field hockey players (Chapters 7 and 8). Our conflicting findings may be explained by our study population of elite athletes who are known to be less URTS prone than previously studied recreational and sub-elite athletes (Hellard et al., 2015; Raysmith & Drew, 2016). As previously discussed, the lower URTS risk in elite athletes has been attributed to the unique traits of elite athletes' immune systems and/or the monitoring and management of stressors by team-sport practitioners to prevent URTS. The latter explanation likely holds true, given factors outside of the team environment such as household illness (Chapters 6 and 8), rest weeks (Chapter 6), domestic weeks (Chapters 6 and 8) sleep quality (Chapter 5) and stress (Chapter 8) were found to influence URTS risk. Therefore, it is critical for team-sport practitioners to consider monitoring and managing athletes' lifestyle and behavioural factors in order to better maintain their health.

9.4 Theme 3: Predictors of URTS risk

Biomarkers

SIgA

Baseline SIgA levels have been shown to predict athletes' 'potential' URTS risk (Gleeson et al., 2012; Gleeson & Bishop, 2013). In support, Chapter 8 identified a significantly lower baseline SIgA concentration in elite field hockey players who experienced at least one URTS episode than those who remained URTS-free during an 8-week training and competition period (Chapter 8). However, Chapter 5 and 6 showed that baseline SIgA concentration did not predict 'potential' URTS risk in elite rugby union or league players. The use of a one-off SIgA measure to predict URTS risk is known to be limited by the high degree of analytical and biological variability associated with this marker (Neville et

al., 2008). Nevertheless, screening of baseline SIgA levels could still be worthwhile for identifying any athletes who are IgA deficient as these athletes may be more susceptible to recurrent URTS (Jorgensen et al., 2013).

To predict athletes' risk for URTS, regular monitoring of SIgA is an arguably more effective approach than a one-off baseline measurement. Indeed, in the exercise immunology literature, declining levels of SIgA have been associated with increased incidence of URTS (Fahlman & Engels, 2005; Neville et al., 2008; Tiernan et al., 2020). In this thesis, a trend for an inverse association between SIgA concentration and URTS incidence was also found in elite rugby union players (Chapter 6). However, alterations in SIgA concentration did not predict URTS risk in elite rugby league (Chapter 5) or field hockey players (Chapter 7). In line with our findings, discrepant evidence has been reported in previous team-sport studies, with an inverse- (Moreira et al., 2014; Tiernan et al., 2020; Yamauchi et al., 2011b) or no- (Cunniffe et al., 2011; Moraes et al., 2017; Moreira et al., 2008) association found between SIgA levels and URTS incidence. These conflicting findings may be explained by methodological differences in measuring SIgA (Pritchard et al., 2017). Indeed, inconsistent results between Chapters 5, 6 and 7 could be explained by the different methods used to analyse SIgA; an in-house sandwich ELISA and point-of-care method was used with rugby union and league players (Chapters 5 and 6) and field hockey players (Chapter 7), respectively. However, the same methodology was used to analyse SIgA in rugby union (Chapter 6) and league players (Chapter 5), yet results still differed. The contrasting results may be explained by the different monitoring periods; SIgA and URTS data were collected for 11-weeks in rugby league players (Chapter 5) and 30-weeks in rugby union players (Chapter 6). The only study in this thesis to observe a trend for an association between SIgA concentration and URTS risk had the longest monitoring period (30-weeks), thus it is possible that longer term monitoring periods are needed to detect a relationship.

The method of expressing SIgA may also influence its association to URTS. In all four studies in this thesis, absolute SIgA concentration was determined. However, different methods of expressing SIgA were also used in Chapters 6 and 7; namely, SIgA secretion rate (Chapter 6), percentage change of SIgA concentration from mean healthy SIgA concentration (Chapter 6) and percentage change of SIgA concentration from baseline SIgA concentration (Chapter 7). Similarly, previous team-sport studies have also expressed SIgA in various ways when exploring the association between SIgA and URTS, including absolute concentration (Cunniffe et al., 2011; Fahlman & Engels, 2005; Morgans et al., 2014; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & José Filho, 2012; Tiernan et al., 2020), secretion rate (Cunniffe et al., 2011; Fahlman & Engels, 2005; Mortatti, Moreira, Aoki, Crewther, Castagna, de Arruda, & José Filho, 2012) and percentage change from mean healthy SIgA (Cunniffe et al., 2011; Sawczuk et al., 2020). Due to the methodological heterogeneity in expressing SIgA, it remains unclear which is approach is best. Therefore, future researches should seek to conduct a meta-analysis to determine which approach is most predictive of URTS risk.

In agreement with previous team sport studies, equivocal evidence on the relationship between SIgA and URTS incidence has been presented in this thesis. Therefore, these findings must be interpreted cautiously as there is not sufficient evidence to suggest that measurement of SIgA alone can predict URTS risk. Nevertheless, if teams have the resources available there may be some benefit in monitoring SIgA concentration, particularly in URTS-prone athletes (i.e., those who experience recurrent URTS episodes). SIgA monitoring has become more accessible in elite sport with the emergence of point-of-care devices, potentially meaning that assessment of an individual athlete's risk of URTS can occur in real time (Dulson et al., 2019). However, to ensure the validity of a SIgA measure, team-practitioners should standardise saliva sampling to control for factors known to influence SIgA (i.e., diurnal rhythm, nutrition and hydration status, caffeine) (Bishop & Gleeson, 2009). In addition, to best predict URTS risk, team-

practitioners should seek to monitor SIgA on a frequent (i.e., weekly basis) and longitudinal basis (i.e., entire sporting season).

Baseline blood biomarkers

It has been reported that resting baseline blood biomarkers, including multi-antigen stimulated cytokine production (Gleeson et al., 2012; Gleeson & Bishop, 2013) and CMV serostatus (He, Handzlik, Muhamad, et al., 2013), can predict athletes' 'potential' URTS risk. However, this thesis observed no association between these biomarkers and URTS incidence in any of the three elite sporting teams (Chapters 5, 6 and 8). There are several factors that may explain our discrepant findings. Firstly, the sample sizes (n=19-28) of this thesis were considerably smaller than previous studies (n=80-236) (Gleeson et al., 2012; Gleeson & Bishop, 2013; He, Handzlik, Muhamad, et al., 2013), and might have reduced the statistical power to detect differences in baseline biomarkers between URTS-free players and those who experienced at least one URTS episode. Secondly, this thesis utilised different sample analysis methodology (i.e., multi-antigen stimulant dilution and analysis methods) compared with previous studies (Gleeson et al., 2012; Gleeson & Bishop, 2013; He, Handzlik, Muhamad, et al., 2013). Finally, the participants of this thesis were elite team-sport athletes rather than recreational endurance-based athletes (Gleeson et al., 2012; Gleeson & Bishop, 2013; He, Handzlik, Muhamad, et al., 2013). Given these methodological differences, it is difficult to directly compare the findings from the present thesis and previous studies. It is acknowledged that the practicality of measuring blood biomarkers in a real-world elite team-sport-setting is limited due to the cost, invasiveness and laboratory expertise required to obtain results. Therefore, based on these constraints and the findings in this thesis, screening of baseline blood immune markers to predict URTS risk may not be warranted in elite team-sport athletes.

Interestingly, a difference in CMV serostatus was reported between contact and non-contact elite team-sport athletes in the present thesis. A higher proportion of elite rugby

union and league players were CMV seropositive (rugby union players: 73%; rugby league players: 96%) (Chapters 5 and 6) than elite field hockey players (25%) (Chapter 8). In fact, the CMV prevalence observed in elite field hockey players is similar to that reported in endurance athletes (23-25%) (Gleeson et al., 2016; He, Handzlik, Muhamad, et al., 2013). CMV is a member of the human herpes virus group that is mostly transmitted through direct contact with bodily fluids (Simpson et al., 2016). The higher CMV seropositivity in rugby union and league players may be explained by the contact-nature of these sports, as the skin-to-skin contact and trauma (i.e., abrasions and lacerations of skin) inherent in playing rugby is known to facilitate the transmission of herpes viral infections (White & Grant-Kels, 1984). To date, research examining the relationship between CMV and URTS risk is still in its infancy. Some researchers suggest CMV seropositivity increases URTS risk (Gleeson & Pyne, 2016), but others show reduced URTS risk in the immunocompetent athlete (He, Handzlik, Muhamad, et al., 2013; Simpson et al., 2016). In contrast, no association between CMV serostatus and URTS incidence was found across all three sporting teams examined in this thesis (Chapters 5, 6 and 8).

