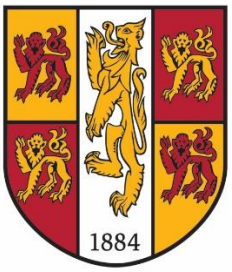
DOCTOR OF PHILOSOPHY

The Influence of Vitamin D, Psychosocial Factors and Sleep on Upper Respiratory Tract Infection and Mucosal Immunity

Harrison, Sophie

Award date:
2020

Awarding institution:
Bangor University



PRIFYSGOL
BANGOR
UNIVERSITY

School of Sport, Health & Exercise Sciences

College of Health & Behavioural Sciences

The Influence of Vitamin D, Psychosocial Factors and Sleep on Upper Respiratory Tract Infection and Mucosal Immunity

---

Sophie Elizabeth Harrison

A thesis submitted to

Bangor University

in fulfilment of the requirements of the degree of

Doctor of Philosophy

Supervisors: Prof. Neil P. Walsh and Dr. Ross Roberts

ACKNOWLEDGEMENTS

First, I must extend my gratitude to my supervisor, Prof Neil Walsh for his support and guidance over the last four years. I have a learnt a lot from Neil about the world of academia. His enthusiasm for science confirmed my desire to continue in scientific research and his high standards for research allowed me to develop as a researcher. I must also extend my gratitude to Dr Ross Roberts for supporting me unofficially and subsequently officially as a supervisor throughout my PhD. His support during the final stages of this PhD have gone over and above, for which I am very grateful.

I would like to extend a big thank you to my fellow PhD candidate, Dr Jason Edwards, for his support and guidance during the many hours of planning, testing and academic discussion. Big thanks also to Kevin Williams for his technical support and for always being a friendly face around the department. I must also thank the undergraduate and postgraduate students for the hours in the lab, and the participants who were willing to take part in this research, without whom this PhD would not have been possible

I would like to extend a huge thank you to my colleagues in the department for fuelling my coffee addiction, providing me with much-needed distractions, listening and empathising with my frustrations, providing me with a constant stream of dog pictures, and for finally dragging me over the finish line. You all contributed in a very important and special way.

To my friends and family outside the department I extend big thanks for your unwavering support and love. To my friends, there are too many to name but special thanks must go to Steph, I have been so lucky to have someone that brings so much light and laughter to my life throughout this process. Further thanks to my friends further afield, in particular, Jenna Anderson, Sheona Dorrian, Clare Mitchell, and to all my weightlifting

friends, who have kept me sane over the last few years. To my Mum and Dad, I can never thank you enough for your unconditional support, guidance and love, and of course for enabling my Starbucks addiction. You have been my rock, and I could not have done this without you. To my sister, Anna, and my brother-in-law, Martin, for their guidance, advice, support and for helping me to always see the lighter side. I am very grateful have such knowledgeable people in my corner. Anna, you have been one of my biggest inspirations. Finally, a massive thank you to my nephews, Finn and Brodie, for the contagious laughter and joy you bring to my life. Thank you for always being a welcome escape.

DECLARATION AND CONSENT

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

SUMMARY OF FINDINGS

The broad aims of this thesis were to investigate the influence of vitamin D supplementation on URTI burden in military recruits (**Chapter 3**), to examine the influence of psychosocial and behavioural factors on URTI risk in marathon runners (**Chapter 4**), and to investigate the influence of psychological stress and anxiety on the mucosal immune response to exercise, in both a controlled, lab-based setting and a field-based marathon (**Chapter 5**).

Firstly, we found that vitamin D sufficiency lowered URTI burden in military recruits during Army training (**Chapter 3**). Specifically, in 1,644 military recruits, we found that vitamin D sufficient recruits were less likely to have a clinician-diagnosed URTI during training, compared to those with serum 25(OH)D <50 nmol·L^{-1} (Study 1). Then, in a randomised-control-trial (RCT), including 249 men, we found that vitamin D supplementation by oral D_3 or simulated sunlight, which achieved vitamin D sufficiency in almost all, reduced URTI burden regardless of supplementation type: 21% lower URTI peak severity and 36% fewer days with URTI (Study 2). And that individuals beginning supplementation with 25(OH)D <50 nmol·L^{-1} benefitted from greater reductions in URTI burden: 33% shorter URTI duration. However, we did not find any influence of vitamin D supplementation on mucosal immunity.

In **Chapter 4**, a prospective, cohort study of 305 marathon runners, we examined the influence of psychosocial factors and sleep on URTI prevalence. We found that runners were more likely to report a URTI during the two weeks before or after a marathon if they reported higher psychological stress, anxiety or neuroticism, early life adverse experience, or lower perceived sleep quality. Runners with early life adverse experience were over two times more likely to report a URTI during the two weeks before the marathon (OR: 2.33) and runners

with poorer perceived sleep quality were two times more likely to report a URTI during the two weeks after the marathon (OR: 0.48).

In the final empirical chapter, we examined the influence of psychological stress and anxiety on the mucosal immune response to a moderate, lab-based bout of exercise (Study 1) and a field-based marathon (Study 2; **Chapter 5**). We found that perceived psychological stress, trait anxiety and state anxiety influenced the mucosal immune response to exercise. In men, perceived psychological stress, trait anxiety and state anxiety were negatively correlated with the mucosal immune response to exercise. In response to a controlled, moderate bout of exercise, in individuals with predominantly low-moderate psychological stress and anxiety, men with lower psychological stress and anxiety experienced an increase in saliva SIgA SR, whereas those with moderate stress and anxiety saw little change. Then, in a field-based marathon, when including individuals reporting high stress and anxiety, men with higher perceived psychological stress and trait anxiety had a greater reduction in mucosal immunity in response to a marathon. These findings support the recommendation that exercise physiologists should account for psychological stress and anxiety when examining the immune response to exercise. Taken together, **Chapters 4 and 5** indicate a key role of psychological factors in immunity in exercising individuals. Specifically, individuals with higher psychological stress and anxiety appear to be at greater risk of URTI and experience greater reduction in mucosal immunity following exercise.

LIST OF PUBLICATIONS

Conference proceedings

Harrison S. E., Edwards J. P., Roberts R. and Walsh N. P. (2019). Sleep quality and psychosocial variables predict common cold in marathon runners. Poster presentation at the American College of Sports Medicine Annual Meeting, Orlando, Florida, May 2019.

Harrison S. E., Edwards J. P., Oliver S. J. and Walsh N. P. (2018). The effect of vitamin D supplementation by sunlight or oral D_3 on mucosal immunity in military recruits. Verbal presentation at the inaugural United Kingdom Society of Exercise Immunology, Loughborough University, April 2018.

TABLE OF CONTENTS

THESIS FORMAT

This thesis contains a general introduction (**Chapter 1)**, which gives a brief overview of the current literature and aims of the thesis. The literature review section (**Chapter 2**) then outlines, in more depth, the background and main research aims. Three experimental chapters, comprised of five research studies, form the main focus of the thesis. The first investigates the influence of vitamin D on URTI burden and mucosal immunity (**Chapter 3**). The second explores the influence of psychosocial factors and sleep on URTI in marathon runners (**Chapter 4**). The third experimental chapter examines the influence of psychological stress and anxiety on the mucosal immune response to exercise (**Chapter 5**). The empirical chapters are largely presented as standalone research papers and as such are formatted for specific journals. **Chapter 3** is in preparation for submission to the journal *Medicine and Sport and Exercise Sciences*, and **Chapter 4** is in preparation for submission to the journal *British Journal of Sports Medicine*, and have therefore been formatted according to the journals' formatting requirements. At times there is some necessary repetition between chapters, although any repetition has been minimised. This approach is in accordance with the research training policy of the School of Sport, Health and Exercise Sciences, Bangor University. Finally, the general discussion (**Chapter 6**) summarises the main findings of the thesis and aims to critically analyse these in relation to the broader literature, as well as recognising limitations and suggesting areas for future research. **Bold type** is used to refer to chapters or sections within this thesis.

LIST OF FIGURES

system (e.g. primary lymphoid tissue) also indicates an autonomic nervous system involvement in the immune response to challenge. Redrawn from (Walsh, 2018).

Figure 2.5: Vitamin D status (25(OH)D nmol·L^{-1}) and respiratory infection (%) across months of the year. Redrawn from (Berry *et al.*, 2011).

Figure 2.6: Proposed mechanisms for the influence of early life adversity on immune dysregulation and subsequent increased URTI susceptibility in adulthood.

Figure 3.1. A schematic of the prospective cohort study (Study 1) that investigated the association between vitamin D status (serum 25(OH)D), upper respiratory tract infection (URTI) and days lost from training, and a randomized controlled trial (Study 2) that investigated the effects of vitamin D supplementation by solar-simulated radiation (SSR), oral vitamin D_3 (ORAL), or placebo (SSR-P or ORAL-P) on URTI and mucosal immune function. Blood and saliva samples were collected at baseline (Study 1 and 2), week 5, and the end of week 12 (Study 2). The syringe icon represents the blood sample; the head and tube icon represents the saliva sample.

Figure 3.2. Seasonal variation in serum 25(OH)D (panel A), vitamin D sufficiency prevalence (serum 25(OH)D ≥50 nmol·L^{-1}; panel B), and the URTI prevalence when serum 25(OH)D ≥50 nmol·L^{-1} or <50 nmol·L^{-1} (panel C) in 1,644 men and women during 12-weeks of military training. a, lower than summer $P < 0.05$. b, lower than autumn $P < 0.05$. c, lower than spring $P < 0.05$, * lower than participants with serum 25(OH)D <50 nmol·L^{-1}, $P < 0.05$. Panel A data are mean ± SD. Panels B and C are percentages represented by vertical bars.

Figure 3.3. Flow diagram of the randomized controlled trial (Study 2) investigating the effects of vitamin D supplementation on upper respiratory tract infection (URTI) and mucosal immune function. Flow diagram indicates the number of participants assessed, randomized, and analyzed at week 12 for URTI, salivary secretory immunoglobulin A (SIgA), and cathelicidin.

Figure 3.4. Serum 25(OH)D in men completing military training whilst receiving 12-weeks of vitamin D supplementation (solar simulated radiation (SSR) or oral vitamin D_3 (ORAL)) or a placebo (solar simulated radiation placebo (SSR-P) or oral placebo (ORAL-P)). Combined vitamin D interventions (SSR and ORAL) vs combined placebo (SSR-P and ORAL-P; panel A), ORAL vs ORAL-P (panel B), and SSR vs SSR-P (panel C). *, greater than placebo, $P < 0.05$. †, greater than baseline, $P < 0.05$. ‡, greater than week 5, $P < 0.05$. Data are mean ± SD.

Figure 3.5. Upper respiratory tract infection duration (URTI; panel A & D), URTI peak severity (panel B & E), and total days with URTI during military training (panel C & F), in the vitamin D supplementation (SSR and ORAL) vs placebo supplementation groups (SSR-P and ORAL-P) in all participants (left-hand column) and participants with a baseline 25(OH)D <50 nmol·L^{-1} ($N = 62$; right-hand column). * and #, lower than placebo, $P < 0.05$ and $P = 0.05$, respectively. Data are mean ± SD. d = Cohen's d effect size. [a] maximum possible peak severity (24 arbitrary units (AU)), [b] total number of days for military training (84 days).

Figure 4.1: Proportion of runners reporting an upper respiratory tract infection during the two weeks before the marathon (pre) if they reported at least one early life adversity compared to those reporting no early life adversities. * higher URTI prevalence compared to no early life adversity, $P < 0.05$.

Figure 4.2: The influence of healthy sleep quality during the two weeks before the marathon (pre; tertile split) on upper respiratory tract infection (URTI) prevalence during the two weeks after the marathon (post). * higher URTI prevalence compared to ≥3.7 group, $P < 0.05$.

Figure 5.1. Flow chart of participants who were recruited, provided saliva samples, and were included in the final data analysis for Study 2.

Figure 5.2. Saliva flow rate, SIgA concentration and SIgA SR before (pre), immediately after (post) and 30 min after (+ 30 min) 1 h of treadmill running at 65% $\dot{V}O_{2peak}$ (EX) or 1 h of seated rest (REST), for men (A, B and C, respectively) and women (D, E and F, respectively) (Study 1). # EX lower than REST, $P < 0.05$. † pre lower than post and + 30 min, $P < 0.05$. †† + 30 min lower than post, $P < 0.05$. Data are mean ± SD.

Figure 5.3. The relationship between saliva SIgA secretion rate (SR) change pre to post 1 h of treadmill running at 65% VO_{2peak} (EX) and perceived psychological stress, trait anxiety and state anxiety in men (A, B & C, respectively) and women (D, E & F, respectively) (Study 1). *, $P < 0.05$; **, $P < 0.01$.

Figure 5.4. Saliva SIgA SR before (pre) and after (post) 1 h of treadmill running at 65% VO_{2peak} (EX) in men with low PPS (LOW; <12 AU) and moderate PPS (MOD; 12-19 AU) (Study 1). † higher at post than pre in the LOW PPS group, $P < 0.05$. * LOW PPS higher than MOD PPS at post, $P < 0.05$. Data are mean ± SD.

Figure 5.5. The relationship between **s**aliva flow rate (FR) change pre to post 1 h of treadmill running at 65% VO_{2peak} (EX) and perceived psychological stress, trait anxiety and state anxiety in men (A, B & C, respectively) and women (D, E & F, respectively) (Study 1). **, $P < 0.01$.

Figure 5.6. Saliva flow rate, SIgA concentration and SIgA secretion rate (SR) before (pre) and after (post) the marathon for men (A, B & C, respectively) and women (D, E & F, respectively) (Study 2). * different to pre marathon, $P < 0.05$. Data are mean ± SD.

Figure 5.7. Saliva SIgA secretion rate (SR) change pre to post marathon in low perceived psychological stress (PPS; <12 AU) vs high PPS (≥20 AU), in men only (Study 2). * greater reduction compared to low PSS, $P < 0.05$. Data are mean ± SD.

LIST OF TABLES

CHAPTER ONE

General Introduction

Illness in athletes can lead to lost training time, reduced volume and intensity of training, increased injury risk, and reduced success at competition (Palmer-Green *et al.*, 2013; Hellard *et al.*, 2015; Svendsen *et al.*, 2016; Timpka *et al.*, 2017). Athletes, military recruits, and those taking part in heavy training, have been reported to experience increased risk of minor illnesses, in particular upper respiratory tract infection (URTI) (Peters and Bateman, 1983; Nieman *et al.*, 1990; Walsh *et al.*, 2011b). Indeed, a reduction in mucosal immunity during heavy training and following prolonged exercise have been implicated in increased URTI risk (Nieman *et al.*, 2006; Neville *et al.*, 2008). Early marathon studies reported an increased risk of URTI following marathons, with those who had faster race times and higher training loads being at greater risk of URTI (Peters and Bateman, 1983; Nieman *et al.*, 1990).

Nutritional deficiencies have often been implicated in immune reduction, and many athletes take nutritional supplements with the aim of reducing the risk of infection (Maughan *et al.*, 2018). Indeed, vitamin D, a sunlight-dependent secosteroid, has been suggested to play a role in immune function, following the discovery of vitamin D receptors in almost all immune cells (Baeke *et al.*, 2010). Government recommendation for vitamin D sufficiency is 25(OH)D ≥50 nmol/L (Institute of Medicine, 2011; European Food Safety Authority, 2016). During the winter months more than half of athletes and military personnel have insufficient vitamin D status, with as many as 35% being considered vitamin D deficient (He *et al.*, 2016a). Lower vitamin D status has been associated with respiratory infection incidence in several cross-sectional and cohort studies (Ginde *et al.*, 2009; Sabetta *et al.*, 2010; Monlezun *et al.*, 2015). Furthermore, a recent meta-analysis found that vitamin D supplementation reduced URTI risk, particularly in those beginning with poorer vitamin D status (Martineau *et*

al., 2017). However, in a new contemporary approach, it has been highlighted that in young, otherwise healthy, athletes, immune tolerance (the ability to dampen defence yet control infection at a non-damaging level) rather than immune resistance (the ability to destroy microbes) may be a more beneficial focus (Walsh, 2019). Indeed, vitamin D supplementation was found to reduce the likelihood of losing training days during military training (Laaksi *et al.*, 2010).

Exercise immunology research has recently recognised the role of prior infection, sleep disruption, environmental extremes, long-haul travel, and psychological stress and anxiety in URTI risk in athletes, alongside physical strain (Ekblom *et al.*, 2006; Svendsen *et al.*, 2016; Drew *et al.*, 2017; Drew *et al.*, 2018; Walsh, 2018). Despite the well-known influence of psychological stress on immunity, and numerous review articles proposing the likely role of these factors in athletic/exercising populations, psychological stress has largely been overlooked in exercise immunology. This omission is surprising given that psychological and physical challenges share pathways and effector limbs for the body's response. Indeed, recent work has shown that psychological stress plays a role in the *in vivo* immune response to exercise (Edwards *et al.*, 2018). Sleep duration and efficiency have also been implicated in increase URTI risk, similarly acting largely through activation of the HPA and SAM axes (Walsh, 2018; Besedovsky *et al.*, 2019). Additionally, early life adverse experiences have received recent attention in immunology literature (Elwenspoek *et al.*, 2017). Providing initial evidence, the offspring of parents who were separated and not speaking to one another had reduced resistance to the common cold, compared to those whose parents were intact, or separated but still communicating (Murphy *et al.*, 2017). Athletes are likely to encounter numerous stressors that can influence immune health, such as psychological stress and anxiety, and sleep disruption, alongside physical strain. However,

little empirical evidence exists that examines the role of these factors in URTI risk in athletic/exercising populations.

Therefore, the broad aims of this thesis were to investigate the influence of vitamin D supplementation on URTI burden, to examine the influence of psychosocial factors and sleep on URTI prevalence in marathon runners, and to investigate the influence of psychological stress and anxiety on the mucosal immune response to exercise.

CHAPTER TWO

Literature Review

2.1 Sport and Exercise Immunology

Athletes and those taking part in heavy training are reported to experience more minor illnesses, in particular upper respiratory tract infection (URTI) (Peters and Bateman, 1983; Nieman *et al.*, 1990; Walsh *et al.*, 2011b). Illness, such as respiratory tract or gastrointestinal infection, in athletes can lead to lost training time, reduced volume and intensity of training, increased risk of injury, and reduced success at competition. Indeed, a study examining 322 Great Britain Olympic athletes showed that ~70% of illnesses recorded resulted in time lost from training, and the remaining illnesses resulted in reduced volume or intensity of training (Palmer-Green *et al.*, 2013). Further, athletes reporting illness symptoms causing anxiety were five times more likely to sustain an in-championship injury (Timpka *et al.*, 2017). Perhaps of greater importance to athletes, those with fewer and shorter illnesses are more likely to compete at a higher level and be successful in major sporting competition (Hellard *et al.*, 2015; Svendsen *et al.*, 2016). For example, cross-country skiers who had shorter URTIs were more successful in major cross-country skiing championships (Svendsen *et al.*, 2016). Indeed, athletes reporting less sick days per year were able to complete more training hours (Martensson *et al.*, 2014). Furthermore, swimmers competing at international level reported fewer upper respiratory tract and pulmonary infections compared to those competing at national level (Hellard *et al.*, 2015). The impact of respiratory infection is not limited to athletic populations, but also has significant implications in the general population. Indeed, 141.4 million workings days were lost because of illness or injury in the UK in 2018, with minor illnesses such as colds and coughs being the most commonly cited reason for days lost, making up 27.2% of all days lost (38.5 million days) (Office for National Statistics, 2019).

Upper respiratory tract infections, such as common colds, are often characterised by coughing, nasal congestion and discharge, sneezing, malaise, and sore throat. Although virology and bacteriology analysis would allow pathological confirmations of URTIs, this is often not feasible, as it is both expensive and time consuming. As such, two key methods of assessing self-reported URTI have emerged; the Wisconsin Upper Respiratory Symptom Survey (WURSS) (Barrett *et al.*, 2002) and the Jackson common cold questionnaire (Jackson *et al.*, 1958). The Jackson common cold questionnaire was chosen for this thesis as it was validated by participants who were nasally inoculated with common cold pathogens, whereas the WURSS validation used semi-structured interviews to determine the prevalence of symptoms, and did not use nasal inoculation or pathology confirmation. Furthermore, while the Jackson common cold questionnaire contains only 8 symptom items, the WURSS contained 44 symptom items, thereby significantly increasing participant burden (Jackson *et al.*, 1958; Barrett *et al.*, 2002). Indeed, pathology confirmation of URTI was previously confirmed in 82% of participants reporting URTI using the Jackson common cold questionnaire (Hanstock *et al.*, 2016).

Acute endurance exercise is reported to induce immune suppression and increase susceptibility to URTI (Walsh *et al.*, 2011b). A positive association between prolonged endurance running and URTI was first shown in ultramarathon runners, of whom 33% had an infection after an ultramarathon, compared to only 15% of age-matched household controls in the same period (Peters and Bateman, 1983). In the week following the LA marathon, 13% of runners were ill compared to only 2% of runners who signed up but did not run the marathon (Nieman *et al.*, 1990). In contrast to these earlier studies, later work showed that there was no difference in the proportion of runners with a URTI in the three weeks before or after a marathon (17% vs. 19%, respectively). However, runners were more likely to have a URTI following the marathon if they reported a URTI in the three weeks before the marathon,

suggesting that factors other than physical strain may play an important role in infection susceptibility under exercising conditions (Ekblom *et al.*, 2006).

Training load and the volume of physical activity (PA) also play a role in immune modulation and URTI susceptibility. Rather than being detrimental for immune health, moderate PA is protective against URTI compared to sedentary behaviour (Matthews *et al.*, 2002). Furthermore, runners with greater training load have been shown to be at increased risk of URTI (Nieman *et al.*, 1990). Taken together, these findings led to the proposal of the J-shaped curve (Figure 2.1), which suggests that moderate PA may lower URTI risk, while a high amount of exercise may increase URTI risk, compared to moderate PA (Nieman, 1994). A number of studies have provided empirical support for this hypothesis. For example, Spence and colleagues showed that, over a four month period, moderately active individuals had fewer URTI episodes than sedentary individuals, and that athletes reported a greater number of URTIs, compared to moderately active individuals (Spence *et al.*, 2007). Further, men and women engaging in ≥ 7 h/week of endurance exercise reported more URTI episodes than those engaging in 3-6 h/week (Gleeson *et al.*, 2013). Further corroborating these findings, professional swimmers monitored over a four year period were 50-70% more likely to have illness during intensive training periods (Hellard *et al.*, 2015).

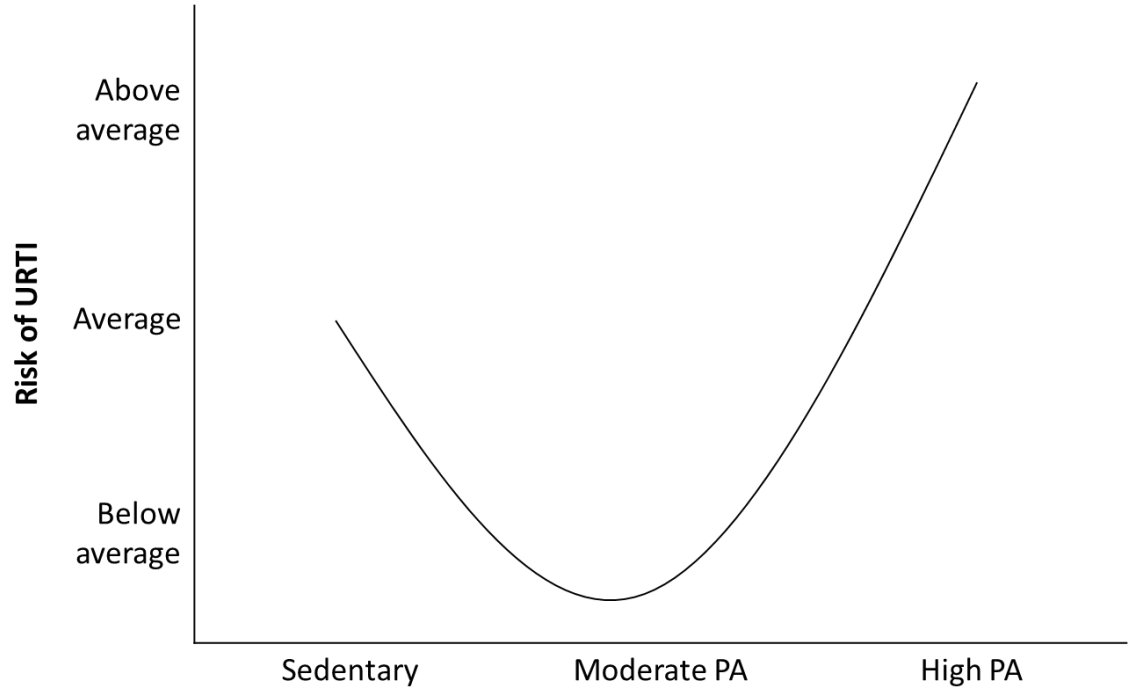


Figure 2.1: J-shaped curve. The relationship between physical activity (PA) load and URTI risk. Redrawn from (Nieman, 1994).

In 2006, the S-shaped curve was proposed, as an extension of the J-shaped curve, in order to explain differences in URTI risk in elite and non-elite athletes (Malm, 2006). The S-shaped curve suggests that elite athletes may be at reduced risk of infection compared to those undertaking similarly high training loads at a non-elite level (Figure 2.2). A number of explanations have been proposed as to why elite athletes may have fewer infections. These include: an underlying genetic predisposition to better resist or tolerate infection; better tolerance of training load increase; and better hygiene, infection avoidance, diet, sleep and stress management (Hellard *et al.*, 2015; Walsh, 2018). Furthermore, elite athletes are more likely to receive funding, they therefore may not have the increased stress of managing a full time job alongside their training and competition schedule (Walsh, 2018). Indeed, by losing less training time due to illness, athletes are able to progress to an elite level.

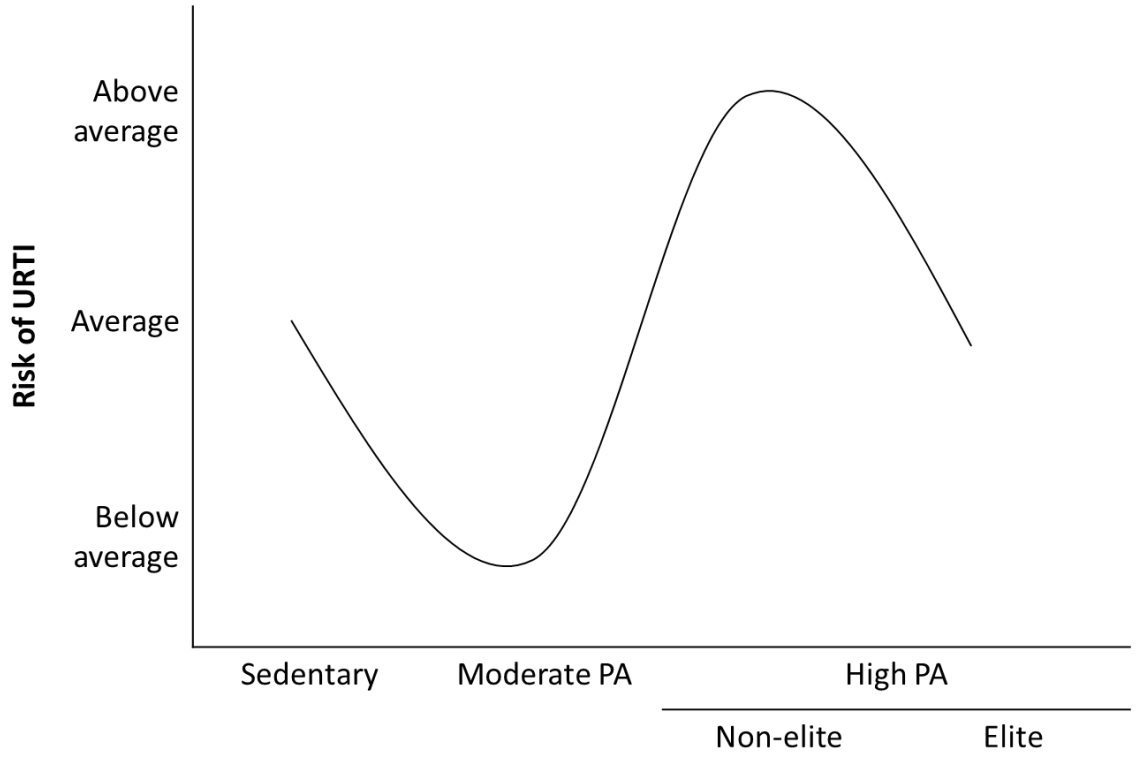


Figure 2.2: S-shaped curve. The relationship between physical activity (PA), and non-elite or elite athlete status, on URTI risk. Adapted from (Malm, 2006).

2.2 Exercise and the human immune system

2.2.1 Overview of the human immune system

The human immune system can be broadly split into two categories, the innate (non-specific) and the adaptive (specific) systems, which interact to function as an effective immune defence. The innate immune system is the first line of defence against infection. Innate immunity does not strengthen upon repeated exposure and has less specific pathogen recognition. The innate branch of the immune system includes both soluble factors, such as complement proteins, interferon α/β and anti-microbial peptides (AMPs), and cells, such as

neutrophils, dendritic cells, macrophages, natural killer (NK) cells and immunoglobulins (Ig). The adaptive system is slower to deploy in response to an infection but has a more specialised response. The adaptive system, which involves T and B cell activity, prevents colonisation of pathogens and destroys invading microorganisms. The innate and adaptive immune systems work in tandem to protect the body; the innate system helps to develop a specific immune response through antigen presentation to the adaptive immune system, enabling pathogen recognition and exclusion (Walsh *et al.*, 2011b).

2.2.2 Overview of the human mucosal immune system

The mucosal surfaces of the gut, urogenital tract, oral cavity and respiratory system are protected by the Common Mucosal Immune System, which is the site of infection or route of access for the majority of viruses and bacteria that cause human disease (Walsh *et al.*, 2011b). Mucosal surfaces are the first line of defence against invading pathogens. Secretory IgA (SIgA) is the most abundant Ig and the main effector of mucosal immunity. Mucosal plasma cells secrete SIgA in a polymeric form, consisting of dimeric IgA molecules joined by a polypeptide J chain and bound to the secretory component, an epithelial derived protein (Brandtzaeg, 1981; Gleeson and Pyne, 2000). Immunoglobulin A provides an effective defence against microbial pathogens by preventing pathogen adherence and penetration of the mucosal epithelium, neutralising viruses within epithelial cells, and excretion of locally formed immune complexes across mucosal epithelial cells to the luminal surfaces (Lamm, 1998). Secretory IgA works in tandem with alpha-amylase, lactoferrin, lysozyme, and to a lesser extent, SIgM and IgG (Gleeson and Pyne, 2000). The current gold standard for measuring mucosal immune markers is through unstimulated saliva samples, however, tear fluid has also received recent attention as a biomarker of mucosal immunity (Hanstock *et al.*, 2016).

2.2.3 Exercise and mucosal immunity

Lowered levels of SIgA, or a decrease from normal SIgA, is associated with increased risk of URTI. Runners who reported a URTI in the two weeks after a 160 km race had a 53% greater reduction in salivary SIgA SR pre to post marathon, compared to those not reporting a URTI (Nieman *et al.*, 2006). Furthermore, lower saliva SIgA was related to increased URTI incidence in elite America's Cup Yacht racers, monitored during 50 weeks of training. Although absolute saliva SIgA concentration was highly variable between individuals and was not related to URTI, when saliva SIgA concentration was relativized to each individuals' mean across the season, athletes had lower relative SIgA concentrations in the week before a URTI; on average a 30% reduction in relative SIgA from healthy values increased the risk of URTI (Figure 2.3), and SIgA concentration was 28% lower during URTI (Neville *et al.*, 2008). Furthermore, when examining the influence of endurance training on mucosal immunity, lower salivary SIgA concentration was related to an increase in the number of sick days during the subsequent training block ($r = -0.76$) (Ihalainen *et al.*, 2016). Tear SIgA has also been shown to have some utility in predicting URTI incidence; tear SIgA concentration was lower the week before and during URTI. Indeed, when tear SIgA SR was <5.5 mg/min participants were at 9x increased risk of URTI, and when tear SIgA SR decreased by more than 30%, participants were at a 6x greater risk of URTI in the following week. Interestingly, salivary SIgA was not predictive of URTI in this study (Hanstock *et al.*, 2016).

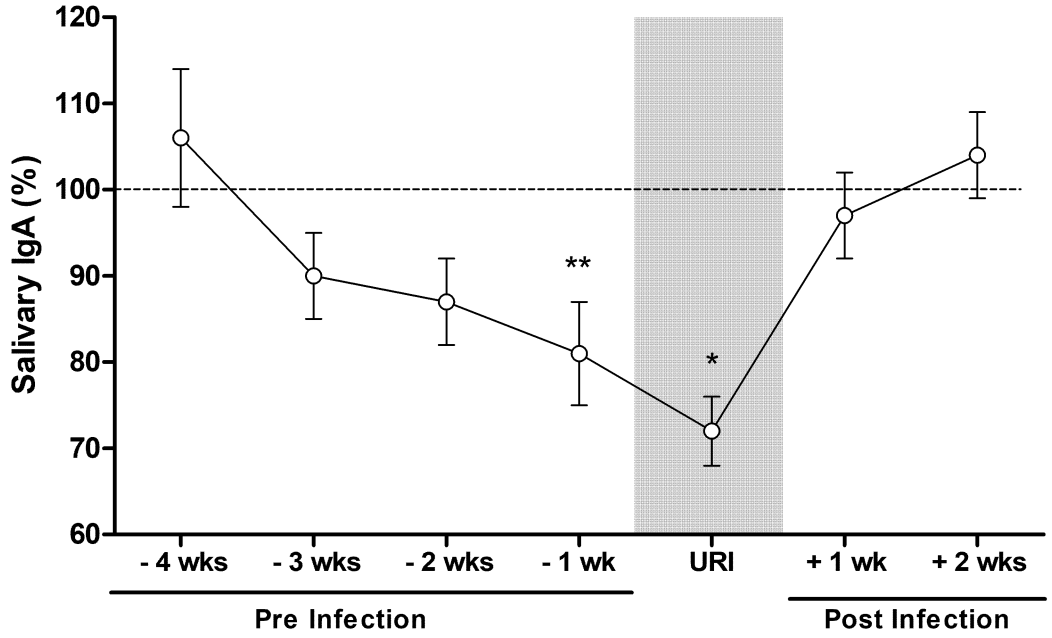


Figure 2.3: SIgA concentration before (pre), during, and after (post) upper respiratory infection (URI) (N = 102). Data are mean ± SEM of individual relative SIgA (% of no URI mean values). *URI significantly lower than –4 wks, +1 wk, and +2 wks, $P < 0.005$; ** –1 wk significantly lower than +2 wks, $P < 0.005$. Redrawn from (Neville *et al.*, 2008).

Prolonged bouts of exercise have classically been shown to lead to a decrease in mucosal immune function, often referred to as the "Open Window Theory" (Nieman, 1994). This transient decreased in mucosal immune markers following exercise has generally been reported to last for one to two hours following an acute exercise bout, before returning to baseline levels (Gleeson and Pyne, 2000; Walsh *et al.*, 2011b). However, research in this area has provided conflicting findings, with a number of studies showing a decrease in mucosal immune markers (Tomasi *et al.*, 1982; Tharp and Barnes, 1990; Steerenberg *et al.*, 1997; Nieman *et al.*, 2002; Walsh *et al.*, 2002; Fahlman and Engels, 2005; Nieman *et al.*, 2006; Usui *et al.*, 2011; Gillum *et al.*, 2013; Gill *et al.*, 2014) and others showing no change following exercise (McDowell *et al.*, 1991; Walsh *et al.*, 1999; Bishop *et al.*, 2000; Laing *et*

al., 2005; Bishop *et al.*, 2006; Allgrove *et al.*, 2008; Davison *et al.*, 2009; Costa *et al.*, 2012; Killer *et al.*, 2015; Leicht *et al.*, 2018; Dulson, 2019). These conflicting results can likely be attributed to a number of factors including varied exercise intensity and duration, differences between lab and field studies, and the likely influence of other factors such as nutrition, sleep and psychological stress, which will be reviewed in further detail in **section 2.3** and onwards.

A relationship has also been reported between SIgA and training load. The majority of studies show that levels of SIgA are not different in athletes compared to non-athletes, except when athletes are engaging in heavy training. For example, it was found that athletes had lower salivary SIgA both pre and post exercise following 7 month of training, compared to recreationally active controls (Gleeson *et al.*, 1995b). American footballers also had lower SIgA SR during a training season when compared to physically active controls (Fahlman and Engels, 2005). Furthermore, lower relative salivary SIgA was related to higher training load in America's Cup sailors (Neville *et al.*, 2008). Recently, higher training load was related to lower SIgA SR in elite para-triathletes, despite no relationship between SIgA and URS. Indeed, training load explained 13% of the variance in SIgA SR (Stephenson *et al.*, 2019). Further, a relationship between military training and a reduction in mucosal immunity has been shown (Carins and Booth, 2002; Tiollier *et al.*, 2005; Whitham *et al.*, 2006). However, it is likely that these reductions are due to multiple factors, such as sleep, hydration, nutrition, and stress, rather than being purely a consequence of physical load. For example, military recruits often experience dietary restriction, stress, and hypohydration. Indeed, mucosal immunity in military recruits under strenuous physical training was associated with dietary restriction, consumption of alcohol, body mass loss, URTI and negative emotions (Carins and Booth, 2002). Shorter periods of training ($\leq$ 4 weeks) do not appear to have negative effects on saliva SIgA SR (Tanner and Day, 2017).