Non-biological self-reported measures

Household illness

In the general population, household illness incidence is a well-established risk factor for respiratory infection transmission, because of the high frequency and intensity of contacts that occur between household members (Tsang et al., 2016). However, prior to this thesis, the influence of household illness on URTS risk had yet to be examined in athletic populations. In Chapters 6 and 8, household illness was found to be the strongest predictor of URTS risk. Elite rugby union and field hockey players were almost three and five times more likely, respectively, to experience an URTS episode when a member in their household was ill. Household illness was measured using a question *"Is anyone in your household ill?"*, and athletes were simply required to provide a 'yes' or 'no' answer to the question. As such, we were unable to ascertain whether household members were

specifically experiencing URTS. More insight would have been gained if we had we asked athletes to report the illness symptoms that their household members had been experiencing, and this information could assist practitioners in applying targeted illness prevention strategies for athletes. Additionally, it could be useful for practitioners to administer a living arrangements questionnaire at the start of the sporting season, given increased risk of household respiratory infection transmission has been associated with a greater number of household occupants (i.e., 4 or more household members) (McIsaac et al., 1998) and living with young children (i.e., under 5 years) (Viboud et al., 2004). Nevertheless, despite the simplicity of the household illness question used in the present thesis, our findings indicated that self-reported household illness monitoring is a novel, cheap and accessible tool and can be used to predict athletes' risk for URTS.

Self-reported wellness indicators

Self-reported wellness indicators in this thesis demonstrated equivocal evidence of predicting URTS risk in elite team-sport athletes. Self-reported total wellness and sleep quality were found to predict URTS risk in elite rugby league players (Chapter 5); while self-reported stress predicted URTS risk in elite field hockey players (Chapter 8). However, no self-reported wellness indicators were predictive of URTS risk in elite rugby union players (Chapter 6). Similar discrepant finings have been reported in the literature where some previous team-sport studies reported an association between self-reported wellness indictors and illness incidence (Fitzgerald et al., 2019; Hamlin et al., 2019; Thornton et al., 2016), and the others not (Ahmun et al., 2019; Buchheit et al., 2013). The contrasting findings may be explained by methodological differences; wellness questionnaires differ considerably between previous studies in terms of questions asked and Likert scales used (Ahmun et al., 2019; Buchheit et al., 2013; Fitzgerald et al., 2019; Hamlin et al., 2019; Thornton et al., 2016). Similarly, in this thesis, a 10-point scale was used in Chapter 5; whereas a 5-point scale was used in Chapters 6 and 8. More work is needed to establish which type of wellness questionnaire is best for predicting URTS risk. Regardless of the methodological heterogeneity, differences still existed between

elite rugby union and field hockey players even when the exact same wellness questionnaire was used. Elite rugby union players were professional (i.e., paid to play), while elite field hockey players were amateur or semi-professional. Given that elite amateur or semi-professional field hockey players had to balance work and/or study alongside sporting commitments it is conceivable that they experienced higher levels of life stress. This additional life stress may explain why stress was identified as a significant predictor of URTS incidence in elite amateur or semi-professional field hockey players but not in elite professional rugby players. Therefore, the effectiveness of self-reported wellness indicators in predicting URTS risk may be sport and/or cohort specific.

As outlined above, individual wellness indicators of self-reported sleep quality and stress were found to predict URTS risk in elite rugby league (Chapter 5) and field hockey players (Chapter 8), respectively. Similarly, in previous studies, increased incidence of URTS in athletes has been associated with inadequate sleep (Fitzgerald et al., 2019) and increased stress (Brink et al., 2010; Drew et al., 2018; Drew et al., 2017; Hamlin et al., 2019). However, this thesis and previous studies did not examine the impact of sleep and stress on immune markers. As such, possible immune mechanisms underpinning increased URTS risk in athletes with inadequate sleep and increased stress remain uncertain. Nevertheless, in the general population, inadequate sleep and high psychological stress levels have been known to have both positive and negative effects on the immune system, depending on the duration of exposure (Dhabhar, 2014). Short-term sleep deprivation (e.g., 1-2 nights) and psychological stress (e.g., minutes to hours) can ‘prime’ immunity (Dhabhar, 2014). For example, acute exposure to sleep deprivation and increased stress have been found to increase the redistribution of immune cells from the blood to peripheral tissues, enhancing immunosurveillance against pathogens (Dhabhar, 2014). Whereas, long term exposure (e.g., weeks to months) to sleep deprivation and high stress can have detrimental effects on immunity, with suppression of T1 T-cell anti-viral activity reported (Dhabhar, 2014). In summary, it is apparent that sleep and stress can influence athletes’ risk for URTS; however, further research

examining the impact of acute and chronic exposure to sleep deprivation and high stress on immunity is needed to elucidate the mechanisms responsible for increased URTS risk.

Summary

Self-reported lifestyle and behavioural factors were found to be more effective than biomarkers in predicting elite team-sport athletes risk for URTS. In all three elite sporting teams, one or more self-reported measure significantly predicted URTS risk: household illness in rugby union players; sleep quality and total wellness in rugby league players; and household illness and stress in field hockey players. Promising evidence was presented for SIgA which had a tendency for an inverse relationship with URTS incidence in rugby union players (Chapter 6). Resting baseline SIgA concentration was also found to predict field hockey players 'potential' URTS risk (Chapter 8). However, alterations in SIgA concentration were not associated with URTS incidence in elite rugby league (Chapter 5) nor field hockey (Chapter 7) players. Finally, across all three sporting teams examined in this thesis, resting blood biomarkers were not predictive of 'potential' URTS risk (Chapters 5, 6 and 8). Collectively, these findings suggest that non-biological self-reported measures can be used in surrogate of biomarkers to predict elite team-sport athletes' risk for URTS. This finding has major implications as monitoring of self-reported lifestyle and behavioural data is an accessible tool for all sports given it is cheap, can be done anywhere and anytime, and does not require a specialist to administer or interpret.

9.5 Theme 4: Strategies to minimise team-sport athletes' risk for URTS

Maintaining athletes' health is critical for optimising training-availability and competition performance. The evidence in the present thesis highlights factors influencing URTS risk

in elite team-sport athletes. Therefore, this section will outline illness prevention strategies that team-sport practitioners should consider to potentially prevent URTS.

Rest weeks (based on evidence from Chapter 6):

- Advise athletes to maintain illness prevention behaviours around rest weeks. For example, sleep at least 8 hours per night, maintain good hygiene-, nutrition- and hydration- practices, and avoid excessive alcohol consumption and ill people (Schwellnus et al., 2016).
- Consider having athletes visit their training facility for a 'check in' during rest-weeks (e.g., Wednesday of the bye week). Illness prevention behaviours (outlined above) can be reinforced and any athletes experiencing illness can visit their team doctor to receive early treatment and necessary medication.
- Consider providing athletes a rest week 'wellness hamper' containing alcohol-based hand sanitiser (Bloomfield et al., 2007), zinc acetate lozenges to take if they do start experiencing URTS (Hemilä, 2017) and infographics informing and reinforcing key illness prevention behaviours (outlined above).

Domestic training weeks (based on evidence from chapters 6 and 8):

- Similar to rest-weeks, educate and encourage athletes to maintain illness prevention behaviours (outlined above).

Low and/or declining SIgA concentration (based on evidence from chapters 6 and 8):

There is limited evidence supporting the use of specific strategies to increase SIgA concentration. Nevertheless, in a case study conducted on an elite English premier league footballer, nutritional and lifestyle support was found to increase SIgA concentration and decrease URTS incidence (Ranchordas et al., 2016). Therefore, a holistic approach should be undertaken, incorporating nutrition, lifestyle and behavioural strategies, including:

- Encourage athletes to eat a well-balanced diet without excessive or inadequate intake of macro and micro nutrients (Bermon et al., 2017).
- Identify any nutritional deficiencies and target nutritional strategies (Soligard et al., 2017).
- Educate athletes on good hygiene (e.g., regularly wash hands, shower, laundry and clean equipment) (Schwellnus et al., 2016).
- Provide athletes a sleep hygiene protocol (see below under the heading *reduced sleep quality*).
- Consider supplementation; chlorella-derived dietary supplementation (Otsuki et al., 2011, 2012) and vitamin D supplementation (He, Fraser, et al., 2016) have been found to increase resting SIgA secretion.