2.3 Upper respiratory tract infection susceptibility in athletic and non-athletic populations

Historically, exercise immunology has predominantly focused on the role of physical strain on immune function. However, a study showing no increase in URTI symptoms following a marathon led to a shift in thinking. Indeed, this study showed that pre-race URTI symptoms increase the risk of URTI after the endurance event (Ekblom *et al.*, 2006). Importantly, athletes and military personnel experience multiple stressors during their everyday lives, around sporting competitions, and through their work environment. Athletes travelling to competitions are likely to experience circadian rhythm disruptions, sleep disruptions, and changes in psychological stress and nutrition, alongside high physical stress. Indeed, military recruits experience sleep disruption, hypohydration, nutritional deficiencies and increased psychological stress, whilst undertaking heavy physical exercise (Walsh, 2018). Long haul travel, competition participation, low energy availability, depression symptoms, higher perceived stress and shorter sleep duration have since been identified as risk factors of illness in athletes and military personnel (Schwellnus *et al.*, 2012; Svendsen *et al.*, 2016; Drew *et al.*, 2017; Wentz *et al.*, 2018). Given the abundance of research regarding psychological stress, sleep, and other risk factors of URTI susceptibility in non-athletic populations, it is surprising that so far these factors have often been overlooked by exercise immunologists.

Physical, psychological and behavioural stressors influence the immune system through similar mechanisms, largely through activation of the sympathetic-adrenal-medullary (SAM) and hypothalamic-pituitary-adrenocortical (HPA) axes (Figure 2.4). Specifically, both physical and psychological stressors are associated with increases in sympathetic nervous system activation, leading to the release of catecholamines. Depending upon the duration and

intensity of the stressor, the HPA axis is also activated leading to an increase in glucocorticoids (Perna *et al.*, 1997). Indeed, hormones released from the SAM and HPA axes under acute stress play an important role in immune function, allowing the immune system to prepare for immune challenges that may be imposed by the stressor. Acute stress can lead to immune-enhancement including changes in dendritic cells, neutrophils, macrophages, SIgA transport, and lymphocyte trafficking and function. Following an acute stressor, some immune markers, such as leukocytes, will often fall below baseline, due to redeployment to tissues such as mucosal linings, skin, lungs, liver and lymph nodes, in preparation for immune challenges that may be imposed by the stressors. Immune markers will then return to baseline levels in the hours following (Walsh *et al.*, 2011a; Dhabhar, 2014). The time taken to return to baseline levels, as well as the direction and magnitude of the immune response, is likely dictate by a combination of stressors, such as physical, psychological and behaviour factors (Dhabhar, 2014; Campbell and Turner, 2018; Walsh, 2018; Simpson *et al.*, 2020). In contrast, chronic stress has been shown to suppress or dysregulate immune function and exacerbate pathological immune response. As well as the HPA and SAM axes, anterior pituitary hormones (e.g., growth hormone (GH) and prolactin), which have known immune-regulatory effects, may be released in response to a stressor. Furthermore, direct sympathetic innervation of immune system organs may occur in response to a stressor, which indicates autonomic nervous system involvement in the immune response to a challenge (Walsh, 2018). Indeed, there are commonalities in the way that physical and psychological stressors influence immunity, moderate levels of physical and psychological stress can upregulate immunity, whereas chronic periods of psychological stress and prolonged exercise or heavy periods of exercise training down regulate immunity (Walsh *et al.*, 2011b; Dhabhar, 2014). It is clear that psychological and physical stressors play a key role in immune function, acting

through similar pathways, and therefore should not be considered in isolation (Perna *et al.*, 1997).

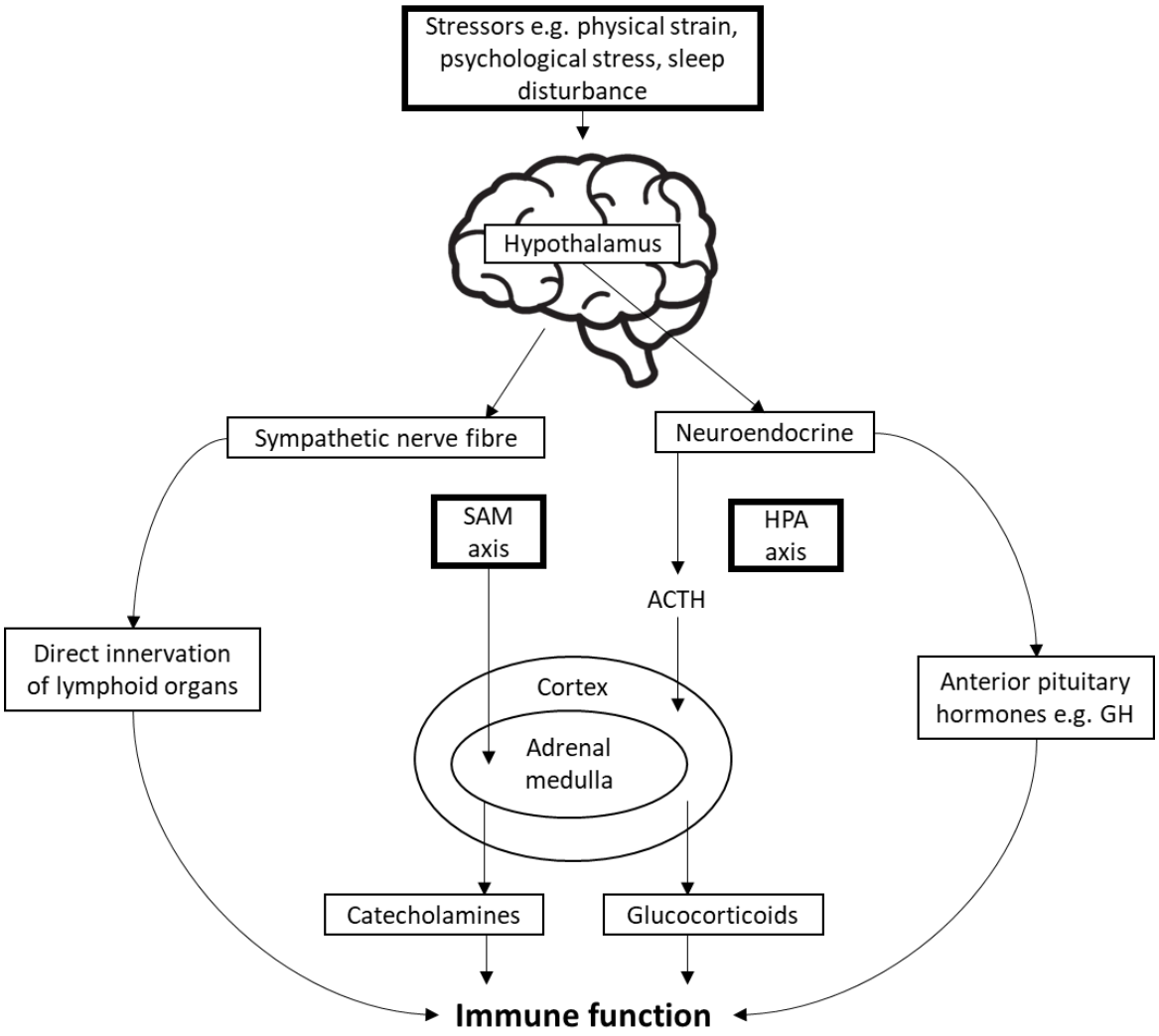


Figure 2.4: Common pathways for the immune response to physical and psychological challenges. Stressors, such as physical strain, psychological stress, and sleep disruption, are characteristically met by a series of coordinated hormonal responses controlled by the central nervous system. The central control station resides within the hypothalamus, with the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic–adrenal–medullary (SAM) axis providing the effector limbs by which the brain influences the body's response to challenge by controlling the production of adrenal hormones known to modulate immune function. The HPA axis regulates the production of the glucocorticoid, cortisol by the adrenal cortex and

the SAM axis regulates the production of catecholamines (epinephrine and norepinephrine) by the adrenal medulla. Aside from these dominant axes, anterior pituitary hormones with known immune-regulatory effects, such as growth hormone (GH) and prolactin, may also be released in response to challenge. Sympathetic nerve innervation of organs of the immune system (e.g. primary lymphoid tissue) also indicates an autonomic nervous system involvement in the immune response to challenge. Redrawn from (Walsh, 2018).

2.4 The role of nutrition in upper respiratory tract infection susceptibility

Given the interest of exercise immunology on the influence of physical strain on immune function, it is unsurprising that research has also explored the role of nutrition in immunity. The immune system requires an adequate supply of energy to function, including from glucose, amino acids and fatty acids. Accordingly, low energy availability is a risk factor for infection (Walsh, 2019). Olympic athletes with low energy availability, reported by questionnaire, were more likely to have an illness (Drew *et al.*, 2017). Furthermore, carbohydrates, proteins and fatty acids have all been implicated in immune function, but with little convincing evidence that additional supplementation is beneficial for immune health in athletic populations (Bermon *et al.*, 2017). A number of micronutrients are also important for maintaining proper immune function; iron, zinc, vitamin D, vitamin E, B6 and B12 play an important role in immune function (Gleeson *et al.*, 2004a). Furthermore, a number of micronutrients have been proposed to reduce the burden of infection in athletes; vitamin D, probiotics, polyphenols, omega-3 PUFAs and Vitamin E (Walsh, 2019). Indeed, micronutrient supplementation is highly prevalent amongst athletes (Maughan *et al.*, 2018).

2.4.1 Vitamin D

Vitamin D refers to a group of fat-soluble secosteroids, which are responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc (Holick, 2007). Historically, vitamin D was primarily associated with bone health. However, the discovery of vitamin D receptors (VDRs) in almost all immune cells has prompted research into the role of vitamin D in immune function (Baeke *et al.*, 2010). An association has been shown between seasonal-sunlight and URTI; when vitamin D status is at its lowest, URTI incidence is at its highest (Figure 2.5) (Berry *et al.*, 2011). Serum 25(OH)D is the gold standard measurement of vitamin D status (Holick, 2009). Government recommendation for vitamin D sufficiency is 25(OH)D $\geq$50 nmol·L^{-1} and individuals with 25(OH)D <30 nmol·L^{-1} are considered vitamin D deficient (Institute of Medicine, 2011; European Food Safety Authority, 2016). Vitamin D deficiency is highly prevalent during wintertime at latitudes >35°, when sunlight is at its annual nadir. Indeed, during wintertime at higher latitudes, such as in the UK, more than half of athletes and military personnel are considered to be below vitamin D sufficiency and as many as 35% could be considered vitamin D deficient (He *et al.*, 2016a).

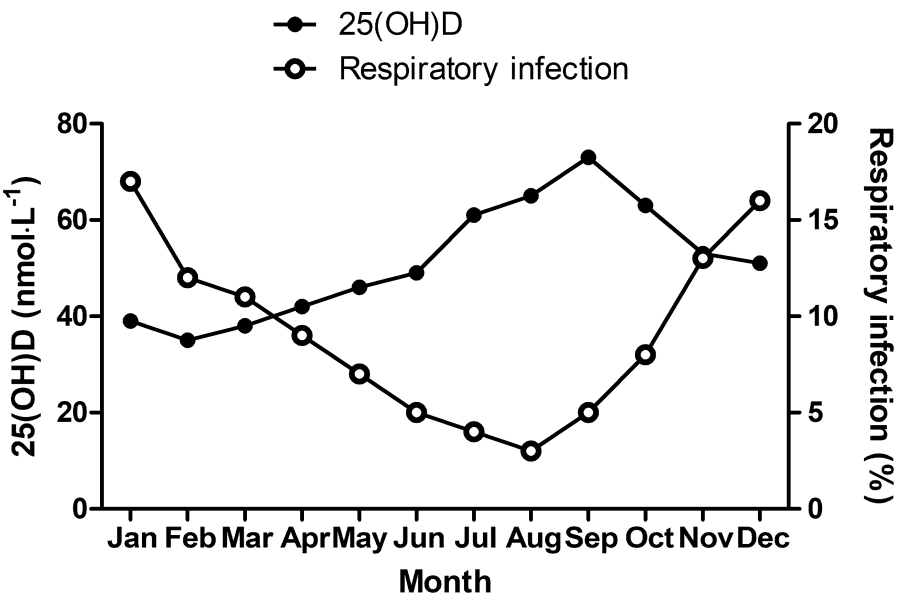


Figure 2.5: Vitamin D status (25(OH)D nmol·L^{-1}) and respiratory infection (%) across months of the year. Redrawn from (Berry *et al.*, 2011).

Vitamin D influences both innate and adaptive immune function, with implications for host defence (Hewison, 2012). Endogenously synthesised vitamin D_3 derived from sunlight UVB exposure of the skin and dietary D_2 and D_3 are hydroxylated in the liver to 25(OH)D (calcidiol or calcifediol). In a second hydroxylation, 25(OH)D is converted in the kidney to the biologically active form, $1,25(OH)_2D$. In target cells, after first binding with VDRs, and subsequently with vitamin D response elements in the nucleus, $1,25(OH)_2D$ exerts its function by acting as a modulator for over 900 genes (Kongsbak *et al.*, 2013). Indeed, $1,25(OH)_2D$ is a vital mediator of innate immune responses, enhancing the antimicrobial properties of immune cells such as monocytes and macrophages through the induction of antimicrobial proteins (Bikle, 2009; Chun *et al.*, 2014). In contrast, many of the reported actions of vitamin D on adaptive immunity are considered anti-inflammatory or 'tolerogenic' in nature (e.g. inhibition of interleukin-2 and interferon-gamma) (Krishnan and

Feldman, 2011; Walsh, 2019). Immune tolerance is described as the ability to dampen defence yet control infection at a non-damaging level (Ayres and Schneider, 2012), which has since been proposed as a key focus of nutritional intervention for immune function in athletes (Walsh, 2019). As such, by limiting excessive inflammation, achieving vitamin D sufficiency in those with low serum 25(OH)D, might reduce the duration and severity of URTI bouts (Thomas *et al.*, 2016; Walsh, 2019). However, few studies have examined the influence of vitamin D supplementation on URTI burden, such as duration and severity of respiratory illness.

There are numerous cross-sectional studies showing an inverse relationship between vitamin D status and URTI incidence. In the National Health and Nutrition Examination Survey (18,883 US citizens), serum 25(OH)D was inversely related to URTI; individuals with 25(OH)D <25 nmol·L^{-1} were 1.36 times more likely to have a URTI compared to those with 25(OH)D ≥75 nmol·L^{-1} (Ginde *et al.*, 2009). Furthermore, individuals with 25(OH)D ≥94 nmol·L^{-1} had a two-fold reduction in respiratory tract infection and fewer days with illness compared to individuals with 25(OH)D <94 nmol·L^{-1} (Sabetta *et al.*, 2010). Indeed, another large study of US citizens found that those with 25(OH)D <75 nmol·L^{-1} were 58% more likely to have an acute respiratory infection compared to those with 25(OH)D ≥75 nmol·L^{-1} (Monlezun *et al.*, 2015). While it is clear that vitamin D status is playing a role in URTI risk, there is ambiguity over the vitamin D status that provides optimal protection from URTI infection. However, the European Food Safety Authority (EFSA) and the Institute of Medicine (IOM) currently recommend vitamin D sufficiency for all population groups to be serum 25(OH)D ≥50 nmol·L^{-1} (Institute of Medicine, 2011; European Food Safety Authority, 2016).

To date, there have been six meta-analyses investigating the influence of vitamin D supplementation on URTI incidence, yet results are somewhat contrasting. Indeed, three out of six of these meta-analyses showed no benefit (Charan *et al.*, 2012; Murdoch *et al.*, 2012; Bergman *et al.*, 2013; Mao and Huang, 2013; Vuichard Gysin *et al.*, 2016; Martineau *et al.*, 2017). However, these variable effects can likely be attributed to the heterogeneity of study participants, and the fact that many study participants were vitamin D sufficient at the onset of supplementation. Vitamin D supplementation reduced the risk of acute respiratory infection in all when examined in a meta-analysis of RCTs (OR (95% CI): 0.88 (0.81 – 0.96)). Specifically, vitamin D supplementation was protective in those receiving daily or weekly vitamin D without additional bolus doses (OR (95% CI): 0.81 (0.72 – 0.91)), but not in those receiving one or more bolus doses (OR (95% CI): 0.97 (0.86 – 1.10). Only a few studies have examined the influence of vitamin D supplementation on URTI risk and mucosal immunity in athletes and military personnel. Finnish military personnel supplemented with vitamin D were less likely to be absent from duty due to respiratory tract infection compared to a control group of recruits (Laaksi *et al.*, 2010). Furthermore, athletes supplemented with 5000 IU of vitamin D daily for 14 weeks had increases in saliva SIgA and cathelicidin secretion rates (He *et al.*, 2016b), although participants in this study began with a median vitamin D status above vitamin D sufficiency and were supplemented with doses much higher than the tolerable upper limit (<4000 IU·day^{-1}), as recommended by EFSA. Additionally, military recruits supplemented with 1000 IU D_3 per day for 12 weeks had subtle increases in saliva SIgA SR (Scott *et al.*, 2019). Adding to recent suggestions that supplementation should only be considered in those who are most in need (Maughan *et al.*, 2018), a meta-analysis also found that individuals who began supplementation with 25(OH)D <25 nmol·L^{-1} had stronger protective benefits compared to those who had baseline 25(OH)D ≥25 nmol·L^{-1} (Martineau *et al.*, 2017).

Vitamin D supplementation studies have largely focussed on dietary vitamin D supplementation, although 90% of our annual vitamin D comes from skin sunlight exposure (Hart *et al.*, 2011; He *et al.*, 2016a). Indeed, it has been shown that the majority of vitamin D can be achieved through short lasting skin exposures during the summer months between latitudes of 30 to 60°N (Rhodes *et al.*, 2010). However, it currently remains unknown whether there are additional benefits for immune function of vitamin D supplementation by UVB exposure, over and above those achieved through oral D_3 supplementation. UVB exposure may be beneficial for immune health, over and above improving vitamin D status, for a number of proposed reasons. UVB exposure produces nitric oxide (NO) locally at the skin surface, which has been associated with cardiovascular health benefits, via the reduction of systemic blood pressure, as well as improving neurotransmission, immune defence, regulation of apoptosis and cell motility (Juzeniene and Moan, 2012; He *et al.*, 2016a). Further, UVB exposure may benefit immune health through mood enhancement and stress reduction (He *et al.*, 2016a).

2.5 The role of sleep in upper respiratory tract infection susceptibility

Sleep is essential for both mental and physical health. Sleep inadequacies can be characterised by shorter sleep duration (<7 hours per night) and poorer sleep quality/ efficiency, for example sleep fragmentation, long sleep-onset latency, and poorer perceptions of sleep (Walsh *et al.*, 2020). Poorer sleep efficiency and shorter sleep duration have been shown to lead to lowered resistance to common cold (Cohen *et al.*, 2009; Prather *et al.*, 2015). Indeed, sleep has been reported to influence numbers and subsets of leukocytes, cytokine levels, concentrations of antibodies and complement factors, as well as functional aspects like cell cytotoxicity, largely through activation of the HPA axis and sympathetic nervous system (Walsh, 2018; Besedovsky *et al.*, 2019). Indeed, numerous studies have reported associations between poorer sleep and infection incidence. In a cohort study of healthy adolescents, individuals with shorter sleep duration had more frequent acute illnesses, and reporting less sleep during the previous week was associated with increased illness prevalence (Orzech *et al.*, 2014). In a large, cohort study of over 22,000 Americans, shorter sleep duration (≤5 h per night) increased risk of infections, and risk of head or chest colds, when examined separately, compared to individuals sleeping 7-8 h per night. Furthermore, effects remained after accounting for age, sex, race/ethnicity, educational attainment, household income, marital status, smoking status, physical activity, body mass index (BMI), and survey year (Prather and Leung, 2016). In contrast, one recent study found no association between sleep and risk of respiratory infection, although this was a slightly smaller study with 2,038 men and women, who were asked about ordinary weekday sleep duration and quality on only a single occasion (Ghilotti *et al.*, 2018).

Athletes have been found to have poorer sleep efficiency compared to non-athletes (81% vs. 89%, elite athletes vs. non-athletes, respectively) (Leeder *et al.*, 2012). Indeed, on average, athletes had sleep efficiency that can be considered abnormal (<85%) (Cohen *et al.*,

2009). Athletes get well below the recommended 8 h of sleep per night (averaging 6.8 ± 1.1 h per night), and sleep is largely influenced by training schedule, evening games, and competition (Sargent *et al.*, 2014; Lastella *et al.*, 2015a; Lastella *et al.*, 2015b; Sargent and Roach, 2016). Furthermore, overreached endurance athletes have been found to have disturbed sleep and increased illness (Hausswirth *et al.*, 2014). Importantly, poorer sleep also has negative effects on both endurance and sprint performance (Oliver *et al.*, 2009; Skein *et al.*, 2011).

Given that athletes are likely to have increased sleep disturbance, surprisingly little research has examined the influence of sleep on infection risk. Australian football athletes with reduced sleep quantity had increased incidence of illness within the next seven days (Fitzgerald *et al.*, 2019). Furthermore, military recruits sleeping less than 6 h per night were four times more likely to have a URTI whilst undergoing arduous military training, and subsequently lost more training days due to URTI, compared to recruits sleeping 7-9 h per night (Wentz *et al.*, 2018). Indeed, sleep disruption has been shown to influence lymphocyte redeployment following an acute bout of exercise (Ingram *et al.*, 2015). Although reductions in mucosal immunity have been shown to precede URTI, two studies examining the influence of acute sleep deprivation on the mucosal immune response to exercise have failed to show a link between sleep and mucosal immunity (Ricardo *et al.*, 2009; Gillum *et al.*, 2015). However, one study of these studies included only eight participants, including four women, for whom they did not account for menstrual phase, and did not measure SIgA, one of the most abundant mucosal immune markers (Gillum *et al.*, 2015). Clearly more research on the influence of sleep on infection risk and immunity in athletic and exercising populations is required.

2.6 The role of psychological stress in upper respiratory tract infection susceptibility

Psychological stress has been described as a constellation of events, consisting of a stimulus (stressor), that precipitates a reaction in the brain (stress perception), that activates physiological fight-or-flight systems in the body (stress response) (Dhabhar, 2014). Anxiety is the reaction to stress characterised by feelings of apprehension, dread, tension, nervousness and worry (Spielberger, 1983). Psychological stress is broadly categorised as either acute or chronic. Acute stress lasts minutes to hours and generally involves the "fight or flight response" first coined in 1932 by Walter B. Cannon (Cannon, 1932). Selection bias caused the "fight-or-flight" response to evolve; individuals responding to environmental threats or stressors were more likely to survive. Although the stressors that humans encounter may have changed, the physiological response to a perceived stressor remains. Multiple physiological systems are activated to prepare the immune system for any acute challenges that are imposed by a stressor, which may lead to short term immune-enhancement. Chronic stress generally lasts several hours per day for weeks or months and can lead to deleterious effects for URTI risk, due to dysregulation in cortisol circadian rhythm and chronic inflammation (Dhabhar and McEwen, 1997). Chronic stressors are largely associated with global immunosuppression and have been found to decrease almost all functional immune measures (Segerstrom and Miller, 2004).

Psychological stress influences the immune system in similar ways to physical stress, through activation of the HPA and SAM axes. Psychological stress leads to increases in norepinephrine and epinephrine through sympathetic activation, and increases in corticotrophin releasing hormone, adrenocorticotropin and cortisol following activation of the HPA axis (Perna *et al.*, 1997). Indeed, similar to exercise stress, acute psychological stress

largely acts through activation of the sympathetic nervous systems, which leads to immune enhancement of innate immune measures. The immune boosting effects of acute stress are largely attributed to mobilisation and redistribution of immune cells (Dhabhar and McEwen, 1997). Acute stress also leads to increased salivary protein exocytosis and IgA transocytosis (Bosch *et al.*, 2002). Chronic stress has negative effects on almost all function measures of the immune system, both innate and acquired branches (Segerstrom and Miller, 2004). Indeed, chronic stress leads to a depression in the secretion of SIgA into saliva due to activation of the HPA axis, which exerts inhibitory effects on IgA synthesis and/or transcytosis (Walsh *et al.*, 2011b). Chronic stress also has longer lasting effects by contributing to the course of chronic diseases involving excessive non-specific inflammation, such as cardiovascular disease, type 2 diabetes mellitus, multiple sclerosis and rheumatoid arthritis. Chronic stress is therefore linked to increased risk of morbidity and mortality (Segerstrom and Miller, 2004). Individuals have different perceptions, processing and appraisal of stressors, and vary in coping mechanisms, which can have significant effects on the kinetics, peak levels and the duration of circulating stress hormones, leading to individualised responses to the same stressor. Indeed, the magnitude and duration of stress-induced increase in stress hormones can have effects on immune cell distribution and function that lead to deleterious effects.

In the general population, psychological stress has been shown to play a role in URTI risk. Indeed, a meta-analysis of 27 prospective studies confirmed that psychological stress is associated with increased upper respiratory infection susceptibility (Pedersen *et al.*, 2010). Furthermore, the effect sizes for the association between stress and infection did not vary significantly according to how respiratory infections were assessed, the type of stress assessed, or whether the studies examined natural exposure or experimental exposure to pathogens (Pedersen *et al.*, 2010). Following common cold exposure, the rates of both

respiratory infection and clinical colds increased in a dose-response manner with increases in the degree of psychological stress (Cohen *et al.*, 1991). Additionally, adults reporting higher life event stress were more likely to have an infectious episode (Cobb and Steptoe, 1996). In a large study of the Dutch working population, individuals who had high psychological job demands were 20% more likely to have a common cold compared to those with low psychological job demands (Mohren *et al.*, 2001). Psychological stress has also been shown to influence vaccination response. Indeed, the chronic stress associated with caregiving for a spouse with progressive dementia was associated with a down-regulation of the immune response to flu virus vaccination (Kiecolt-Glaser *et al.*, 1996). Further adding to the evidence that stress-induced suppression of immune function may have clinical significance, individuals higher in negative affect had a lower antibody response to hepatitis B vaccination (Marsland *et al.*, 2001).

Mucosal immunity is also influenced by both chronic and acute psychological stress. Mucosal immunity is generally seen to increase in response to an acute stressor before returning to baseline levels within the following hours. For example, a number of studies have shown these effects using examination stress as the experimental model. Indeed, salivary SIgA concentration increased immediately post examination in nursing students compared to pre exam, and then returned to pre levels two hours after the exam (Takatsuji *et al.*, 2008). In other studies, students also had higher SIgA SR before an exam compared to salivary SIgA SR measured two and six weeks later (Bosch *et al.*, 1998). Exam stress has also been used to examine the effects of more chronic psychological stress, with salivary lysozyme found to be lower during a high stress period (immediately before an exam) when compared to a low stress period (after the completion of all exams) (Perera *et al.*, 1997). In addition, studies examining chronic stress on mucosal immunity in caregivers show that individuals who experience chronic stress are likely to have lowered IgA SR (Phillips *et al.*,

2006; Gallagher *et al.*, 2008). Further, it has been suggested that chronic stress may influence the immune response to an acute stressor. Indeed, personality traits associated with high levels of chronic stress, such as neuroticism, are associated with smaller cortisol and cardiovascular reactions to an acute stressor, indicating diminished stress reactivity (Bibbey *et al.*, 2013).

Given the vast amount of research confirming a link between psychological stress and URTI risk in non-athletic populations, it is surprising that very few exercise immunologists have reported or accounted for psychological stress in their studies. Furthermore, despite numerous review articles proposing the likely important role of psychological stress on immune function in exercising individuals (Gleeson *et al.*, 1995a; Clow and Hucklebridge, 2001; Gleeson *et al.*, 2004b; Keaney *et al.*, 2018; Walsh, 2018), there is little empirical evidence supporting this position. However, providing initial evidence, a recent study found that an individual's state anxiety and perceived psychological stress prior to an exercise bout played an important role in determining the strength of the *in vivo* immune response after exercise (Edwards *et al.*, 2018). Furthermore, in an observational study of athletes nine months out from the Olympics, individuals with depression symptoms and higher perceived stress were more likely to report an illness. Unfortunately, it appears measures of psychological stress paralleled illness reporting, making it difficult to discern causality. Furthermore, only 16 illnesses were recorded in athletes (Drew *et al.*, 2017). Interestingly, in a follow-on from this study, which examined illness in athletes three months before the Olympics, poor mental health was not associated with illness incidence. The authors in this study propose that this may be due to survival bias, as those individuals with poorer mental health may not have made it through the selection process to the three-month pre-Olympic period, which aligns with the S-shaped curve of infection (Malm, 2006; Drew *et al.*, 2018). Importantly for athletes, pre-championship illnesses causing anxiety are associated with a

five times increased likelihood of sustaining an in-championship illness (Timpka *et al.*, 2017). Given the shared biological mechanisms of psychological and physical stress, and recent research suggesting a link between illness and psychological stress and anxiety in athletic populations, it is important we strive to understand and explore the role of psychological stress in URTI susceptibility and mucosal immunity.

2.7 The role of early life adverse experiences in upper respiratory tract infection susceptibility

Early life adversity (ELA) is an overarching term for a multitude of early life adverse experiences, for example physical and emotional neglect, low socioeconomic conditions, family conflict, and peer victimisation (Fagundes *et al.*, 2013a; Elwenspoek *et al.*, 2017). ELA has recently been recognised as having the potential to exert immune dysregulation that persists across the lifespan (Elwenspoek *et al.*, 2017). ELA prevalence is astonishingly high, with a recent study reporting that 39% of the global population have likely been exposed to one or more childhood adversity (Kessler *et al.*, 2010). ELA impacts both the innate and acquired branches of the immune system, and has therefore been implicated in URTI susceptibility. The key factors that are thought to play a role in ELAs' influence on immune dysregulation into adulthood are increased circulatory inflammation, impaired cellular immunity, accelerated immunoscenescence, blunted physiological responses and increased risky health behaviours (Figure 2.6) (Elwenspoek *et al.*, 2017).

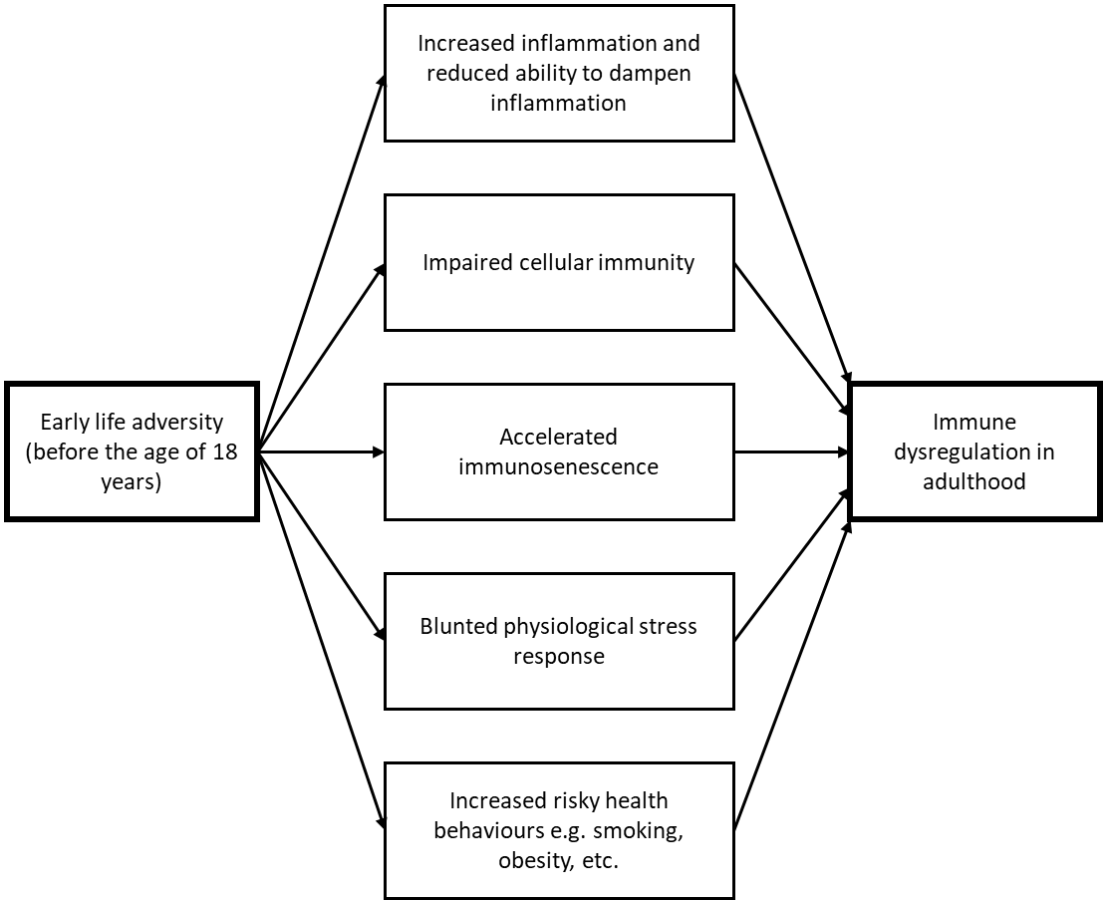


Figure 2.6: Proposed mechanisms for the influence of early life adversity on immune dysregulation and subsequent increased URTI susceptibility in adulthood.

Early life adversity increases circulatory inflammation and reduces the ability to dampen inflammation (Fagundes *et al.*, 2013a). Indeed, this overactive and unresolved inflammation leads to chronic low-grade inflammation, which is associated with the pathogenesis of a number of chronic diseases. Individuals who have experienced adverse childhood experiences have higher levels of inflammation, including higher white blood cell count (Surtees *et al.*, 2003), circulating pro-inflammatory cytokine levels and C-reactive protein (CRP) (Baumeister *et al.*, 2016). ELA also influences adulthood immune function through accelerated immunosenescence, which leads to characteristics seen in the aged immune system, such as increased susceptibility to infection and chronic diseases, such as

cardiovascular disease and type two diabetes mellitus, low-grade inflammation, and decreased NK cell cytotoxicity (Elwenspoek *et al.*, 2017). Indeed, numerous studies have found an association between ELA and shortened telomere length (Kananen *et al.*, 2010; Tyrka *et al.*, 2010; Kiecolt-Glaser *et al.*, 2011; Surtees *et al.*, 2011; Puterman *et al.*, 2016; Tyrka *et al.*, 2016). Individuals with a history of ELA have also been shown to have attenuated stress reactivity; the HPA and SAM axes are less responsive to acute stress (Lovallo *et al.*, 2012; Lovallo, 2013; Schwaiger *et al.*, 2016). Furthermore, individuals with adverse childhood experiences may engage in more risky health behaviours such as smoking, obesity, alcohol abuse, drug abuse, and sexual risk behaviour, which may mediate the relationship between ELA and disease susceptibility (Surtees *et al.*, 2003; Ramiro *et al.*, 2010). Indeed, individuals with a blunted stress response have more social and behavioural problems (Ouellet-Morin *et al.*, 2011) and increased adiposity (Carroll *et al.*, 2008). Finally, individuals with early life adverse experiences have been shown to have impaired immunity. Breast cancer survivors who experienced more childhood adversities had higher Epstein-Barr virus (EBV) and cytomegalovirus (CMV) antibody titers than those with fewer childhood adversities, indicating poorer cellular immune system control of the latent virus' (Fagundes *et al.*, 2013b). Individuals with a history of sexual or physical abuse had lower salivary SIgA levels (Waldron *et al.*, 2016). Interestingly, individuals who have experienced ELA may have more pronounced psychological changes when they encounter life stressors in adulthood. Women with a history of childhood sexual abuse were more likely to suffer from depression after a stressful life event in adulthood (Dougherty *et al.*, 2004). Furthermore, adults who were abused as children, or had a lower socioeconomic background, were more likely to perceive ambiguous situations as more threatening (Miller *et al.*, 2011). Indeed, individuals who have adverse childhood experiences may have fewer social and psychological resources

to manage stress, making encountering stress during adulthood more impactful on immune function (Fagundes *et al.*, 2011).

Currently, there is little empirical research linking ELA to URTI. However, a recent study provides initial evidence, showing that the offspring of parents who were separated and never spoke were more susceptible to common cold, compared to those whose parents were intact, or separated but still communicated (Murphy *et al.*, 2017). However, the role of early life adversity in URTI susceptibility and immune function in athletes is yet to be explored.