Household illness incidence (Based on evidence from chapters 6 and 8):

In the general population, pharmaceutical and non-pharmaceutical interventions have been examined for their efficacy in reducing the spread of illness within the household (Tsang et al., 2016). Previous research suggests that these strategies are most effective if they are applied within 36 h of the household members' symptom onset (Tsang et al., 2016). Thus, if feasible, practitioners should monitor household illness on a daily basis through simply adding the 'yes' or 'no' question '*Is anyone in your household ill?'* within existing wellness questionnaires. The pharmaceutical and non-pharmaceutical strategies listed below can be used by the ill household member and/or prophylactically by non-infected household members:

- Pharmaceutical interventions: Zinc acetate lozenges (Hemilä, 2017) and anti-viral drugs (e.g., Oseltamivir and Zanmivir) (Tsang et al., 2016).
- Non-pharmaceutical interventions: Hand hygiene, wash/disinfect surfaces, avoid self-inoculation, cough and sneeze into tissue or elbow (not the hands), isolate the ill household member (i.e., sleep in a room alone) and face masks (Tsang et al., 2016).

Reduced sleep quality (Based on evidence from chapter 5):

- During the first week of pre-season training, administer a validated sleep questionnaire (e.g., Athlete Sleep Behaviour Questionnaire) to identify poor sleepers (Driller et al., 2018).
- Throughout the sporting season, regularly monitor athletes' self-reported sleep quality using a customised wellness questionnaire (Buchheit et al., 2016; Buchheit et al., 2013; Fitzgerald et al., 2019; Thornton et al., 2016).
- Encourage athletes to adopt sleep hygiene strategies, including: maintaining a regular bed and wake time; ensuring a quiet, cool, and dark bedroom environment (19-22°C); avoiding stimulants (e.g., caffeine) prior to sleep; and limiting light-emitting technology devices in the hours prior to sleep (O'Donnell & Driller, 2017).

Increased perceived stress (Based on evidence from chapter 8):

- During the first week of pre-season training, identify athletes with high anxiety, stress and/or depression using a validated questionnaire; for example the Depression, Anxiety, Stress Scale (DASS-21) (Henry & Crawford, 2005) or Recovery Stress Questionnaire (REST-Q-Sport-52) (Kellmann & Kallus, 2001). Additionally, assess each athlete's lifestyle factors/circumstances (e.g., work and/or study commitments, young family etc.) to understand their life-stressors and to identify periods during the season when they may experience high(er) stress and anxiety (e.g., exams for athletes studying).
- Throughout the sporting season, regularly monitor athletes' self-reported stress using a customised wellness questionnaire (Buchheit et al., 2016; Buchheit et al., 2013; Fitzgerald et al., 2019; Thornton et al., 2016).
- Encourage athletes to consult a sport psychologist (Walsh, 2018).
- Advise athletes to keep unnecessary life-stress to a minimum (Walsh, 2018).
- Encourage athletes to engage in mindfulness practices including meditation,

breathing awareness, walking and yoga (Barrett et al., 2012; MacDonald & Minahan, 2018).

9.6 Thesis Limitations

It is acknowledged that there are inherent limitations with some aspects of the research presented as part of this thesis. Accordingly, the limitations in this thesis have been considered from a design, sampling, measurement and analysis perspectives.

Measurement of URTS incidence

URTS can result from infectious (viral, bacterial or fungal etiology) or non-infectious and inflammatory causes (e.g., allergies, asthma and trauma to respiratory epithelial membranes) (Gleeson & Pyne, 2016). In this thesis, it was not possible to determine the aetiology of URTS episodes, given the logistical difficulties and associated costs of pathology analyses. Therefore, only athletes reported perceived symptoms were determined. It is possible that there was a self-reporting bias where athletes did not declare illness, due to not wanting to be ruled out from match selection. Indeed, in Chapter 6, professional rugby union players had a financial incentive to be selected for matches; players earned additional money for each match played. As such, future research should examine professional team-sport athletes' attitudes to reporting illness to elucidate the number of athletes downplaying or hiding illnesses.

Constraints of research with elite athletes

Each study in this thesis was conducted within an elite team-sport setting, thus a restricted sample size was inevitable. However, in all studies, a repeated measures design was used whereby data were regularly collected over extended periods of time to increase statistical power (Guo et al., 2013). Another constraint of conducting research in elite athlete populations was that laboratory experiments were not performed owning to the confines of highly demanding training competition and travel schedules in elite sport. Laboratory studies have been favoured in the exercise immunology literature;

however, this approach may lack ecological validity, given it is difficult to accurately replicate elite athletes' real-world sporting demands and practices. Therefore, an applied observational study design was applied in this thesis, whereby several measures (e.g., URTS, biomarkers, self-reported) were collected from elite team-sport athletes during real-life training and competition periods. Though observational studies cannot provide the same level of evidence as laboratory experiments do, the findings from this thesis are more likely to be applicable to elite team-sport athletes and still provide useful information on factors influencing URTS risk.

Supplementation use

In the exercise immunology literature, researchers tend to prohibit supplementation, given certain supplements (e.g., probiotics, vitamin C, vitamin D, quercetin, etc.) have been proposed to alter specific aspects of the immune system and reduce the incidence, severity and/or duration of URTS episodes (Maughan et al., 2018; Walsh, 2019). For this reason, the studies conducted in elite field hockey players (Chapters 7 and 8) prohibited use of supplementation. However, in Chapters 5 and 6, rugby union and league players were prescribed several supplements. While good compliance was reported by the rugby union team's dietitian and the rugby league team's trainer, supplement use was not quantified (e.g., dietary surveys, interviews and questionnaires) (Knapik et al., 2016). Therefore, the relationship between supplementation and URTS risk could not be investigated in elite rugby union and league players.

Measurement of wellness indicators

Although numerous validated psychometric tools are available, many sporting teams prefer using customised questionnaires due to their simplicity and shorter time required for completion (Saw et al., 2015). Indeed, this thesis employed customised wellness questionnaires, whereby athletes provided a rating on a 5-point scale (Chapters 6, 7 and 8) or 10-point scale (Chapter 5) for wellness indicators (e.g., stress, mood, muscle soreness, fatigue, sleep quality, motivation to train). However, these customised

questionnaires were not validated; thus, it is unknown if they measured what was intended to be measured. Nevertheless, a recent study tested the measurement reliability and sensitivity of a customised wellness questionnaire (1-5 Likert scale), very similar to the questionnaires employed in this thesis, and demonstrated acceptable to good sensitivity in elite team-sport athletes (Ryan et al., 2019).

Measurement of training load

Internal training load was measured in the studies conducted in elite rugby union and league players (Chapters 5 and 6) where no association was found between internal training load and URTS risk. Unfortunately, internal training load was not monitored in elite field hockey players (Chapters 7 and 8); instead, the team's sport scientist used global positioning systems (GPS) to quantify athletes' external training loads when they trained or competed for the national team. However, the GPS data were not collected for players' training sessions or matches outside of the national team (i.e., club and/or regional teams), conditioning/fitness sessions, or gym sessions. Therefore, the relationship between training load and URTS risk could not be examined in elite field hockey players.

Clinical relevance of immune markers

As there is a high level of redundancy to the immune system, it is difficult to draw conclusions about athletes' immune function status as a whole based on the immune markers examined in the present thesis. In vivo immune markers are considered more clinically relevant than the immune markers examined in this thesis (Davison et al., 2016). Emerging studies have used cutaneous measures of in vivo immunity, such as delayed-type hypersensitivity responses to intradermal injection of antigens or contact hypersensitivity responses to epicutaneous application of antigens (Davison et al., 2016; Diment et al., 2015; Smith et al., 2011). In vivo immune models assess immune responses to a whole-body challenge by initiating an integrated and highly coordinated immune response in the normal tissue environment (Davison et al., 2016; Diment et al.,

2015; Smith et al., 2011). However, the practicality of measuring in vivo immune markers is limited with large numbers of elite team-sport athletes. The immune markers examined in this thesis were selected on the basis that they appear to have some clinical relevance, with previous studies showing that multi-antigen stimulated cytokine production (Gleeson et al., 2012), SIgA concentration (Gleeson et al., 2012; Neville et al., 2008) and CMV serostatus (He, Handzlik, Muhamad, et al., 2013) can predict athletes risk for URTS.

9.7 Directions for future research

Monitoring the impact of URTS on training and competition performance

Of the three elite teams investigated in this thesis, URTS episodes only impacted training availability in elite field hockey players, with three players missing a training session each. Whereas, in rugby union and league players who experienced 35 and 18 URTS episodes, respectively, no trainings or matches were modified or missed as a result of URTS. It was observed that team-practitioners tended to only advise athletes to avoid training and competition if they were reporting 'below-the-neck' symptoms; as such, athletes often continued training and competing when experiencing URTS. Therefore, in addition to assessing training and competition availability, future studies should seek to quantify the impact of URTS on training and competition performance. This could be achieved by examining subjective (i.e., internal training load) and/or objective (e.g., GPS, heart rate) performance indicators and comparing them between URTS-free and URTS episode periods.

Relationship between herpes viruses, SIgA and URTS

A potential area for future study is to explore the relationships between herpes viruses (i.e., EBV and CMV), SIgA and URTS. The relationship could not be assessed in this thesis as CMV reactivation was not examined regularly. Nevertheless, a previous study found that URTS and EBV reactivation were inversely correlated with SIgA in rugby players during an intensive training camp (Yamauchi et al., 2011b). A significant decrease in SIgA secretion rate was observed one day before EBV reactivation

(Yamauchi et al., 2011b), and higher EBV reactivation was observed in players experiencing URTS, compared with healthy players. These findings suggest that when SIgA is reduced (e.g., during intensified training), latent herpes virus can reactivate and elicit URTS. Therefore, to better elucidate the mechanisms underlying URTS in team-sport athletes, further research is required to understand the interaction of CMV and/or EBV reactivation, SIgA and URTS.

Illness prevention studies

All sporting teams examined in this thesis used illness prevention strategies (e.g., hand hygiene, training load monitoring and management, face masks on planes, etc.). Nonetheless, it was beyond the scope of this thesis to quantify the effectiveness of these strategies in reducing URTS risk. The efficacy of illness prevention strategies has been predominately investigated in epidemiology and general population research (Aiello et al., 2010; Cowling et al., 2010; Jefferson et al., 2011). Fewer illness prevention studies have been conducted in athletic populations, with the previous studies in athletes focusing on nutrition and supplement interventions (Bermon et al., 2017). This thesis identified several risk factors for URTS in elite team-sport athletes; however, further research is needed to determine which illness prevention strategies are the most effective in maintaining athletes' health.

Female athletes

All studies in this thesis were conducted in male team-sport athletes. Our findings are not necessarily applicable to female team-sport athletes, as previous research demonstrated differences in immune responses and URTS incidence between male and female athletes (He, Bishop, et al., 2014). Sex chromosome genes and sex hormones (e.g., oestrogens, progesterone and androgens) are known to contribute to the differential regulation of immune responses and illness susceptibility between the sexes (Klein & Flanagan, 2016). In addition, it has yet to be examined but there may be differences in lifestyle and behavioural factors (e.g., stress, sleep) that have the potential

to influence URTS risk between male and female athletes. Given female athletes appear to be more susceptible to URTS than their male counterparts (Drew et al., 2017; He, Bishop, et al., 2014), further research examining factors influencing URTS risk in female team-sport athletes is needed.

9.8 Conclusions and practical recommendations

The findings in this thesis further the understanding of factors influencing URTS risk in elite team-sport athletes. In contrast to previous exercise immunology studies, sport-related stressors (i.e., training, competition and international travel) did not appear to increase URTS risk in elite team-sport athletes. The management of athletes by team-sport practitioners and the application of illness prevention strategies may have contributed to reduced URTS risk during sport-related stressor exposure. Interestingly, rest weeks (i.e., a period in which athletes were not exposed to sport related stressors), domestic training weeks, household illness incidence, reduced sleep quality and increased stress were identified as risk factors for increased URTS. Therefore, it appears that lifestyle and behavioural factors outside of the team environment may influence athletes' risk for URTS to a greater extent than sport-related stressors.

The findings in this thesis also suggest that self-reported measures are more effective than biomarkers in predicting elite team-sport athletes' URTS risk. In all three elite sporting teams, self-reported measures were significantly associated with athletes' URTS risk: household illness in rugby union players; total wellness and sleep quality in rugby league players; and household illness and stress in field hockey players. However, equivocal evidence was presented for SIgA. A trend for an inverse association between SIgA concentration and URTS incidence was observed in rugby union players, and resting baseline SIgA concentration was found to predict 'potential' URTS risk in field hockey players. Whereas, alterations in SIgA concentration were not associated with URTS risk in rugby league or field hockey players. Finally, across all three sporting teams

examined in this thesis no resting blood biomarkers were found to predict 'potential' URTS risk.

Based on the findings from this thesis, the following information and recommendations are provided to team-sport athletes, dietitians, nutritionists, sport scientists and coaches:

- Athletes may be more at risk for URTS during periods in which they are outside of the team environment, and not as closely monitored or managed by team-practitioners, including rest weeks (i.e., Christmas break and bye weeks) and domestic training weeks.
- To predict athletes' risk for URTS, practitioners should monitor athletes' self-reported household illness and wellness indicators, particularly sleep quality, stress and total wellness (i.e., summation of stress, sleep quality, motivation to train, fatigue and muscle soreness).
- If teams have the necessary funding, there may be benefit in monitoring SIgA alongside self-reported measures to predict URTS risk. However, SIgA measurement may be logistically impractical in a team-sport setting due to the number of athletes, cost and rigorous pre-sample standardisation required for valid results.
- Illness prevention strategies should be applied when team-sport athletes are exposed to factors that increase risk for URTS, including: rest weeks, domestic training weeks, decreased SIgA concentration, household illness incidence, reduced sleep quality, reduced total wellness, and increased stress levels.

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APPENDICES

Participant Information Sheet

02/11/2016

Project Title

Immune function and illness incidence in New Zealand team sport athletes over the course of a sporting season.

An invitation

Hi, my name is Lauren Keaney and I am a PhD student at AUT. Along with Assoc. Prof Dr Andrew Kilding, Senior Lecturer Dr Deborah Dulson and Senior Lecturer Dr Fabrice Merien, I invite you to help with a project that examines immune functions and illness incidence in elite team sport athletes.

Purpose of this research?

A strong body of evidence suggests that athletes are at increased risk for upper respiratory tract infections i.e. the common cold (e.g. runny nose, cough etc.) due to intensive exercise depressing immune cell functions. The presence of upper respiratory tract infections is of great concern for athletes as it can impair their ability to train and perform optimally. Studies examining illness and immunity have tended to focus on endurance athletes. Research on team sport athletes is required as training demands vary greatly between the sports. Team sport athletes engage in a diverse range of training activities such as running-based conditioning, resistance training, skill drills and team drills. Along with undergoing significant amounts of training stress, elite team sport athletes are exposed to additional stressors such as travel, competition and adverse environmental conditions. Our current knowledge on how these stressors individually and collectively influence a team sport athletes' immune system is limited. It important to determine how these sport related stressors effect immunity as the immune system is responsible for defending the body from infection.

This study will seek to answer the following research questions:

1. Are there periods during the season when team sport athletes are more susceptible to illness?

2. Are there any markers that can be used to identify an athletes' susceptibility to infection during periods of increased risk?
3. How does an athlete's immune system cope with additional stressors of heat and travel during training & competition?

The findings of this study will be used in my PhD thesis, in addition they may be published into a research journal and/or presented at a conference. However you will not be named in any of this research, therefore your identity will remain confidential.

How was I identified and why am I being invited to participate in this research?

As a male rugby player, you have been invited to be part of this research.

However, you will not be able to take part in this research if:

- ✓ *You are under 18 years of age or over 35 years of age*
- ✓ *You have ever had an injury or medical condition that you think may affect your ability to sense pain or discomfort*
- ✓ *You have a serious medical condition*
- ✓ *You have cultural or religious sensitivities about human body measurements*

What will happen in this research?

If you wish to participate in this study you will need to sign an informed consent form. This is a monitoring study; data will be collected throughout the sporting season. You will be required to give two blood samples; one preseason and one postseason. You will also be required to provide saliva samples throughout the season. To obtain a blood sample a qualified phlebotomist will insert a small needle into a vein in your forearm. To collect saliva samples, you will be required to dribble into a tube for 2-3 minutes. These samples will be taken to a lab where they will be analysed for immune markers. If you experience sickness over the monitoring period you will be required to complete an illness questionnaire, this will involve you rating illness symptoms (e.g. runny &/or blocked nose, sore throat, cough, fever etc.) as light, moderate or severe.