2.8 Thesis aims

A wide variety of factors influence human immune health, but empirical research examining the influence of these factors in athletes is lacking. With this information in mind, the objectives of this thesis were to investigate: 1) the influence of vitamin D on URTI burden and mucosal immunity (**Chapter 3**); 2) the role of psychosocial factors and sleep on URTI risk in marathon runners (**Chapter 4**); 3) the influence of psychological stress and anxiety on the mucosal immune response to exercise (**Chapter 5**).

CHAPTER THREE

Vitamin D Supplementation by Sunlight or Oral D_3 on Respiratory Infection Burden

3.1 Abstract

Purpose: To determine the relationship between vitamin D status and upper respiratory tract infection (URTI) in a prospective cohort study of active men and women across seasons (Study 1). Then, in a randomized, placebo-controlled trial, to investigate the effects on URTI and mucosal immunity of achieving vitamin D sufficiency (25(OH)D ≥50 nmol·L^{-1}) by a unique comparison of safe, simulated-sunlight or oral D_3 supplementation in winter (Study 2). **Methods:** Study 1, involved 1,644 military recruits. In Study 2, 249 men received either placebo, simulated-sunlight (1.3x standard erythemal dose, three-times-per-week for 4-weeks and then once-per-week for 8-weeks) or oral vitamin D_3 (1,000 IU·day^{-1} for 4-weeks and then 400 IU·day^{-1} for 8-weeks). URTI was diagnosed by physician (Study 1) and Jackson common cold questionnaire (Study 2). We measured serum 25(OH)D by LC-MS/MS and salivary secretory immunoglobulin A (SIgA) and cathelicidin by ELISA. **Results:** In Study 1, vitamin D sufficient participants were 40% less likely to suffer URTI than participants with 25(OH)D <50 nmol·L^{-1} (OR (95% CI) = 0.6 (0.4–0.9), $P < 0.05$); an association that remained after accounting for season and smoking. Each URTI resulted in 3 ± 3 days lost from training. Only 21% of recruits were vitamin D sufficient during winter. In Study 2, vitamin D supplementation strategies achieved vitamin D sufficiency in almost all (≥95%) and reduced the URTI burden compared to placebo: 15% lower URTI peak severity and 36% fewer days with URTI ($P < 0.05$). In recruits with low baseline 25(OH)D (<50 nmol·L^{-1}) vitamin D supplementation also shortened URTI duration by 33% ($P = 0.05$). Supplementation did not

affect SIgA or cathelicidin. **Conclusion:** Vitamin D sufficiency reduced URTI burden during arduous training.

3.2 Introduction

Athletes and military personnel experience arduous training, psychological stress, and nutritional inadequacy that may compromise host defense and increase their susceptibility to respiratory illness such as the common cold, particularly during the autumn-winter (Walsh *et al.*, 2011b; Walsh, 2018). The immunomodulatory effects of vitamin D are also considered to play a role in the seasonal stimulus for upper respiratory tract infections (URTI) (Ginde *et al.*, 2009; Walsh, 2019), which has fuelled considerable interest in the potential for prophylactic benefits of vitamin D supplementation on URTI; stoked further by studies reporting vitamin D sufficiency in less than half of all athletes and military personnel during the winter, when skin exposure to sunlight ultraviolet-B (UVB) radiation is negligible (Thomas *et al.*, 2016; Carswell *et al.*, 2018; Maughan *et al.*, 2018). Vitamin D is widely accepted to influence both innate and adaptive immunity with implications for host defense (Hewison, 2012; He *et al.*, 2016a). For example, 25(OH)D is converted in the kidney to the biologically active form $1,25(OH)_2D$, which enhances the innate immune response by the induction of antimicrobial proteins like cathelicidin. Antimicrobial proteins help to prevent URTI as part of the first line of defense. The actions of vitamin D on adaptive immunity may also be anti-inflammatory or 'tolerogenic' (Walsh, 2019). Immune tolerance is described as the ability to dampen defense yet control infection at a non-damaging level and has been proposed as a new focus for nutritional supplementation to reduce URTI burden (Ayres and Schneider, 2012). As such, maintaining vitamin D sufficiency (serum 25(OH)D $\geq$50 $nmol \cdot L^{-1}$) may reduce URTI burden, by preventing URTI (i.e. reducing URTI prevalence) or improving immune tolerance (i.e.

reducing URTI duration, or severity) (Thomas *et al.*, 2016; Maughan *et al.*, 2018; Walsh, 2019).

Large cross-sectional and randomized, placebo-controlled supplementation studies in the general population highlight that vitamin D shows promise in protecting against URTI (Ginde *et al.*, 2009; Bergman *et al.*, 2013; Martineau *et al.*, 2017). In contrast, cross-sectional studies in young healthy and athletic populations present conflicting findings (Laaksi *et al.*, 2007; Halliday *et al.*, 2011; He *et al.*, 2013), which might be explained by small samples with few URTI, a limited range of vitamin D status due to single-season data collections, and a lack of control for factors known to independently influence URTI (i.e. season and smoking). The findings from randomized, placebo-controlled trials investigating the influence of vitamin D supplementation on respiratory infection and immune responses in active persons are extremely limited and also present a mixed picture (Laaksi *et al.*, 2010; He *et al.*, 2016b; Jung *et al.*, 2018; Scott *et al.*, 2019), showing reduced URTI symptoms (Jung *et al.*, 2018), improved mucosal immunity (i.e. cathelicidin and IgA) (He *et al.*, 2016b; Scott *et al.*, 2019), and less missed training days due to URTI (Laaksi *et al.*, 2010), as well as, no effect on URTI symptoms (Laaksi *et al.*, 2010) or mucosal immunity (Jung *et al.*, 2018; Scott *et al.*, 2019). The significant heterogeneity reported in these trials may stem from variations in participant baseline vitamin D status and dosing regimens; these factors are considered to modify the effect of vitamin D on immunity to respiratory pathogens (Martineau *et al.*, 2017). The participants in these studies (Laaksi *et al.*, 2010; He *et al.*, 2016b) were vitamin D sufficient at baseline (Laaksi *et al.*, 2010; He *et al.*, 2016b), and studies gave greater than current IOM and EFSA recommended oral vitamin D doses (He *et al.*, 2016b; Jung *et al.*, 2018); which likely limits the need and potential benefit of vitamin D supplementation (Maughan *et al.*, 2018), and raises the risk of adverse outcomes (tolerable upper intake 4000 $IU \cdot d^{-1}$), respectively (Institute of Medicine, 2011; European Food Safety Authority, 2016). The

answer to whether vitamin D supplementation has measurable and meaningful effects on URTI in healthy and active populations is unclear but eagerly awaited, given the negative impact of URTI on training and performance (Thomas *et al.*, 2016; Maughan *et al.*, 2018; Walsh, 2018). Vitamin D can be obtained from dietary sources but is primarily synthesized by skin exposure to sunlight UVB radiation (He *et al.*, 2016a). UV radiation has a range of vitamin D-dependent and -independent effects on immunity (Hart *et al.*, 2019). However, whether there are additional benefits of safe sunlight exposure, compared to oral vitamin D supplementation, is unknown.

With this information in mind, we first determined the relationship between vitamin D status and URTI prevalence in a large, prospective cohort study of young men and women commencing military training across all seasons (Study 1). We hypothesized that fewer vitamin D sufficient individuals would suffer URTI, compared to individuals who had serum 25(OH)D <50 nmol·L^{-1}. Then, in a randomized, placebo-controlled trial (Study 2), we investigated the effects, on URTI burden (prevalence, duration, and severity) and mucosal immunity, of achieving vitamin D sufficiency by either simulated sunlight, following recommendations on safe, low-level sunlight exposure (Advisory Group on Non-ionising Radiation, 2017), or oral D_3 supplementation, in wintertime. In study 2, we also assessed URTI duration and severity to examine the potential beneficial tolerogenic effects of vitamin D on URTI. Vitamin D sufficiency was targeted because maintaining serum 25(OH)D concentration ≥50 nmol·L^{-1} has been recommended for multiple health outcomes by the Institute of Medicine (IOM) and European Food Safety Authority (EFSA) and is achievable using safe doses (Institute of Medicine, 2011; European Food Safety Authority, 2016). We hypothesized that achieving vitamin D sufficiency during winter by vitamin D supplementation would reduce URTI burden, and improve mucosal immunity, compared to placebo supplementation.

3.3 Methods

These studies received ethics approval from the UK Ministry of Defence Research Ethics Committee that was conducted following the Declaration of Helsinki (2013), and are registered at www.clinicaltrials.org [NCT02416895, NCT03132103]. British Army recruit volunteers participated in Study 1 and Study 2 after providing fully informed written consent and passing a clinician-screened medical assessment. Men (Study 1 and Study 2) were located at Infantry Training Centre Catterick, UK (latitude 54°N), and women (Study 1) were located at Army Training Centre Pirbright, UK (latitude 51°N). All volunteers were studied during 12 weeks of Basic Military Training that follows a syllabus of basic military skills including physical training, weapon handling, map reading, and fieldcraft. The progressive, structured, physical training program included: endurance training, circuit training, agility-based gymnasium work, assault course practice, and marching with a load.

3.3.1 Study one

3.3.2 Participants and study design

1,644 men and women (n = 1,220 men: 95% white ethnicity, age 21 ± 3 years; body mass 75.3 ± 9.9 kg, height 1.77 ± 0.06 m, BMI 24.0 ± 2.7 $kg \cdot m^{-2}$, 38% smokers, n = 424 women: 95% white ethnicity, age 22 ± 3 years, body mass 64.8 ± 8.2 kg, height 1.65 ± 0.06 m, BMI 23.7 ± 2.4 $kg \cdot m^{-2}$, 24% smokers) participated in this prospective cohort study between January 2014 and September 2015. Participants were included if they gave baseline blood samples and URTI data throughout military training.

3.3.3 Experimental procedures

We collected baseline measures from each participant during initial medical assessment; including a venous blood sample for determination of serum 25(OH)D; height

and body mass; ethnicity and smoking history by questionnaire (Figure 3.1). Medical records were accessed to obtain data on physician-diagnosed URTI and lost training days due to URTI. A lost training day denoted a recruit was unavailable for normal military training.

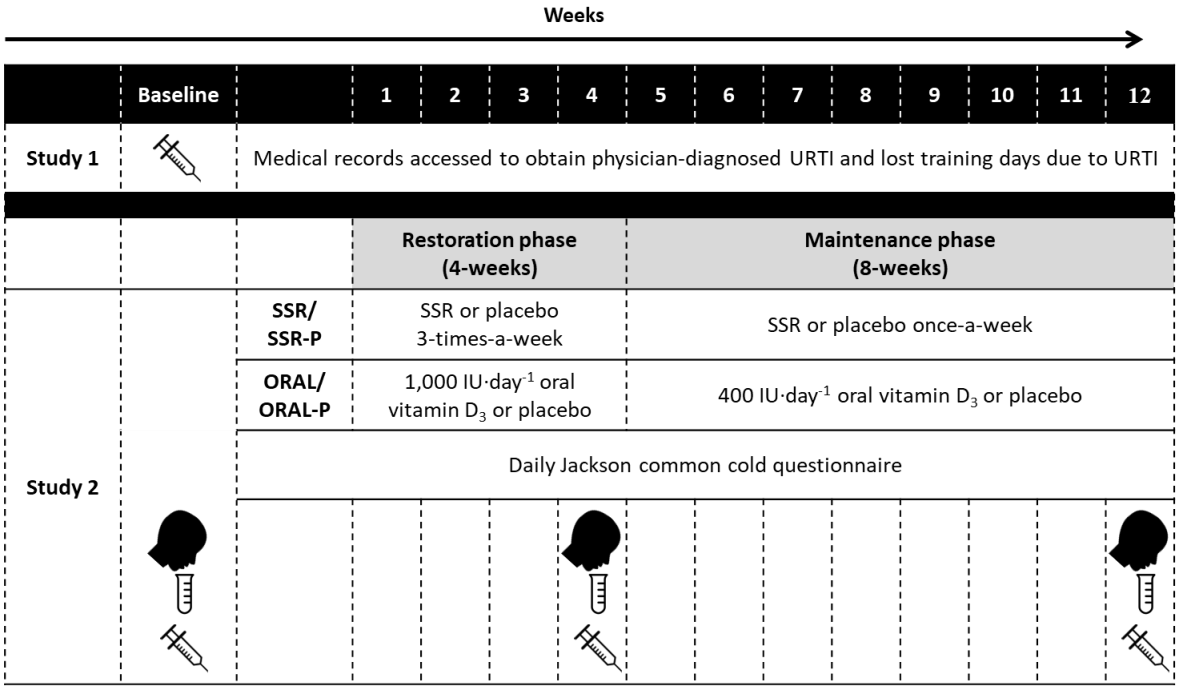


Figure 3.1. A schematic of the prospective cohort study (Study 1) that investigated the association between vitamin D status (serum 25(OH)D), upper respiratory tract infection (URTI) and days lost from training, and a randomized controlled trial (Study 2) that investigated the effects of vitamin D supplementation by solar-simulated radiation (SSR), oral vitamin D_3 (ORAL), or placebo (SSR-P or ORAL-P) on URTI and mucosal immune function. Blood and saliva samples were collected at baseline (Study 1 and 2), week 5, and the end of week 12 (Study 2). The syringe icon represents the blood sample; the head and tube icon represents the saliva sample.

3.3.4 Study two

3.3.5 Participants and study design

249 men (age 22 ± 7 years, body mass 76.3 ± 10.8 kg, height 1.77 ± 0.06 m, BMI 24.2 ± 3.0 $kg \cdot m^{-2}$) volunteered and gave written informed consent to participate in a double-blind, randomized, placebo-controlled trial, gave a baseline blood sample and were assigned to one of four intervention groups (Figure 1). Participants were recruited at the start of 12 weeks of Basic Military Training during January and February of 2016 and 2017; when ambient UVB is negligible at UK latitudes (50–60°N), and serum 25(OH)D is at its annual nadir. Participants were excluded if they were >4 on the Fitzpatrick Skin Type Scale (Fitzpatrick, 1988), used sunbeds, consumed supplements containing vitamin D, or had travelled to a hot sunny climate in the previous three months. There was no difference between treatment or control groups in demographics, anthropometrics, and serum total 25(OH)D at baseline (Table 3.1).

Table 3.1. Study 2 baseline participant demographics, anthropometrics and lifestyle behaviors in solar simulated radiation (SSR), SSR placebo (SSR-P), oral vitamin D_3 (ORAL) and oral placebo (ORAL-P) supplemented groups.

	SSR	SSR-P	ORAL	ORAL-P
Demographics				
Age (years)	21 ± 3	22 ± 3	21 ± 3	23 ± 12
Ethnicity (Caucasian) [*n* (%)]	61 (98)	57 (97)	63 (100)	65 (100)
Skin type (I, II, III, IV) [*n* (%)]	4 (7), 16 (26), 33 (53), 9 (15)	4 (7), 16 (27), 28 (48), 11 (19)	5 (8), 18 (29), 33 (52), 7 (11)	3 (5), 19 (29), 29 (45), 14 (22)
Anthropometrics				
Height (m)	1.78 ± 0.06	1.78 ± 0.06	1.77 ± 0.07	1.78 ± 0.06
Body mass (kg)	76 ± 11	77 ± 11	75 ± 11	77 ± 10
BMI (kg/m^2)	24 ± 3	24 ± 3	24 ± 3	24 ± 3
Lifestyle behaviors				
Alcohol user [*n* (%)]	51 (82)	47 (80)	55 (87)	51 (78)
Smoker [*n* (%)]	23 (37)	25 (42)	26 (41)	21 (32)

Data are presented as mean ± SD unless otherwise stated. There were no differences in demographics, anthropometrics, or lifestyle behaviors between groups ($P > 0.05$).

3.3.6 Experimental procedures

Participants were randomized within platoons to one of four intervention groups: 1) oral vitamin D_3 supplementation (ORAL); 2) oral placebo supplementation (ORAL-P); 3) solar simulated radiation (SSR); or, 4) solar simulated radiation placebo (SSR-P). We used block randomization (by www.randomiser.org) to achieve equal distribution of intervention groups within each platoon so any differences in training conditions between platoons did not influence the outcomes of the study. The intervention strategy for the SSR and ORAL groups was to restore and then maintain IOM and EFSA recommended vitamin D sufficiency (serum 25(OH)D ≥50 $nmol \cdot L^{-1}$). Participants completed a 4-week restoration phase, necessary because serum 25(OH)D was at its annual wintertime nadir, followed by an 8-week

maintenance phase. Height and body mass were measured during the routine initial medical assessment. Participants filled in a Lifestyle Questionnaire that included information on supplementation use, sunbed use, and outdoor sun exposure. A venous blood sample was collected for the determination of serum vitamin D metabolites at baseline, week 5, and week 12. Participants were excluded from analysis if they did not achieve ≥80% compliance with the intervention. Vitamin D from the diet was estimated in week 12 using a food frequency questionnaire, and solar UVR exposure was measured in weeks 4 and 11 using polysulphone badges, worn on the upper chest/anterior shoulder region on the outer clothes, as described (Webb *et al.*, 2010; Carswell *et al.*, 2018). The change in absorbance of the badges due to exposure was measured using a spectrophotometer and related to the erythemal effective UVR (sunburning) through a standard polynomial relationship; data are expressed as standard erythemal dose (SED) per day (Webb *et al.*, 2010). Participant dietary vitamin D intake was calculated excluding the oral D_3 supplement participants received in the ORAL group. On completion of the study, participants were asked to guess the intervention they had received.

3.3.7 Simulated sunlight intervention

Simulated sunlight was provided following guidelines on safe, low-level sunlight exposure for vitamin D synthesis (Scientific Advisory Committee on Nutrition, 2016); described previously to achieve serum 25(OH)D ≥50 nmol·L^{-1} in the majority of white skinned persons (Rhodes *et al.*, 2010). Those assigned to the SSR intervention were exposed three-times-a-week during the restoration phase to an experimenter-controlled constant UVR dose using a whole body irradiation cabinet (Hapro Jade, Kapelle, The Netherlands) fitted with Arimed B fluorescent tubes (Cosmedico, Stuttgart, Germany). The fluorescent tubes emitted a UVR spectrum similar to sunlight (λ: 290–400 nm; 95% UVA: 320–400 nm, 5% UVB: 290–320 nm) that was characterized by a spectroradiometer (USB2000+, Ocean Optics BV, Duiven, The Netherlands) radiometrically calibrated with traceability to UK national

standards. During each exposure, participants received a 1.3x SED whilst wearing shorts and a T-shirt to expose ~40% skin surface area. This dose is equivalent to ~15 minutes, midday summer sun exposure six-times-per-week for a casually dressed individual in northern England (latitude 53.5°N) (Rhodes *et al.*, 2010). This dose can be related to exposure times at other world locations (Webb *et al.*, 2011). For example, the equivalent exposure times in Philadelphia, Pennsylvania, USA (40°N), and Oslo, Norway (60°N) would be approximately 12 and 18 minutes, respectively. During the maintenance phase, we exposed SSR participants to the same 1.3x SED dose only once-a-week: pilot investigations confirmed the required dose to maintain sufficiency (serum 25(OH)D ≥50 nmol·L^{-1}). A constant SSR dose was maintained during the study by monitoring irradiance using a spectroradiometer (USB2000+, Ocean Optics BV) and adjusting for any decrease in measured irradiance emitted by increasing exposure time, as described (Rhodes *et al.*, 2010) (mean duration of SSR exposures was 222 ± 23 s). We controlled the exposure time by using an electronic timer on the irradiation cabinet. For the SSR-P participants the number of intervention exposures each week and the exposure duration were the same as SSR except the irradiation cabinet fluorescent tubes were covered with transparent UVR blocking film (DermaGard UV film, SunGard, Woburn, Massachusetts, USA). A spectroradiometer confirmed the UVR blocking film was effective at preventing transmission of 99.9% of UVR.