If you have any personal or cultural issues regarding the above procedures please let the primary researcher know of these prior to the study so that these can be accommodated for.

What are discomforts and risks?

You may experience discomfort during the blood collection in the form of a small sting from the needle prick. Only qualified experienced phlebotomists will take your blood. Therefore, the risk and discomfort associated with this procedure will be minimal. If any complications arise, a certified researcher will always be present to perform first aid.

What are the benefits?

This research will identify when during the sporting season the team and you as an individual are more susceptible to illness. This study will also identify which sport related stressors (training, travel, competition, adverse environmental conditions etc.) cause the greatest immune depression. Once we have identified periods of increased risk, strategies (e.g. supplementation) can be applied to minimise your risk for illness. Maintaining your health is of high importance as athletes cannot train or perform optimally when they are sick.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details to satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected?

If the data is published in the public domain your name as a subject will not be revealed and all subjects will remain de-identifiable.

Will data be passed onto my coach and influence my selection in the team?

This study is independent of the team you are involved in. A summary of the collective team findings will be shared with your coach. The coaches summary sheet will only contain general information, it will not contain any of your identifiable information. Your individual data will only be shared with your coach if you explicitly agree to it by ticking ‘yes’ on the consent form.

What are the costs participating in this research?

There will be no financial costs associated with this study, however if you choose to participate you will be required give up approximately 2 hours and 10 minutes; 10 minutes for two blood collections and 2 hours for saliva samples (2-5 minutes per sample).

Appendix A: Participant information sheet for Chapters 5 and 6

What If I want to withdraw from the study?

If you do decide to participate you can withdraw from the study at any time.

Will I receive feedback on the results of this research?

At the end of the study, verbal feedback will be given to you and a 1-2 page summary of the study research findings will be provided. As mentioned, your individual results from the study will only be shared with your coach if you grant permission. Analysis of your blood and saliva samples may reveal that your immune markers do not fit within normative ranges. If this situation arises the researchers are not qualified to determine why your results are abnormal. In this instance you will be provided with sufficient information to consult your general practitioner.

What do I do if I have concerns about this research?

If you have any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor; Dr Andrew Kilding, andrew.kilding@aut.ac.nz, +64 921 9999 x 7056. Any concerns regarding the conduct of the research should be notified to the Executive Secretary of AUTEC, Kate O'Connor, ethics@aut.ac.nz, 09 921 9999 ext 6038.

How do I join this study:

Contact Lauren Keaney if you are interested in participating or if you have any questions about the study.

Researcher Contact Details:

Name: Lauren Keaney

E-mail: Lauren.ck.92@gmail.com

Project Supervisor Contact Details:

Name: Dr Andrew Kilding

E-mail: andrew.kilding@aut.ac.nz

Approved by the Auckland University of Technology Ethics Committee on 27/09/2016, AUTEC Reference number 16/319.

Appendix B: Consent form for Chapters 5 and 6

Consent form

Project title: ***Immune function and illness incidence in New Zealand team sport athletes over the course of a sporting season***

Project Supervisor: ***Dr Andrew Kilding***

Researcher: ***Lauren Keaney***

- ○ I have read and understood the information provided about this research project in the Information Sheet dated 02/11/2016.
- ○ I agree that the data collected from this study may be published provided my name is not used. I also agree that the collected data may be used in future studies, other than the one titled above, that have been approved by the ethics committee.
- ○ I have had an opportunity to ask questions and to have them answered.
- ○ I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without being disadvantaged in any way.
- ○ I understand that if I withdraw from the study then I will be offered the choice between having any data or tissue that is identifiable as belonging to me removed or allowing it to continue to be used. However, once the findings have been produced, removal of my data may not be possible.
- ○ I am not suffering from any serious medical condition.
- ○ I agree to provide blood and saliva samples.
- ○ I agree to take part in this research.
- ○ I wish to receive a summary of the research findings (please tick one):

  Yes○ No○
- ○ I understand that a general summary of the team's collective data will be shared with the coach
- ○ I agree to allow the use of my individual data to be shared with my coach (please tick one): Yes○ No○

Appendix B: Consent form for Chapters 5 and 6

○ I wish to have my blood and/or saliva samples returned to me in accordance with right 7 (9) of the *Code of Health and Disability Services Consumers' Rights* (please tick one): Yes○ No○

Participant signature:

Participant name:

Date:

Approved by the Auckland University of Technology Ethics Committee on 27/09/2016 AUTEC Reference number 16/319.

Appendix C: Health screen questionnaire

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name:_____ **Date: / /**

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to (i) ensure their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

1. **At present,** do you have any health problems which you are:

a)	on medication, prescribed or otherwise	Yes	☐	No	☐
b)	attending you general practitioner	Yes	☐	No	☐
c)	on a hospital waiting list	Yes	☐	No	☐

2. **Have you ever had** or **presently suffer** from any of the following:

a)	Convulsions/epilepsy	Yes	☐	No	☐
b)	Asthma	Yes	☐	No	☐
	*If **'yes'** are you on any medication relating to such*	Yes	☐	No	☐

Appendix C: Health screen questionnaire

c)	Known exercise induced bronchoconstriction Does your chest *'tighten up'* during/after exercise	Yes	☐	No	☐
d)	Head injury	Yes	☐	No	☐
e)	Problems with bones or joints	Yes	☐	No	☐
f)	Disturbance of balance/coordination	Yes	☐	No	☐
g)	Numbness in hands or feet	Yes	☐	No	☐
h)	Disturbance of vision	Yes	☐	No	☐
i)	Any pain or injuries (ankle, knee, hip, shoulder etc.)	Yes	☐	No	☐
j)	Eczema	Yes	☐	No	☐

k)	Hypoglycaemia (Suffer from low blood sugar levels)	Yes	☐	No	☐
l)	Diabetes	Yes	☐	No	☐
m)	A blood disorder	Yes	☐	No	☐
n)	Heart problems	Yes	☐	No	☐
o)	Thyroid problems	Yes	☐	No	☐
p)	Digestive problems	Yes	☐	No	☐
q)	Post viral fatigue	Yes	☐	No	☐

If ***'yes'****, for how long?*

……

……

……

Flu in the last 3 months

r) Yes ☐ No ☐

*If **'yes'**, were you on any medication relating to such?* Yes ☐ No ☐

s) Kidney or liver problems Yes ☐ No ☐

t) History of blood clots/poor circulation Yes ☐ No ☐

u) Known allergies Yes ☐ No ☐

*If **'yes'** please describe the allergy (E.g. rhino–conjunctivitis, hay fever):*

……

……

……

If you answered YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short lived, insignificant or well controlled.):

……

……

……

……

……

Appendix C: Health screen questionnaire

3. **Has any,** otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise: Yes ☐ No ☐

4. Are you **presently** taking any 'antibiotics'? Yes ☐ No ☐

*If **'yes'**, what for and for how long*

……

……

……

……

5. Have you had a cold or feverish illness in the past month? Yes ☐ No ☐

6. Do you frequently suffer from upper respiratory infections (Symptoms such as sore throat, runny/or blocked nose, cough, headache, weakness, mucus in the throat, repetitive sneezing, fever etc.): Yes ☐ No ☐

*If **'yes'** please indicate which symptoms you experience and how often (e.g. sore throat, every few months):*

……

……

Appendix C: Health screen questionnaire

……

……

7. Are you accustomed to vigorous exercise? Yes ☐ No ☐

Thank you for your cooperation

Participant Information Sheet

27/04/2018

Project Title

The impact of repeated heat exposure on male athletes' thermoregulation, wellbeing, immunity and health.

An Invitation

Hi, my name is Lauren Keaney, I am an assistant physiologist at High Performance Sport New Zealand (HPSNZ) and a PhD candidate at AUT. Along with Dr Deborah Dulson, Assoc. Prof Andrew Kilding, Dr Fabrice Merien, I invite you to help with a project that examines how heat training influences thermoregulation, wellness, immunity and illness in elite male team sport athletes. The information obtained from this study may be used for my PhD thesis and for academic research outputs (e.g. publications, presentations) and could help to guide heat acclimation recommendations for New Zealand athletes in the lead up to the Tokyo Olympics.

What is the purpose of this research?

Exercise in hot environments (≥ 27^{o}C) results in an increase in core body temperature, which is known to impair exercise performance over a variety of sporting events, including hockey. To attenuate heat-induced performance impairments, elite athletes commonly engage in heat acclimation (i.e. training in hot conditions). Heat acclimation prior to competing in hot conditions leads to thermoregulatory and physiological adaptations which allow athletes to cope better in the heat and preform more optimally. At present heat acclimation studies have mostly been involved endurance sport athletes, findings from these studies are not necessarily applicable to team sport athletes. Research on team sport athletes is required given the forthcoming pinnacle event of the 2020 Tokyo Olympics where it's expected to extremely hot and humid (30-40°C with 60-80% humidity). To guide heat acclimation recommendations for NZ team sport athletes, this research project seeks to answer the following questions:

1. How does training and playing in hot conditions affect elite male team sport athletes thermoregulatory responses and ability to cope in the heat.

2. How does the addition of 'heat' during training & competition influence athlete's wellness (i.e. sleep, stress, fatigue etc.), immunity and risk for illness?

Furthermore, the findings of this research may be used for my PhD thesis, academic publications and presentations.

How was I identified and why am I being invited to participate in this research?

As a member of the NZ Black Sticks Mens hockey team you have been invited to be part of this research.

However, you will **not** be able to take part in this research if:

- ✓ *Playing overseas and not living in NZ over the study period.*
- ✓ *You have any known heart or cardiovascular condition or if a member.*
- ✓ *You have engaged in heat training in the last 6 weeks.*
- ✓ *You have ever had an injury or medical condition that you think may affect your ability to sense pain or discomfort.*
- ✓ *You have cultural or religious sensitivities about human body measurements.*
- ✓ *You are sick or injured.*
- ✓ *You are taking prescribed medication (e.g. antibiotics) at the start of study. If you get sick and require medication during the study you will have to withdraw from the study.*
- ✓ *You have any other reason to consider that you are not in good health and of average, or better than average fitness. If you wish to participate in the study you will be required to complete a health screen questionnaire, if we identify any red flags (e.g. injury, illness, cardiovascular condition) you will not be able to participate.*
- ✓ *You are under 18 years of ager or over 35 years of age.*

How do I agree to participate in this research?

After reading this information form you will be given a consent from. Please provide informed consent by signing the consent form and returning it to Lauren. Once you have provided consent, you will be given an helath screen questionnaire to complete, if any information gathered on this questionnaire raises a red flag (e.g. injuries or illness) you will not be invited to participate. Your participation in this research is voluntary (it is your choice) and whether you choose to participate will neither advantage nor disadvantage you. You can withdraw from the study at any time. If you choose to withdraw from the study, then you will be offered the choice between having any data that is identifiable as belonging to you removed or allowing it to continue to be used. However, once the findings have been produced, removal of your data may not be possible.

Appendix D: Participant information sheet for Chapters 7 and 8

What will happen in this research?

Involvement in this research will involve 1. Monitoring; 2: Heat acclimation intervention; and 3: Heat response testing.

1. Monitoring (June – July):

Wellness, immune and illness data will be collected over a 2-month period to examine how you cope with the additional stressor of 'heat' during training & competition.

Late-May: Normal training

Data will be collected over 2-weeks of 'normal training' (i.e. no heat added) to establish a baseline.

Mid-June (18th -27th): Heat acclimation

Data will be collected over 2-week heat acclimation intervention.

July: Overseas tour in hot conditions

Data will be collected over 3-week training and competition tour in Japan and India where it is expected to be hot and humid (30-40°C with 60-80% humidity).

Data to be collected over the monitoring period:

- *Resting blood samples 3x (Sample 1: Day 1 of normal training block; Sample 2: After heat acclimation intervention; & Sample 3: After overseas tour):* To obtain a blood sample a qualified phlebotomist will insert a small needle into a vein in your forearm. These samples will be taken to a lab where they will be analysed for immune markers. Specifically, blood samples will be analysed for markers that have been associated with upper respiratory illness symptoms including white blood cell counts, cytomegalovirus serostatus and immune cell functions (cytokine gene expression).
- *Resting saliva samples 2x per week over monitoring period (14 samples total over 7 -weeks):* You will place a swab on your tongue, the swap will change colour when enough saliva is collected for analysis (collection time ~ 1 min). This sample will be analysed for immune markers and stress hormones.
- *Subjective measures to be collected over the 7 week monitoring period (Illness, wellness & training load data):* You will be required to complete a simple illness symptoms questionnaire once a week. This will involve you rating illness symptoms (e.g. runny nose, sore throat, headache, fever) as non-existent, light, moderate or severe. You will also be required to complete a wellness

questionnaire where you will be asked to rate your sleep quality, mood, muscle soreness, stress levels and fatigue. Finally, you will be asked to rate the intensity of all exercise sessions you perform using scales of 0-10 from rest (0) to maximal (10).

2. Heat acclimation (HA) intervention:

You will complete heated training in NZ to prepare you for the hot playing conditions expected in Japan and India. You will perform 8x HA sessions in total, consisting of 4x active and 4x passive HA sessions. Active HA sessions will require you to perform 1 h of circuit based resistance training in a heated gym (30°C & 40%RH) while wearing long sleeves and pants to increase heat storage. Passive HA sessions will involve you sitting in a sauna (30 min at 70°C) within 5 minutes of completing a training session.

3. Heat response testing

To examine how respond to exercise in the heat, you will perform an intermittent spring cycling protocol in hot conditions (35°C). The cycling protocol is 46-min in total and involves 23 x 2-min activity blocks. Each 2-min activity block will consist of a 5-sec sprint, 105-sec active recovery and 10-sec of seated rest. This protocol has been selected as the ability to repeatedly produce maximal, brief/short-term efforts (i.e., sprinting) is a key performance parameter in hockey. Various measures will be collected pre-, during- and post- the intermittent spring cycling protocol:

Measures collected pre- and post- HRT:

You will be required to provide saliva and blood samples pre-, immediately post- and 1 hour post- the HRT.

Measures collected during exercise:

During each HRT core temperature, skin temperature, heart rate, sweat loss, thermal comfort and sensation, rate of perceived exertion and plasma volume will be measured. Core temperature is measured using a small rectal probe which you will self-insert ~ 10 cm past the rectal sphincter. At the opposite end of the rectal probe there is a wire attachment that will sit outside of your cycling shorts/pants and will plug into a data logger which will record your core temperature while you are exercising in the hot conditions. This is the gold standard technique for measuring core temperature in athletes when exercising. Further details on the intermittent sprint cycling protocol can be found below

What are the discomforts and risks?

- Blood sampling: You may experience discomfort during the blood collection in the form of a small sting from the needle prick.
- Heat response testing: The discomforts you may experience during heat response testing are the same you would experience during a sporting match (heavy legs and breathing). You may experience more discomfort than normal when exercising in hot conditions due to elevated body temperatures and sweat rate. There is greater risk for heat illness (e.g. heat stroke) when exercising in hot compared to temperate conditions.
- Heat response testing – rectal probe: The concept of a rectal probe may sound uncomfortable, however, once inserted many athletes report that they don't notice it while exercising.

How will these discomforts and risks be alleviated?

- Blood sampling: Only qualified experienced phlebotomists will take your blood.
- Heat response testing: There is no risk of heat illness as your core temperature and thermal perception will be closely monitored and you will be able to leave the heat chamber or heated gym at any time. If any complications arise, a certified researcher will always be present to perform first aid.
- Heat response testing – rectal probe: Rectal probes are widely used with elite athletes as it is the gold standard technique for measuring core temperature in athletes when exercising. There is no risk having your core temperature measured this way. For your safety, it is very important core temperature is measured accurately, as if you become too hot and reach hyperthermia (core temperature > 39.5°C) you will need to stop the test and be removed from the heat chamber.

What are the benefits?

- Gain an in depth understanding on how you respond to heat stress, this is known to be highly individual.
- Learn how well you adapt to training in the heat. This information could guide you and your coach (if you have granted permissio results to be shared with your coach) in making you an individualised heat management plan.
- Understand how intensive exercise in the heat effects your immune system.
- Learn how training in the heat influences your wellness, immunity and risk for illness.

This experience and information you gain through participating in this study will assist you when future competitions are held in hot environments.