3.3.8 Oral vitamin D_3

Participants receiving the ORAL intervention consumed a vitamin D_3 capsule daily, containing 1,000 IU and 400 IU during the restoration and maintenance phases, respectively (Pure Encapsulations, Sudbury, Massachusetts, USA). The restoration dose was based on previous predictive modeling to achieve serum 25(OH)D ≥50 nmol·L^{-1} (Cashman *et al.*, 2008), and pilot investigations that showed it achieved similar serum 25(OH)D concentrations to SSR; and was less than the tolerable upper intake recommended by the

IOM and EFSA (Institute of Medicine, 2011; European Food Safety Authority, 2016). The ORAL maintenance dose was also in agreement with recommendations (Institute of Medicine, 2011; Scientific Advisory Committee on Nutrition, 2016). For 12 weeks, ORAL-P participants consumed an identical-looking cellulose placebo capsule daily (Almac Group, County Armagh, UK). Independent analysis found the vitamin D_3 content of the 1,000 and 400 IU capsules to be 1,090 and 460 IU, respectively, and confirmed the placebo did not contain vitamin D (NSF International Laboratories, Ann Arbor, Michigan, USA).

3.3.9 Blood collection and analysis

In studies 1 and 2, whole blood samples were collected by venipuncture from an antecubital vein into plain vacutainer tubes (Becton Dickinson, Oxford, UK) and left to clot for 1 hour. Subsequently, samples were centrifuged at 1500 *g* for 10 minutes at 4°C and the serum aliquoted into universal tubes before being immediately frozen at -80°C for later analysis. Total serum 25(OH)D was measured with high-pressure liquid chromatography-tandem mass spectrometry (Tang *et al.*, 2017). Analyses were performed in a Vitamin D External Quality Assurance Scheme certified laboratory (Bioanalytical Facility, University of East Anglia, Norwich, UK).

3.3.10 URTI diagnosis

As in Study 1, medical records were accessed to obtain data on physician-diagnosed URTI and lost training days due to URTI. However, in study 2 due to the smaller sample size and consequently the anticipated smaller number of total URTI, we chose to monitor URTI principally by self-reported daily symptoms of the common cold over the 12-week training period (Study 2), using the Jackson common cold questionnaire (Jackson *et al.*, 1958). A strength of the Jackson common cold questionnaire compared to physician-diagnosed URTI is that URTI duration and severity, as well as prevalence, can be assessed. Participants were

asked to rate eight symptoms (sneezing, headache, feeling generally unwell, runny nose, blocked nose, sore throat, cough, chilliness) on a 4-point Likert scale (Not at all = 0, Mild = 1, Moderate = 2, Severe = 3). Data were included when participants completed ≥80% of their daily Jackson questionnaires. A URTI was defined by a total symptom score of greater than or equal to six each day for two or more consecutive days (Hanstock *et al.*, 2016). For each URTI we determined the duration with symptoms (URTI average duration) and the peak severity of symptoms (URTI peak severity). We also calculated the total number of days with a URTI during basic training for each participant (total days with URTI).

3.3.11 Saliva collection and analysis

In study 2, at baseline, week 5 and week 12 **s**aliva samples were collected in the evening, between 6:00 and 9:30 pm, at least 15 minutes postprandial. Saliva was collected for 5 min in a pre-weighed 30 ml tube using the passive dribble method. Samples were weighed immediately after collection, centrifuged at 1500 *g* and 4°C for 10 minutes, aliquoted and then stored at -80°C. Samples were analyzed by enzyme-linked immunosorbent assay for secretory immunoglobulin-A (SIgA) and cathelicidin concentration (Salimetrics, Pennsylvania, USA, and Hycult Biotech, Pennsylvania, USA). The mean intra-assay CV was 2.3% and 10.2%, respectively. Assuming the density to be 1.00 $g \cdot mL^{-1}$ for saliva, the secretion rate was calculated by multiplying the flow rate by concentration, as described previously (Oliver *et al.*, 2007).

3.3.12 Statistical analysis

For Study 1, we estimated a minimum required sample size of 1,286, using a type 1 error (one tailed) of 5%, a power of 80% and an anticipated odds ratio of 1.5, equivalent to a small effect size. This was based on previous literature describing the difference in URTI prevalence between individuals with low and high vitamin D status where 20% of individuals

with high vitamin D status reported a URTI (Ginde *et al.*, 2009), whilst also anticipating that 20% of individuals would have low vitamin D status across the whole year (Davies and Shaw, 2011). We used logistic regression to compare vitamin D status (25(OH)D ≥50 vs <50 nmol·L^{-1} and ≥75 vs <30, ≥50–<75 and <75 nmol·L^{-1}) with URTI prevalence during 12-weeks military training, and the first three weeks of military training; 25(OH)D has a 2–3 week half-life (Holick, 2009). Sex and smoking were included as covariates as they are known to influence URTI susceptibility. We used Chi-square tests to compare URTI prevalence between vitamin D sufficient participants and those with serum 25(OH)D <50 nmol·L^{-1}, and the proportion of vitamin D sufficient participants between seasons. We used one-way ANOVA to compare vitamin D status (25(OH)D) between seasons. For study 2, we estimated a minimum required sample size of 37 in each group, using the anticipated odds ratio of 0.3 for URTI prevalence between vitamin D and placebo supplemented individuals with low vitamin D status (Martineau *et al.*, 2017), and that 60% would report a self-report URTI during basic military training (Laaksi *et al.*, 2007; Hanstock *et al.*, 2016; Davison *et al.*, 2020), with a type 1 error (one-tailed) of 5%, and a power of 80%. We compared URTI prevalence between vitamin D (SSR and ORAL) and placebo (SSR-P and ORAL-P) supplementation groups using logistic regression. We used independent samples *t*-tests (2 groups (SSR and ORAL combined, SSR-P and ORAL-P) to compare vitamin D and placebo supplementation effects on average URTI duration, total days with URTI, URTI peak severity, saliva flow rate, SIgA, and cathelicidin. Serum 25(OH)D, total days with URTI, URTI duration, URTI severity, saliva flow rate, SIgA, and cathelicidin were compared between vitamin D strategies, and placebo groups, by mixed-model ANOVA ((4 groups (SSR, ORAL, SSR-P, and ORAL-P) × 3-time points (baseline, week 5 and 12)). Sunlight exposure and dietary vitamin D between SSR, ORAL, SSR-P, and ORAL-P groups were compared by one-way ANOVA. Data points that were more than three-times the interquartile

range were deemed as outliers and removed. Where data was not normally distributed it was transformed using square-root calculation. Significance was set at $P < 0.05$. Statistical analyses were performed using SPSS Version 25 (IBM Corp, NY, US). Cohen's *d* effect sizes (*d*) are presented to indicate the meaningfulness of group differences for URTI duration, URTI severity, and total days with URTI; whereby, values greater than 0.2, 0.5, and 0.8 represent small, medium and large effects, respectively (Cohen, 1988).

3.4 Results

3.4.1 Study one

3.4.2 Low proportion of wintertime vitamin D sufficiency in healthy young men and women

Baseline serum 25(OH)D was lower in winter than all other seasons ($P < 0.01$, Figure 3.2A); when only 21% of participants were vitamin D sufficient (baseline serum 25(OH)D ≥50 nmol·L^{-1}; Figure 3.2B).

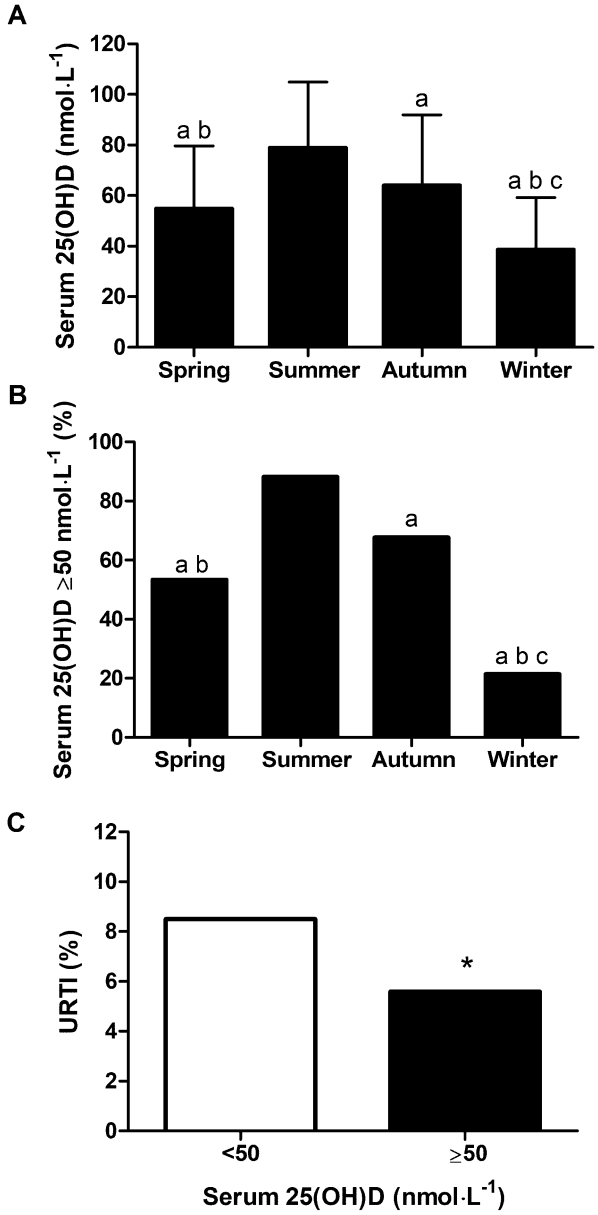


Figure 3.2. Seasonal variation in serum 25(OH)D (panel A), vitamin D sufficiency prevalence (serum 25(OH)D ≥50 nmol·L^{-1}; panel B), and the URTI prevalence when serum 25(OH)D ≥50 nmol·L^{-1} or <50 nmol·L^{-1} (panel C) in 1,644 men and women during 12-weeks of military training. a, lower than summer $P < 0.05$. b, lower than autumn $P < 0.05$. c, lower than spring $P < 0.05$, * lower than participants with serum 25(OH)D <50 nmol·L^{-1}, $P < 0.05$. Panel A data are mean ± SD. Panels B and C are percentages represented by vertical bars.

3.4.3 Vitamin D sufficiency associated with reduced URTI prevalence

A total of 110 URTI episodes were recorded with 7% of participants having at least one physician-diagnosed URTI. On average, each URTI resulted in 3.4 ± 3.3 lost training days (4.0% of total training days). Only six recruits had two URTI episodes and no recruits had more than two URTI episodes. Vitamin D sufficient participants at baseline were 40% less likely to have a physician-diagnosed URTI, during 12 weeks of training, than participants with baseline serum 25(OH)D <50 nmol·L^{-1} (6% vs 9%, respectively, OR (95% CI) = 0.6 (0.4–0.9), $P < 0.05$, Figure 3.2C). Moreover, vitamin D sufficient participants at baseline were half as likely to have a URTI within the first three weeks of training than participants with a baseline serum 25(OH)D <50 nmol·L^{-1} (2% vs 5%, OR (95% CI) = 0.5 (0.3–0.8), $P < 0.05$). This was during the period of training when approximately half of all URTI episodes occurred (47%, 52 URTI episodes). The association between vitamin D status and URTI prevalence remained when controlling for sex and smoking ($P < 0.05$). URTI prevalence was not different between participants with a baseline serum 25(OH)D ≥75 nmol·L^{-1} and baseline serum 25(OH)D of <30, ≥50–<75, or <75 nmol·L^{-1} ($P > 0.05$).

3.4.4 Study two

A flow diagram detailing the number of participants assessed, recruited, and excluded from the analysis is provided in Figure 3.3. During the 12-week intervention, daily sunlight exposure (0.35 ± 0.56 SED·d^{-1}) and dietary vitamin D were not different between groups (153 ± 136 IU·d^{-1}, $P > 0.05$). Participants were sufficiently blinded to the intervention since only 38% correctly guessed their allocated group, 27% were incorrect, and 34% said they did not know whether they had received an active or placebo intervention.

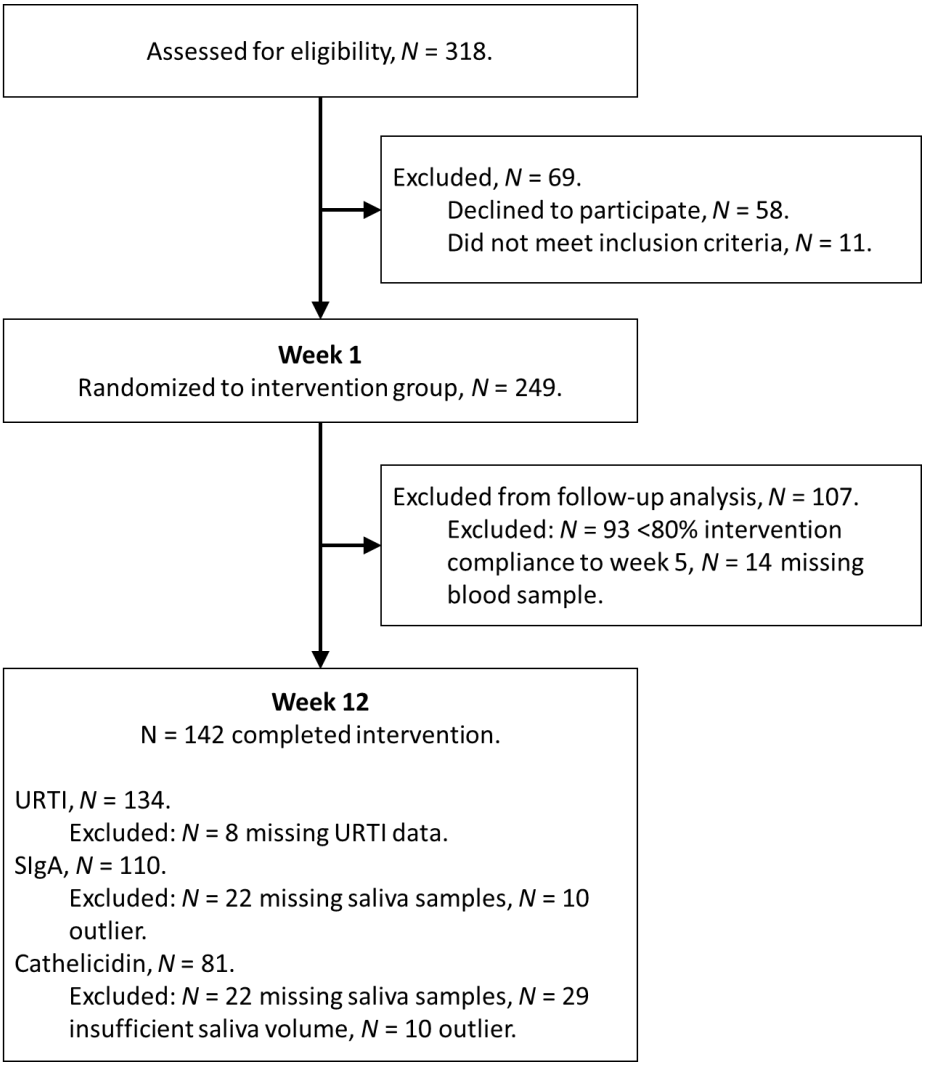


Figure 3.3. Flow diagram of the randomized controlled trial (Study 2) investigating the effects of vitamin D supplementation on upper respiratory tract infection (URTI) and mucosal immune function. Flow diagram indicates the number of participants assessed, randomized, and analyzed at week 12 for URTI, salivary secretory immunoglobulin A (SIgA), and cathelicidin.

3.4.5 Winter simulated sunlight and oral vitamin D_3 increased vitamin D sufficiency

At baseline, before wintertime vitamin D supplementation began, only one-quarter (27%) of volunteers were vitamin D sufficient. Both SSR and ORAL supplementation

strategies were successful to achieve vitamin D sufficiency in almost all (≥95%); week 5 and 12 serum 25(OH)D concentrations in the SSR and ORAL groups were higher than in the respective placebo groups ($P < 0.001$, Figure 3.4).

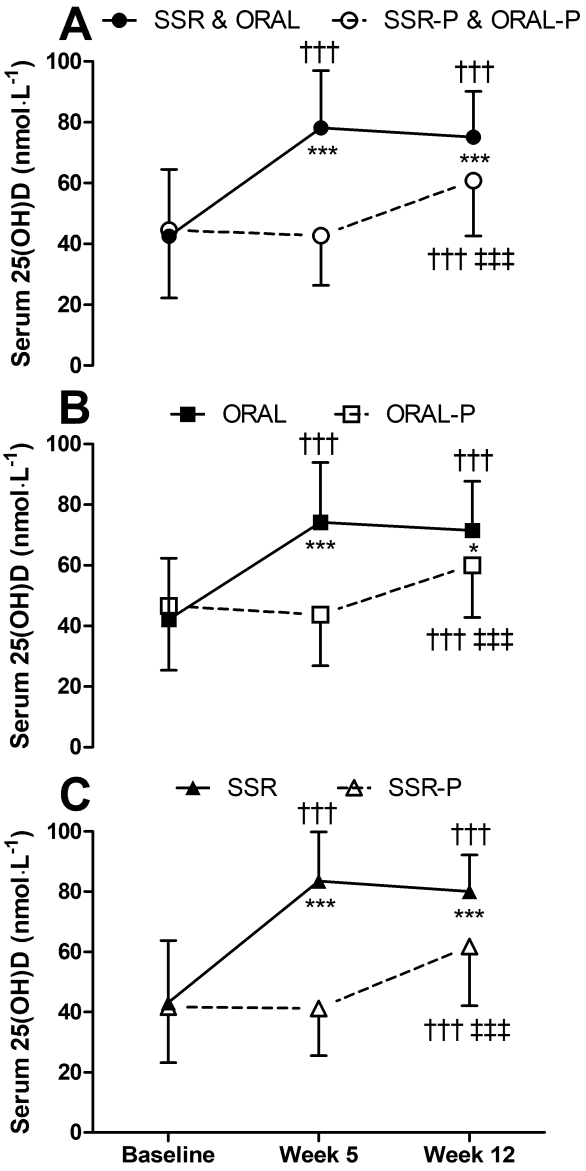


Figure 3.4. Serum 25(OH)D in men completing military training whilst receiving 12-weeks of vitamin D supplementation (solar simulated radiation (SSR) or oral vitamin D_3 (ORAL)) or a placebo (solar simulated radiation placebo (SSR-P) or oral placebo (ORAL-P)). Combined vitamin D interventions (SSR and ORAL) vs combined placebo (SSR-P and ORAL-P; panel A), ORAL vs ORAL-P (panel B), and SSR vs SSR-P (panel C). *, greater than placebo, $P < 0.05$. †, greater than baseline, $P < 0.05$. ‡, greater than week 5, $P < 0.05$. Data are mean ± SD.