The researchers will also benefit from completing this research as the findings will be used for submission to peer review journals and conferences.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected?

All data collected during this study will only be available to the researchers involved, If the data is published in a public domain, your name as a subject will not be revealed as all reported data will remain de-identifiable.

Will the data be passed onto my coach and influence my selection in the team?

This study is independent of the team you are involved in. A summary of the collective team findings will be shared with your coach. The coaches' summary sheet will only contain general information (team averages), it will not contain any identifiable information. You can choose to share your individual data with coach, your data will remain de-identifiable (i.e. instead of using your name a number will be used). Your de-identified data will only be shared with your coach if you explicitly agree by ticking 'yes' on the consent form.

What are the costs of participating in this research?

There will be no financial costs associated with this study, however if you choose to participate you will be required to give up approximately 13 hours spread over a 7 week period; 150 min for each heat response test and accompanying blood and saliva collections (2 x HRTs: 5 h total), 1 h for each heated resistance training session (4x: 4 h total), 30 min for each post exercise sauna (4x: 2 h total), 10 min for resting blood sample collection (3x: 30 min total), 2 min for resting saliva sample collection (14x: 28 min total), 2 min to complete wellness questionnaires (21x: 42 min in total) and 5 min to complete weekly illness questionnaire (7x: 35 min total).

What opportunity do I have to consider this invitation?

You have 2 weeks decide whether you wish to participate in the study, if you do decide to participate you can withdraw from the study at any time.

Will I receive feedback on the results of the research?

Appendix D: Participant information sheet for Chapters 7 and 8

At the end of the study, verbal feedback will be given to you and a 1-2 page summary of the research findings will be provided. Analysis of your blood and saliva samples may reveal that your immune markers do not fit within normative ranges. If this situation arises the researchers are not qualified to determine why your results are abnormal, however you will be provided with sufficient information to consult your general practitioner.

What happens to any left-over blood and saliva samples?

Following laboratory analysis, if any of your samples are left-over you can choose to have them returned to you by ticking 'yes' on the consent form. If you wish not to have your samples returned they will be discarded.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, Dr Deborah Dulson, *deborah.dulson@aut.ac.nz,* 09 921 999 ext 7417.

Concerns regarding the conduct of the research should be notified to the Executive Secretary of AUTEC, Kate O'Connor, *ethics@aut.ac.nz* , 921 9999 ext 6038.

Whom do I contact for further information about this research?

Please keep this Information Sheet and a copy of the Consent Form for your future reference. You are also able to contact the research team as follows:

Researcher Contact Details:

Lauren Keaney – *lauren.keaney@hpsnz.org.nz*

Project Supervisor Contact Details:

Dr Deborah Dulson – deborah.dulson@ut.ac.nz

Approved by the Auckland University of Technology Ethics Committee on *21/06/2018*, AUTEC Reference number *18/196*.

Appendix E: Participant consent form for Chapters 7 and 8

Consent form

Project title: ***The impact of repeated heat exposure on male athlete thermoregulation, wellbeing, immunity and health.***

Project Supervisor: ***Dr Deborah Dulson***

Researcher: ***Lauren Keaney***

- ○ I have read and understood the information provided about this research project in the Information Sheet dated 27/04/2018.
- ○ I have had an opportunity to ask questions and to have them answered.
- ○ I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without being disadvantaged in any way.
- ○ I understand that if I withdraw from the study then I will be offered the choice between having any data or tissue that is identifiable as belonging to me removed or allowing it to continue to be used. However, once the findings have been produced, removal of my data may not be possible.
- ○ I am not suffering from heart disease, high blood pressure, any respiratory condition (mild asthma excluded), any illness or injury that impairs my physical performance, or any infection.
- ○ I agree to provide blood and saliva samples.
- ○ I agree to take part in this research.
- ○ I agree that a general summary of the findings will be shared with the coach, however this will not contain any identifiable information.
- ○ I agree to allow my individual de-identified data to be shared with my coach (please tick one): Yes○ No○
- ○ I wish to receive a summary of the research findings (please tick one): Yes○ No○
- ○ I wish to have my blood and saliva samples returned to me in accordance with right 7 (9) of the *Code of Health and Disability Services Consumers' Rights* (please tick one): Yes○ No○

Participant signature:

Participant name:

Date:

Approved by the Auckland University of Technology Ethics Committee on 21/06/2018 AUTEC Reference number 18/196.

Appendix F: Coach letter of endorsement for Chapters 7 and 8

Hockey New Zealand Inc.
Sport Central, Eden Business Park, Level 1
14 Normanby Rd, Mt Eden 1024
PO Box 67-088, Mt Eden, Auckland 1349
T: (09) 630 2932 E: support@hockeynz.co.nz
www.hockeynz.co.nz

13 June 2018

RE: Lauren Keaney, Heat acclimation, acclimatisation and response research

To Whom It May Concern:

This is a letter of support for the study being conducted by Lauren Keaney with the Black Sticks Men's team looking at 'the impact of heat acclimation and acclimatisation on athlete wellbeing, mucosal immunity and illness' and 'the influence of acclimation status on immune responses at rest and in response to exercise in the heat'.

This information is critical as we come within 2 years of what will be the hottest Olympic Games in the modern times and the Black Sticks ability to perform in this environment. To further aid the study Lauren will travel with the team to Japan and India from 4-24 July 2018 following on from extensive preparation and data collection by her in May/June in Auckland. This information will guide our individual and team strategies to perform in Tokyo in 2020.

If there are areas that you would like to discuss please contact me direct.

Kind regards

Darren Smith
National Coach – Black Sticks Men
+64 225879241
darren.smith@hockeynz.co.nz

Appendix G: High Performance Sport New Zealand Research Committee letter of endorsement for Chapters 7 and 8

HIGH PERFORMANCE
SPORT NEW ZEALAND

Subject: Feedback on Proposed Research

Title of Research projects:
1) The influence of acclimation status on immune responses at rest and in response to exercise in the heat.
2) The impact of heat acclimation and acclimatisation on athlete wellbeing, mucosal immunity and illness.

Presentation Date:
18/06/2018

Dear Lauren,

On behalf of the HPSNZ Athlete Performance Support Research Committee, I'd like to thank you for the recent presentation of your proposed research topic(s).

Congratulations, having reviewed your proposed initiative, the Committee have ***endorsed*** your research projects listed above.

We wish you all the best with the project and look forward to hearing the outcomes of this important work in the future.

Thank you again for your efforts thus far and please don't hesitate to contact us if you have any questions or would like any further support or advice.

Kind Regards,

Dr. Matt Driller
Research Support Committee Chair, HPSNZ

HIGH PERFORMANCE
SPORT NEW ZEALAND

Appendix H: Prime ministers scholarship application (Funding for Chapter 7)

PRIME MINISTER'S SUPPORT TEAM SCHOLARSHIP

Application Form

Section 1:

PERSONAL DETAILS

Name	Lauren Catherine Keaney
Are you known by any other name	No
Email	Lauren.keaney@hpsnz.org.nz
Gender	Female
DOB	12/12/1992
Phone:	n/a
Mobile	0274990593
Postal Address	44 Middleton Road, Remuera, Auckland, 1050

Region	Auckland

Section 2: Endorsement

a. Has the application has been developed in consultation with your HPSNZ Sport Performance Manager, Discipline Lead or Performance Consultant? **Yes/**No

b. Has the application been discussed with your NSO? **Yes**/No

Section 3: Scholarship Type: **Individual** or Group

If this is a group scholarship you will need the name, role and email address for each of the group members

Appendix H: Prime ministers scholarship application (Funding for Chapter 7)

First Name	Last Name	Role	Email Address
Lauren	Keaney	Assistant physiologist	Lauren.keaney@hpsnz.org.nz

Section 4: Biography of Applicant:

What is your current role in High Performance Sport?
Assistant physiologist
List the key athletes you work with:
Black Sticks Women (Covering Katherine Oberlin-Brown's maternity leave)
List and describe your greatest contribution to High Performance Sport:
▪ Implementation of heat response testing and heat acclimation with the NZ Football Ferns (2017) and the Black Sticks Women team (2018). As a result of this work we have been able to identify how athletes in these teams respond and adapt to heat stress. This information will assist coaches in preparing for the 2020 Tokyo Olympics. ▪ Implementation of heat response testing with Sam Gaze who recently came first in an elite UCI Mountain Bike World Cup. ▪ Worked alongside Julia Casadio in a heat project with the Men's endurance track cycling team. The purpose of this project was to assess thermoregulatory and performance responses to a 6-week training block in a hot climate (Florida). Coaches were provided with recommendations to optimise future heat camps that will take place in the lead up to the 2020 Tokyo Olympics. ▪ Contributed to the Tokyo 2020 environmental report that was distributed to NSO's at the end of 2017.