3.4.6 Winter vitamin D supplementation reduced URTI burden

A total of 93 Jackson-defined URTI episodes were recorded with 69% of participants having at least one self-reported URTI. The URTI prevalence and duration were similar in vitamin D and placebo supplementation groups (ORAL and SSR vs ORAL-P and SSR-P 71% vs 68%, Figure 3.5A, $P > 0.05$). However, winter vitamin D supplementation reduced URTI burden compared to placebo: participants had 15% lower URTI peak severity ($P < 0.05$; Figure 3.5B), and 36% fewer total days with a URTI ($P < 0.05$; Figure 3.5C). Moreover, participants beginning supplementation with serum 25(OH)D <50 nmol·L^{-1} had 33% shorter average URTI duration ($P = 0.05$; Figure 3.5D), 21% lower URTI peak severity ($P < 0.05$; Figure 3.5E) and 43% fewer total days with URTI ($P < 0.05$; Figure 3.5F), when receiving vitamin D rather than placebo supplementation. There was no difference in URTI prevalence, duration, severity or total days with URTI between vitamin D supplementation strategies, or between the different placebo groups ($P > 0.05$). Specifically, the ORAL and SSR vitamin D supplementation strategies effect on URTI burden was similar (ORAL vs SSR, URTI prevalence 70% vs 72%, total days with URTI 9.2 ± 8.4 vs 8.4 ± 6.7 days, URTI average duration 6.9 ± 5.0 vs 6.5 ± 5.7 days, URTI peak severity 10.8 ± 3.0 vs 12.3 ± 3.8 AU, all $P > 0.05$). A physician-diagnosed URTI was recorded for 8% of recruits (10 participants), which was comparable to 8% prevalence in the same seasonal period in Study 1, and resulted in 3.3 ± 1.3 training days lost.

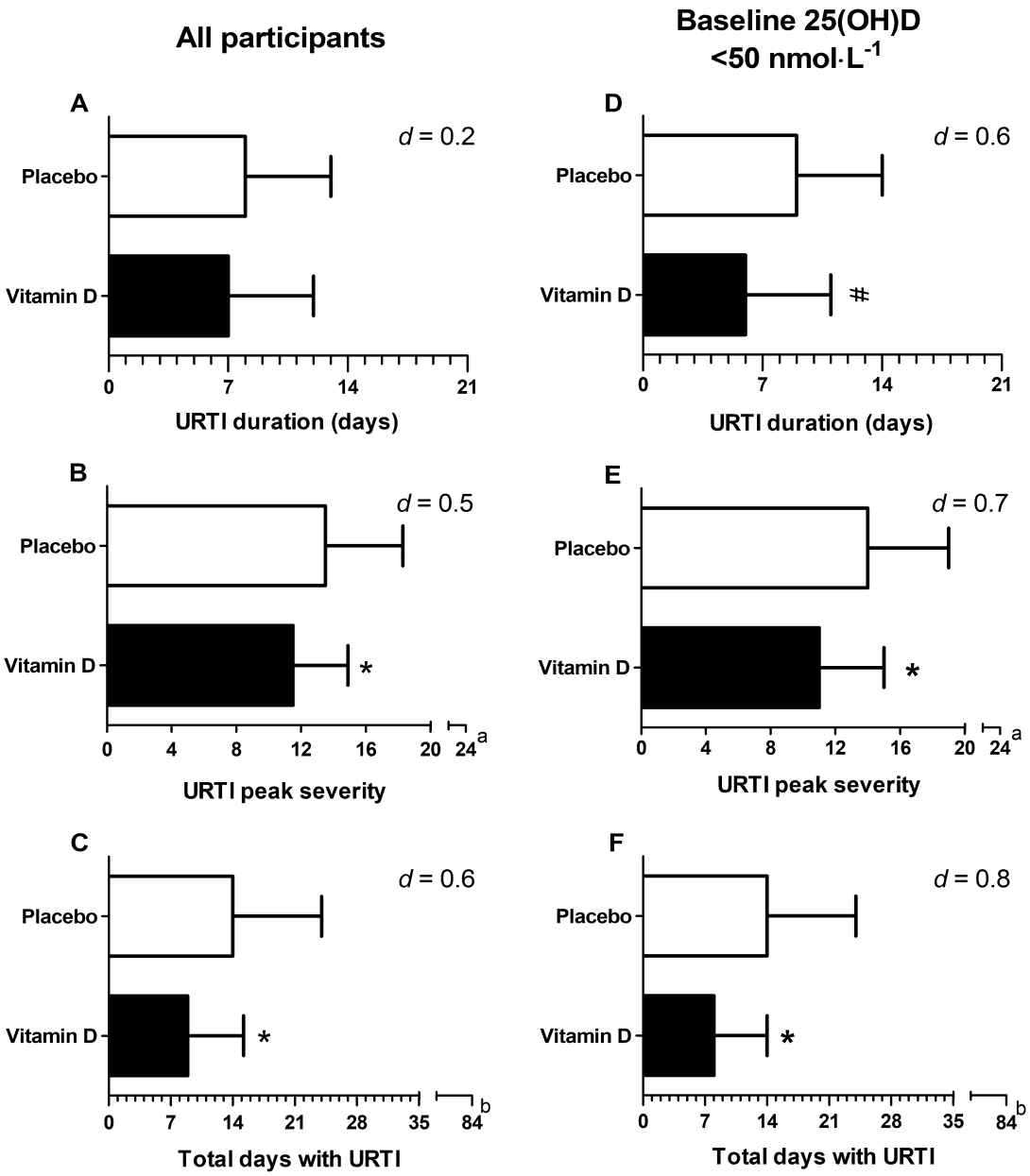


Figure 3.5. Upper respiratory tract infection duration (URTI; panel A & D), URTI peak severity (panel B & E), and total days with URTI during military training (panel C & F), in the vitamin D supplementation (SSR and ORAL) vs placebo supplementation groups (SSR-P and ORAL-P) in all participants (left-hand column) and participants with a baseline 25(OH)D <50 nmol·L^{-1} (N = 62; right-hand column). * and #, lower than placebo, $P < 0.05$ and $P = 0.05$, respectively. Data are mean ± SD. d = Cohen's d effect size. [a] maximum possible peak severity (24 arbitrary units (AU)), [b] total number of days for military training (84 days).

3.4.7 Vitamin D supplementation and mucosal immunity

Vitamin D supplementation and placebo groups were similar at baseline, and weeks 5 and 12 for saliva FR, SIgA concentration, SIgA SR, cathelicidin concentration, and cathelicidin SR ($P > 0.05$; Table 3.2).

Table 3.2. Influence of 12-weeks solar simulated radiation (SSR), placebo solar simulated radiation (SSR-P), oral vitamin D_3 (ORAL), and oral placebo (ORAL-P) on saliva flow rate (FR), SIgA concentration, SIgA secretion rate (SR), and cathelicidin concentration and cathelicidin SR.

		SSR	SSR-P	ORAL	ORAL-P
FR ($\mu l \cdot min^{-1}$)	Baseline	205 ± 128	184 ± 181	260 ± 214	241 ± 173
	ΔBaseline to week 5	+5 ± 124	+26 ± 160	-36 ± 159	-5 ± 208
	ΔBaseline to week 12 † ‡	+69 ± 125	+124 ± 207	+24 ± 243	+64 ± 201
SIgA concentration ($mg \cdot ml^{-1}$)	Baseline	0.14 ± 0.08	0.12 ± 0.06	0.13 ± 0.06	0.12 ± 0.05
	ΔBaseline to week 5 †	+0.01 ± 0.08	+0.04 ± 0.09	+0.02 ± 0.09	+0.02 ± 0.07
	ΔBaseline to week 12 †	+0.00 ± 0.05	+0.03 ± 0.06	+0.03 ± 0.1	+0.03 ± 0.09
SIgA SR ($\mu g \cdot min^{-1}$)	Baseline	27 ± 17	18 ± 11	26 ± 19	25 ± 17
	ΔBaseline to week 5	-2 ± 22	+12 ± 16	+1 ± 18	+1 ± 20
	ΔBaseline to week 12 † ‡	+9 ± 16	+25 ± 31	+10 ± 22	+14 ± 24
Cathelicidin concentration ($\mu g \cdot L^{-1}$)	Baseline	14 ± 11	14 ± 14	13 ± 13	12 ± 11
	ΔBaseline to week 5	-8 ± 16	+6 ± 18	-2 ± 10	-1 ± 15
	ΔBaseline to week 12	-5 ± 14	+1 ± 19	-4 ± 16	-1 ± 17
Cathelicidin SR ($ng \cdot min^{-1}$)	Baseline	3.25 ± 3.04	1.69 ± 1.91	2.42 ± 2.28	3.13 ± 4.79
	ΔBaseline to week 5	-0.82 ± 3.82	+0.96 ± 1.81	-0.54 ± 1.78	-1.35 ± 4.25
	ΔBaseline to week 12	-0.70 ± 4.10	+2.15 ± 3.61	+0.14 ± 2.45	-0.64 ± 5.60

Main effect of time vs baseline, † $P < 0.05$. Main effect of time vs week 5, ‡ $P < 0.05$. Data are mean ± SD. Saliva SIgA (N = 110), and saliva cathelicidin (N = 81).

3.5 Discussion

The principal finding of these two studies was that vitamin D sufficiency reduced the burden of URTI in healthy young adults completing arduous training. In Study 1, in 1,644 young, healthy men and women, we demonstrated that vitamin D sufficient men and women were 40% less likely to suffer a physician-diagnosed URTI during training than those with serum 25(OH)D of <50 nmol·L^{-1} (Figure 3.2). This finding can be considered robust as it was observed after accounting for sex and smoking, which is a strength of Study 1 when compared to previous research that has not controlled for factors known to independently influence URTI (Laaksi *et al.*, 2007; Halliday *et al.*, 2011; He *et al.*, 2013). We also showed the association between baseline vitamin D status and URTI was stronger during the first three, rather than twelve, weeks of military training, which might be expected as 25(OH)D has a two to three-week half-life (46). Study 1 extends previous vitamin D and URTI understanding in active populations by collecting data in a large sample, throughout all seasons, and including a full range of serum 25(OH)D. The burden of URTI was evident in Study 1 as each URTI resulted in 3 days of missed training. Given these findings, and that only 21% of participants were vitamin D sufficient during winter, in Study 2 we examined the effect of winter vitamin D supplementation on URTI prevalence and mucosal immunity. To additionally examine the potential beneficial tolerogenic effects of vitamin D on URTI in Study 2, we used the Jackson self-report URTI questionnaire to monitor URTI duration and severity, as well as prevalence.

Study 2 was a double-blind, randomized, placebo-controlled trial, involving a unique comparison of safe, simulated, casual skin sunlight exposure and oral vitamin D_3 supplementation, specifically designed to achieve vitamin D sufficiency. We found that vitamin D supplementation by simulated-sunlight and oral D_3 were similarly effective to achieve IOM and EFSA recommended vitamin D sufficiency in most persons (≥95%, Figure

3). Vitamin D supplementation did not reduce the self-reported URTI prevalence or benefit mucosal immunity compared to the placebo (Table 2). However, in line with our hypothesis, vitamin D supplementation reduced URTI burden compared to placebo, which broadly agrees with the majority of the limited previous research in this area (Laaksi *et al.*, 2010; Jung *et al.*, 2018), i.e. vitamin D supplementation reduced URTI symptoms (Jung *et al.*, 2018), and absence from duty due to respiratory infection (Laaksi *et al.*, 2010). Indeed, in Study 2, participants receiving vitamin D reported 15% lower URTI peak severity and 36% fewer days with URTI compared to placebo (Figure 3.5). These reductions in URTI burden can be considered meaningful as effect sizes were medium to large. Study 2 findings are notable as they highlight that vitamin D supplementation may reduce URTI burden, rather than prevent URTI. Indeed, vitamin D supplementation did not influence the innate mucosal antimicrobial proteins SIgA and cathelicidin, which form an important part of the first line of defense that prevents URTI. Instead, the benefits of vitamin D supplementation on URTI burden in Study 2 may be attributable to the known anti-inflammatory effects of vitamin D, which have been shown to inhibit inflammatory cytokines and signalling pathways (Krishnan and Feldman, 2011). Future research investigating vitamin D supplementation effect on URTI tolerance and circulating inflammatory cytokine is needed (Walsh, 2019). Based on the current study findings it is reasonable to hypothesize that vitamin D may improve immune tolerance by limiting inflammation in response to an infection (i.e. controlling infection at a non-damaging level), which subsequently leads to a reduction in self-report severity and duration of the URTI (Ayres and Schneider, 2012).

In Study 1 and 2, we confirmed URTI by different methods, which might be considered a limitation. However, when considered carefully, the different methods provide a fuller understanding of vitamin D's effect on URTI, than when either study findings are considered in isolation. Indeed, Study 1 and 2 findings are largely complementary, supporting

the notion that vitamin D sufficiency, compared to a serum 25(OH)D <50 nmol·L^{-1}, reduced the burden of URTI on the recruit, and military medical and training services (Figures 3.2 and 3.5). Specifically, improved URTI tolerance with vitamin D supplementation in Study 2 would be expected to translate to vitamin D sufficient individuals reporting less to medical services, and consequently having fewer physician-diagnosed URTI than those individuals with 25(OH)D <50 nmol·L^{-1}, which is entirely consistent with the main finding of Study 1 (Figure 3.2): URTI prevalence was lower in vitamin D sufficient individuals than those with 25(OH)D <50 nmol·L^{-1}. The disparity in prevalence between Study 1 and 2 indicate that the different URTI methods probably identify URTI at different ranges of severity (Study 1 physician URTI 7%, Study 2 physician URTI 8% vs 69% self-report URTI). The overall lower URTI prevalence in Study 1 compared to Study 2 suggests a physician-diagnosed URTI in Study 1 was only identified after symptoms became intolerable and the individual sought medical help, whereas, in Study 2, daily self-report enabled a fuller range of mild to severe URTI to be identified. That individuals tolerated a URTI in Study 1 before reporting to medical services is also evident in the longer self-reported URTI duration in Study 2 than training days lost to physician-diagnosed URTI in Study 1 (8 ± 8 vs 3 ± 3 days, respectively). In summary, vitamin D sufficiency, compared with 25(OH)D <50 nmol·L^{-1}, did not reduce the prevalence of self-reported URTI but did lower self-reported URTI severity and days with URTI (Figure 3.5), which likely improved tolerance to URTI that consequently lead to reduced physician-diagnosed URTI (Figure 3.2). A limitation of Study 1 and 2 is that we did not assess the pathology of the URTI, which would help to confirm the presence of a pathogen and URTI prevalence findings. However, previous research has shown that pathogens were confirmed in 82% of URTI identified using the same daily self-report Jackson common cold questionnaire method as in this study (Hanstock *et al.*, 2016). Replacing self-report with pathogen recognition would also create methodological

limitations, for example, the assessment of URTI severity would not be possible. Therefore, future research should consider a blended approach in the assessment of URTI.

Currently, there is no consensus for the optimal vitamin D threshold or dose for immune health (He *et al.*, 2016a). Participants beginning supplementation with serum 25(OH)D <50 nmol·L^{-1} reported shorter URTI duration when receiving vitamin D compared to placebo supplementation. Further evidence that participants with serum 25(OH)D <50 nmol·L^{-1} benefitted more from vitamin D supplementation than the entire sample is clear when examining the effect sizes between vitamin D and placebo for URTI outcomes; small-medium effect sizes for the entire sample, compared to medium and large effect sizes for participants with serum 25(OH)D <50 nmol·L^{-1} (Figure 3.5). Alongside other findings from this research program that show benefits of vitamin D sufficiency on *in vivo* immunity (Kashi *et al.*, 2020), Study 1 and 2 findings support IOM and EFSA recommended vitamin D threshold for bone and general health (25(OH)D ≥50 nmol·L^{-1}). Compared to IOM and EFSA recommended vitamin D sufficiency, we revealed no additional protection from URTI of higher vitamin D status, including a previously proposed optimal threshold (serum 25(OH)D >75 nmol·L^{-1}) (Holick *et al.*, 2011). Although recent research, including from this research program, questions a direct benefit of vitamin D on exercise performance (Carswell *et al.*, 2018), the current studies highlight that exercise performance may indirectly benefit from maintaining vitamin D sufficiency by reducing lost training days to URTI. Indeed, research has found that athletes who have less URTI are more likely to perform better in a sporting competition (Svendsen *et al.*, 2016). These findings are timely as the recent IOC and American College of Sports Medicine nutrition and athletic performance position stands highlight vitamin D insufficiency is widespread in athletes, and that vitamin D has the potential to influence exercise performance and training (Thomas *et al.*, 2016; Maughan *et al.*, 2018). Study 2 is the first to demonstrate the benefits of vitamin D supplementation, in

line with IOM and EFSA guidelines, on URTI in an active population. We have shown no additional benefit of SSR compared to oral vitamin D_3 supplementation on URTI, immune function (this study and (Kashi *et al.*, 2020)), or exercise performance (Carswell *et al.*, 2018). Therefore, we advise the 400 $IU \cdot d^{-1}$ oral vitamin D_3 dose, from the maintenance phase of Study 2, to maintain vitamin D sufficiency when UV exposure is inadequate: between early autumn and late winter, and for those that live indoors for the majority of sunlight hours or cover-up from the sun. This oral vitamin D_3 supplementation approach corresponds with current IOM and EFSA recommendations and, unlike simulated sunlight, there is no time burden for an individual; no requirement for bulky irradiation cabinets; and oral vitamin D_3 supplementation is effective regardless of sun-reactive skin type.