Section 5: Scholarship Activity:

A scholarship is composed of one or more activities e.g. attend a competition, go to a conference or workshop, do a course etc. For each objective, please provide the following information:

Provide a title/brief description for your scholarship activity
Developing and testing Heat Strategies for Men's Hockey World Cup (Dec 2018) Tokyo Olympic Performance (2020)
What is the Specific Performance Question you are trying to answer with this scholarship?

Appendix H: Prime ministers scholarship application (Funding for Chapter 7)

1. Understand the Tokyo environment in July to better define preparation for the Black sticks Men's 2020 Olympic performance 2. Collect environment and performance data to enable us to refine heat preparation strategies for the Team in the lead-up to the World Cup in India (December 2018) and Tokyo 2020 3. Test specific heat management strategies in Tokyo to refine Team and Individual preparation plans. 4. Test how the stressors of heat, travel and impaired sleep during a tour may influence immunity and illness 5. To identify individual responses and 'at risk' players to ensure we can individualize our planning and preparation and optimize health and readiness for performance in the Heat by 2020.
What exactly are you trying to achieve with this activity?
1. Confirm Tokyo environmental demands in July (same timeframe as Tokyo 2020). 2. Assess the players responses to performance in the heat to define individual plans and refine preparation strategies for the Team. 3. Test heat management strategies to confirm most appropriate for India and Tokyo. 4. Test the impact of stressors; heat, travel and sleep across a 20-day tour on immunity and illness to confirm specific opportunities to promote health and wellbeing in India and Tokyo environments.
How will this be achieved (what is your action plan)?
Plan Pre- Tour (May-June) 1. Coaches will select a team of 20 to attend Japan/India Tours, these players will be tracking positively toward World Cup and Tokyo performances and likely contenders for Team selection.

Appendix H: Prime ministers scholarship application (Funding for Chapter 7)

2. Perform heat-stress testing on the athletes in NZ before the tour – to help us to identify athletes more likely to struggle in a hot environment and allow us to monitor these athletes more closely in Japan and India (we will target more cooling strategies/alternate warm-ups etc. for these athletes).
3. Heat acclimation program implemented before they go to prepare athletes for conditions
4. Confirm the appropriate strategies to trial on tour with Physiology and Nutrition personnel
5. Collect baseline data (ie sleep, wellness, illness, immune (salivary immunoglobulin A) and stress hormones (cortisol)).

While in Tokyo and India

1. Collect data on environmental conditions
2. Monitor sweat rates/body mass throughout tour (pre/post trainings and games)
3. Collect information on core temperatures during games (ideally using ingestible pills or tympanic thermometers), HR/GPS and perceptual measures (thermal comfort/thermal sensation etc.)
4. Trial pre-cooling strategies (includes; cold water immersion/slushy/ice vests)
5. Trial cooling strategies during the matches (including menthol-flavoured slushies)
6. Trial post-cooling strategies (cold water immersion)
7. Monitor sleep, wellness, illness, immune markers and stress hormones.

On return from India

1. Complete another heat-stress test to confirm athletes that adapted well to the heat, or didn't.

Appendix H: Prime ministers scholarship application (Funding for Chapter 7)

2. Refine strategies, protocols and preparation requirements for India WC in the first instance 3. Clarify health and wellbeing strategies to optimise; heat, travel, and sleep for performance 4. Prepare heat management plan for 2018-2020
How and with whom will you circulate your new knowledge?
Knowledge will be circulated in report and/or through a presentation to: • Hockey NZ Coaches, management, athletes • HPSNZ High Performance Network, particularly Physiology team • NZOC key partners • Other HPDs/NSOs preparing Tokyo performances and athletes

Start Date	04/07/2018	End Date	23/07/2018

Budget:

Activity	Location	Cost Item	Date	Unit Details	Unit Price	Quantity	Total
International Flights	Tokyo, Bhubaneswar, Auckland	Airfares	O4/07/18 16/07/18 23/07/18	Return		1	$3,500
International Accommodation	Tokyo, Bhubaneswar,	Hotel	04/07/18 – 16/07/18 17/07/18 23/07/18	20 nights	$	12 8	$3,600
Food	Tokyo,	Food	04/07/2018	20 days	$	12	$1,500

Appendix H: Prime ministers scholarship application (Funding for Chapter 7)

	Bhubaneswar,					8	
Miscellaneous Eg Visa							$400
Consumables		Ingestible core temperature pills			$80	32 (16 players x 2 games)	$2560
Consumables	n/a	Menthol, Ice slurry equipment (e.g. Gatorade, Ice)					$500
Time	Tokyo - Bhub	Day rate	4/7-23/7	20 days	$150	20	$3000
						Project-total	$15,060
Will there be any funds contributed by someone else? e.g. NSO, other scholarship, personal contribution							
						If yes, what is the amount of the contributed funds?	$
						Total Application Amount	$

Appendix I: Letter of endorsement for prime ministers scholarship application (Funding for Chapter 7)

Hockey New Zealand (Inc.)
Sport Central, Eden Business Park, Ground Floor
14 Normanby Rd, Mt Eden 1024
PO Box 67-088, Mt Eden, Auckland 1349
T: (09) 630 2932 E: support@hockeynz.co.nz
www.hockeynz.co.nz

23 March 2018

Tracey Paterson
HPSNZ APS Consultant for Hockey NZ
High Performance Sport New Zealand
AUT Millennium

Dear Tracey

Please find this letter as endorsement from Hockey New Zealand and the Vantage Black Sticks Men's programme for Lauren Keaney's application for a Prime Minister's Support Team Scholarship to develop Heat Strategies for the Tokyo 2020 Olympics and the FIH Men's Hockey World Cup in India in December 2018.

The tour targeted is that of Japan then India in July this year. The Japan leg involves a test series in the full heat conditions that will be experienced in the Tokyo 2020 Olympics, hence it will be the beginning, two years out, of preparing to perform in this pressurised environment.

It will enable Lauren and the team to understand the Tokyo environment from a hockey performance perspective in July, to define optimal heat preparation needed for 2020 success, collect relevant data, test and refine heat preparation and management strategies for Tokyo and the World Cup this year in India.

This Prime Minister's Support Team Scholarship for Lauren Keaney is therefore a critical part of the programme for our Vantage Black Sticks Men, as they fully prepare for Tokyo, two years out.

Thank you for your support.

Yours sincerely

Paul MacKinnon
High Performance Director
Hockey New Zealand
paul.mackinnon@hockeynz.co.nz

Appendix J: Prime ministers scholarship award (Funding for Chapter 7)

HS

HPSNZ PM Scholarships
Thu 17/05/2018 16:38
Lauren Keaney; Matt Driller; Michael Flynn; Tracey Paterson

Dear Lauren,
Thank you for your recent application to the Prime Minister's Support Team Scholarship programme. Please find below confirmation of the outcome of your application.

Recipient	Scholarship Type	Activity	Decision	Awarded Amount	Code	Comments / rationale
Lauren Keaney	Individual	Developing and testing Heat Strategies for Men's Hockey World Cup (Dec 2018) Tokyo Olympic Performance (2020)	Awarded	$15,060	LK18A	Nil

When organising any scholarship related activity please use the reference code above. Contact your admin support person to arrange booking and logistics for your activity.

IMPORTANT
Keep note of your approved budget to ensure that you are within your appropriate limit. If you find that costs have changed since the time of application please contact the scholarship team prior to proceeding.

INTERNATIONAL TRAVEL
If your activity involves any international travel you will still need to complete the Memo for International SCHOLARSHIP Paid Travel. For the process to arrange this travel please refer here.

Any queries regarding the management of your scholarship please don't hesitate to contact me or refer to the programme guide.

Kind regards
Travis

PM Scholarships
High Performance Sport New Zealand
Physical Address: Millennium Institute of Sport & Health, 17 Antares Place, Mairangi Bay 0632
Postal Address: PO Box 302 563, North Harbour, Auckland 0751
Email: pmscholarships@hpsnz.org.nz
Web: www.hpsnz.org.nz