3.5.1 Conclusions

Vitamin D sufficiency reduced URTI burden in military recruits during arduous training. In Study 1, vitamin D sufficient recruits had fewer URTI than those with serum 25(OH)D <50 $nmol \cdot L^{-1}$. In Study 2, winter vitamin D supplementation, which achieved vitamin D sufficiency in almost all, reduced URTI peak severity and total days with URTI compared to a placebo. To avoid the burden of URTI, including missed training days, maintaining year-round vitamin D sufficiency is recommended for military personnel and other active populations such as athletes that participate in arduous training.

CHAPTER FOUR

Risk Factors for Upper Respiratory Tract Infection in Marathon Runners; Stress, Anxiety, Early Life Adversity and Perceived Sleep Quality

4.1 Abstract

Objectives Upper respiratory tract infection (URTI) in athletes increases the likelihood of experiencing lost training time, reduced volume and intensity of training, injury, and reduced success in competition. Recently, it has been suggested that sleep and psychosocial factors likely play a substantive role in athlete URTI susceptibility. Here we examine the influence of psychological stress, anxiety, neuroticism, early life adversity (ELA), and sleep on URTI in marathon runners. **Methods** In a prospective, cohort, observational study, 305 marathon runners self-reported daily URTI in the two-weeks before and after an arduous marathon, during the common cold season in Wales, UK. Participants completed questionnaires assessing trait anxiety, perceived psychological stress, state anxiety, neuroticism, perceived life hassles, ELA, sleep duration, and perceived sleep quality. We used logistic and linear regression to determine predictors of URTI. **Results** Runner URTI prevalence was similar pre and post marathon (13.6 *vs*. 14.4%, respectively; $N = 81$ URTI bouts; $P > 0.05$). Runners were at greater risk of URTI during the two weeks before or after a marathon if they reported higher trait anxiety, greater perceived psychological stress, higher levels of neuroticism, higher state anxiety, greater perceived life hassles, early life adverse experience, or lower perceived sleep quality ($P < 0.05$). Runners who reported experiencing ELA were over two times more likely to report a URTI in the two weeks before the marathon (OR 2.33; $P < 0.05$). Runners reporting lower perceived sleep quality were two times more likely to report post-marathon URTI (OR: 0.48; $P < 0.05$). Effects remained after accounting for sex, age and

prior illness. **Conclusion** Psychological stress, anxiety, ELA and poorer perceived sleep quality are predictors of URTI in marathon runners.

4.2 Introduction

Illness in athletes leads to lost training time, reduced volume and intensity of training, increased injury risk, and reduced success at competition (Palmer-Green *et al.*, 2013; Hellard *et al.*, 2015; Svendsen *et al.*, 2016; Timpka *et al.*, 2017). Historically, exercise immunology research largely focused on infection risk following prolonged endurance exercise, in line with the Open Window Theory, finding that greater training load and faster race time increased URTI risk (Peters and Bateman, 1983; Nieman *et al.*, 1990). However, following research showing that pre-marathon infection was a key risk factor for post-marathon infection (Ekblom *et al.*, 2006), exercise immunologist proposed that factors, such as psychological stress, anxiety, and sleep disruption, play a role in infection risk in athletic populations (Campbell and Turner, 2018; Walsh, 2018; Simpson *et al.*, 2020). Indeed, physical exertion, psychological stress and sleep disturbances share mechanisms by which they alter immunity and infection susceptibility, largely through activation of the HPA and SAM axes (Perna *et al.*, 1997; Walsh, 2018; Besedovsky *et al.*, 2019).

Empirical research in athletes has recently provided initial evidence that factors other than physical training increase respiratory infection susceptibility, such as long haul travel, sleep disruption, and psychological stress and anxiety (Svendsen *et al.*, 2016; Drew *et al.*, 2017; Wentz *et al.*, 2018; Fitzgerald *et al.*, 2019). Early life adversity (ELA) has also received recent attention in immunology literature (Elwenspoek *et al.*, 2017). ELA is an overarching term for a multitude of early life adverse experiences occurring before the age of 18 years, including physical and emotional neglect, low socioeconomic conditions, family

conflict, and peer victimisation (Fagundes *et al.*, 2013a; Elwenspoek *et al.*, 2017). Providing initial evidence, children of parents who were separated and not speaking to one another had reduced resistance to common cold exposure in adulthood, compared to individuals whose parents were intact, or separated but still communicated (Murphy *et al.*, 2017). Illness timing is particularly important for athletes; illness before competition reduces success in competition, increases the chances of not competing, and increases the risk of in-competition injury (Svendsen *et al.*, 2016; Van Tonder *et al.*, 2016; Timpka *et al.*, 2017). Furthermore, risk factors are likely to exert different effects on infection risk before and after competition. As athletes experience increases in anxiety and sleep disturbances around sporting events (Juliff *et al.*, 2015; Ford *et al.*, 2017), it is reasonable to hypothesise that measurement timing will impact results.

Given the lack of empirical research into the influence of psychosocial factors and sleep on infection prevalence in athletic populations, the aim of this study was to investigate the role of psychological stress, anxiety, ELA, and sleep in URTI susceptibility, in marathon runners. Then, to understand whether these psychosocial or sleep variables had different effects on URTI risk at different time points: in the lead up to or following a sporting event. We hypothesised that runners with higher anxiety, greater psychological stress, who had experienced ELA, or reported poorer sleep, would be more susceptible to URTI.

4.3 Methods

This study received local ethical approval and was conducted in accordance with the Declaration of Helsinki (2013).

4.3.1 Participants and study design

In this prospective, cohort, observational study, 336 marathon runners (*N* = 227 men: age = 46 ± 9 years, height = 177 ± 13 cm, body mass = 78 ± 11 kg, BMI = 24 ± 3 kg·m^{-2}, *N* = 109 women: age = 43 ± 8 years, height = 166 ± 6 cm, body mass = 63 ± 8 kg, BMI = 23 ± 3 kg·m^{-2}) agreed to participate in the study and gave informed consent. Participants completed questionnaires at baseline and during the two weeks before and after the Snowdonia Marathon (www.snowdoniamarathon.co.uk), which took place in October 2017, in Wales, UK. Participants completed questionnaires remotely, using Qualtrics online software (Qualtrics, San Francisco, USA). This particular marathon is known to be arduous as it has a total ascent of 939 m and is run on mixed terrain (road and trail). Average finish times were 4.4 ± 0.8 h for men and 4.9 ± 0.8 h for women respectively.

4.3.2 Upper respiratory tract infection incidence, duration and severity

Participants self-reported daily symptoms of the common cold during the two weeks before and after the marathon, using the Jackson common cold questionnaire (Jackson *et al.*, 1958). They rated the severity of eight symptoms (sneezing, headache, feeling generally unwell, runny nose, blocked nose, sore throat, cough, chilliness) on a 4-point Likert scale (Not at all = 0, Mild = 1, Moderate = 2, Severe = 3). Data were included when participants completed ≥80% of their daily Jackson questionnaires. We defined a URTI episode by a total symptom score ≥6 each day for two or more consecutive days (Hanstock *et al.*, 2016). To be categorised as a new URTI the participant had a minimum of five consecutive days with a symptom score of zero before again reporting two or more consecutive days with a symptom score ≥6. We calculated URTI duration and peak severity as the average URTI duration of all URTI episodes and the highest severity score of any URTI episode, respectively. At commencement of daily monitoring, participants were asked to nominate a household member who was over 18 years of age and not running the 2017 Snowdonia Marathon. Participants were asked to report the sex and age of a household member who was closest in

age to them, to be used as a household control for the entirety of the study. A total of 226 household controls were nominated (25% men, age = 45 ± 10 years). During biweekly questionnaires participants were asked whether their nominated household control had a common cold or the flu during the previous time period.

4.3.3 Predictor variables

Baseline questionnaires

At baseline, participants reported demographic information about their sex, age, height and body mass. Participants also answered questionnaires that assessed their trait anxiety, using the trait portion of the State-Trait Anxiety Inventory (STAI-T) (Spielberger, 1983) and neuroticism, using the Ten Item Personality Index (TIPI) (Gosling, 2003).

Questionnaires during the two weeks before and after the marathon

At the beginning of the monitoring period, participants reported their perceived psychological stress, using the perceived stress scale (PSS) (Cohen *et al.*, 1983) and whether they felt they had a URTI during the previous month. During the two weeks before and after the marathon, participants gave weekly information about their training distance. Participants reported the time they went to sleep and awoke to allow calculation of sleep quantity and rated their sleep quality on a 5-point Likert scale (1 = very poor, 5 = very good). We calculated average sleep duration and perceived sleep quality when participants were healthy (days with URTI removed). We assessed state anxiety using the state portion of the State Trait Anxiety Inventory (STAI-S) (Spielberger, 1983) on the morning of the marathon. Participants also reported the degree to which they had experienced any life hassles the day before and on the day of the marathon (e.g. travel, accommodation, delays, etc.) on a 0 – 100 mm visual analogue scale (VAS). At the end of the monitoring period, participants again rated their perceived psychological stress over the last month. Finally, participants answered

a questionnaire regarding their ELA experiences before the age of 18, including questions about family financial problems, food insecurity, homelessness, psychological abuse, peer victimisation, physical neglect, emotional neglect, household mental illness, household alcohol abuse, household crime, parental divorce, parental absence, death of a parent or sibling and violent crime victimisation, as previously described (Mersky *et al.*, 2017). Unfortunately, due to ethical constraints, we could not include questions about physical abuse, domestic violence or sexual abuse.

4.3.4 Statistical analysis

We used logistic regression to examine variables relating to URTI prevalence over the whole period, and during pre and post marathon periods, separately. We ran analyses as simple models without covariates and then with the inclusion of sex, age and prior illness, as these variables have been shown to influence URTI susceptibility (Ekblom *et al.*, 2006; Drew *et al.*, 2017). To further illustrate our findings, chi-square analysis was used to compare URTI prevalence between runners with ELA and those with no ELA, and between runners with perceived sleep quality of <3.0, 3.0–3.6, and ≥3.7 (tertile split). We also used multiple linear regression to examine the influence of trait anxiety, perceived psychological stress and neuroticism on URTI duration and URTI peak severity. Chi-squared analysis compared the proportion of runners and household controls with a URTI pre vs post marathon. Significance was set at $P < 0.05$ and we performed all statistical analyses using SPSS Version 25 (IBM Corp, NY, USA).

4.4 Results

4.4.1 Upper respiratory tract infections

Seventy-eight runners (26%) reported at least one URTI episode during the two weeks before or after the marathon. Ten runners had two URTIs, two runners had three URTIs, and all others reported only one URTI during the four-week monitoring period. Only four runners had a URTI both pre and post marathon. Of those runners who reported a URTI, the average duration was 6 ± 5 days and the average peak severity was 11 ± 4 AU. The proportion of runners reporting a URTI pre and post marathon was similar ($P > 0.05$; 39 *vs.* 42 bouts, 13.6 *vs*. 14.4%, respectively). The proportion of household controls reporting a URTI pre and post marathon was also similar ($P > 0.05$; 23.7% *vs*. 20.4%, respectively). Average state anxiety on the morning of the race was 40 ± 11 AU, which is similar to pre competition levels reported in collegiate athletes (Wilson *et al.*, 2000) and higher than norms reported for working adults of a similar age (Spielberger, 1983), indicating the significance of this sporting event to participants.

4.4.2 Predictors of URTI across the whole monitoring period

Higher trait anxiety, greater perceived psychological stress, higher levels of neuroticism, and lower perceived sleep quality, were predictors of URTI in marathon runners ($P < 0.05$; Table 4.1). Lower perceived sleep quality was the strongest predictor of URTI; runners with lower perceived sleep quality were 64% more likely to report a URTI ($P < 0.05$; Table 4.1). Effects remained after accounting for sex, age and prior illness ($P < 0.05$). Furthermore, runners with higher trait anxiety and greater perceived psychological stress reported longer URTI duration ($P < 0.05$, $R^2 = 0.079$ and 0.075, unstandardized B = 0.144 and 0.191, respectively). There was no effect of trait anxiety, perceived psychological stress or neuroticism on URTI peak severity, or of neuroticism on URTI duration ($P > 0.05$). Training distance was not a predictor of URTI prevalence in marathon runners ($P > 0.05$).

Table 4.1: Variables predicting upper respiratory tract infection (URTI) prevalence during the two weeks before and after the marathon. * $P < 0.05$.

		OR (95% CI)
Variable timing	Predictor variable	Simple model
Stable measure	**Neuroticism**	1.22* (1.01–1.47)
	Trait anxiety	1.04* (1.01–1.07)
Before monitoring	**Perceived psychological stress**	1.04* (1.00–1.09)
During monitoring	**Perceived psychological stress**	1.05* (1.01–1.09)
	Perceived sleep quality	0.61*[a] (0.40–0.95)

[a] odds ratio below 1 indicates that lower perceived sleep quality increases likelihood of URTI; Effects remained after account for sex, age and prior illness.

4.4.3 Predictors of pre and post marathon URTI

When examining pre and post marathon periods separately, early life adverse experience, higher trait anxiety, greater perceived psychological stress and higher levels of neuroticism were predictors of pre-marathon URTI ($P < 0.05$; Table 4.2). Specifically, runners who reported at least one early life adverse experience (58% of runners) were over two times more likely to report a URTI in the two weeks before the marathon compared to those who had not experienced any ELAs ($P < 0.05$; Table 4.2). Indeed, runners with early life adverse experience had a higher prevalence of pre marathon URTI than those with no early life adversity experience ($P < 0.05$; Figure 4.1). Greater perceived psychological stress, higher levels of neuroticism and higher trait anxiety were predictors of runners reporting a URTI during the pre-marathon period ($P < 0.05$; Table 4.2), but not the post marathon period ($P > 0.05$). Pre-marathon effects remained after accounting for sex, age and prior illness ($P < 0.05$).

Table 4.2: Variables predicting upper respiratory tract infection (URTI) prevalence during the two weeks before (pre) and after (post) the marathon. Early life adversity (ELA). * $P < 0.05$.

			OR (95% CI)
URTI timing	Variable timing	Predictor variable	Simple model
Pre marathon	*Stable measure*	**Neuroticism**	1.35* (1.06–1.72)
		Trait anxiety	1.05* (1.02–1.09)
		ELA[b]	2.33* (1.05–5.18)
	Before monitoring	**Perceived psychological stress**	1.08* (1.02–1.13)
	During monitoring	**Perceived psychological stress**	1.08* (1.02–1.14)
Post marathon	*Two weeks before marathon*	**Perceived sleep quality**	0.55*[a] (0.36–0.89)
	Two weeks after marathon	**Perceived sleep quality**	0.48*[a] (0.27–0.84)
	24h before marathon	**Hassles**	1.02* (1.00–1.03)

[a] odds ratio below 1 indicates that lower perceived sleep quality increases likelihood of URTI; [b] No ELA = 0; Effects remained after account for sex, age and prior illness

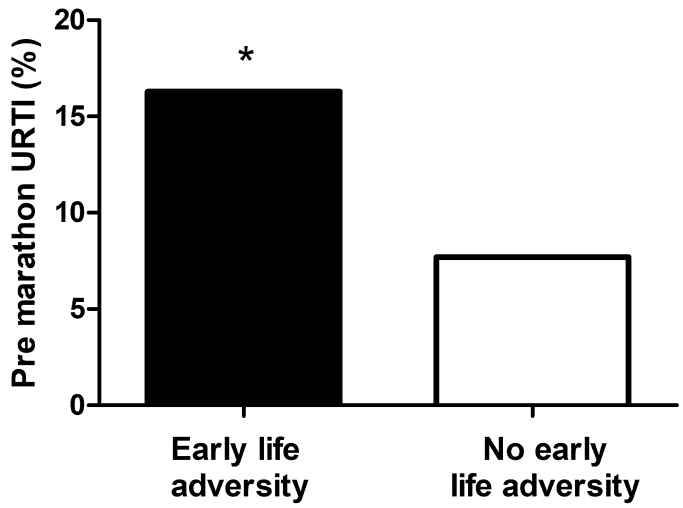


Figure 4.1: Proportion of runners reporting an upper respiratory tract infection during the two weeks before the marathon (pre) if they reported at least one early life adversity compared to those reporting no early life adversities. * higher URTI prevalence compared to no early life adversity, $P < 0.05$.

Lower perceived sleep quality, greater perceived life hassles and higher state anxiety were predictors of post-marathon URTI ($P < 0.05$; Table 4.2). Although sleep quality was not a predictor of pre-marathon URTI ($P > 0.05$), runners were over two times more likely to report a post-marathon URTI if they reported lower perceived sleep quality during either the two weeks before or after the marathon ($P < 0.05$; Table 4.2). Indeed, runners with perceived sleep quality of <3.0 and 3.0–3.6 had a higher prevalence of URTI post marathon, compared to runners with perceived sleep quality of ≥3.7 ($P < 0.05$; Figure 4.2). Furthermore, runners who felt they had experienced greater life hassles in the 24 h before the race (e.g. disrupted travel) were more likely to report a URTI in the post-marathon period ($P < 0.05$; Table 4.2). Effects remained after accounting for sex, age and prior illness ($P < 0.05$). Interestingly, after controlling for sex, age and prior illness, runners with higher state anxiety on the morning of

the marathon were more likely to have a URTI post-marathon ($P < 0.05$; OR (95% CI): 1.04 (1.00–1.07)).

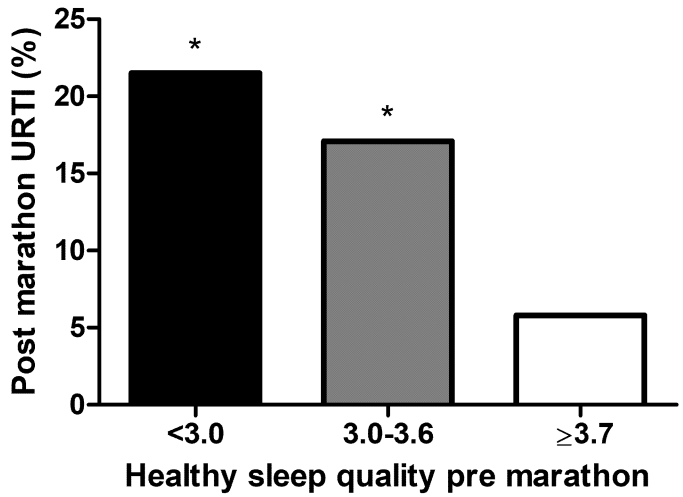


Figure 4.2: The influence of healthy sleep quality during the two weeks before the marathon (pre; tertile split) on upper respiratory tract infection (URTI) prevalence during the two weeks after the marathon (post). * higher URTI prevalence compared to ≥3.7 group, $P < 0.05$.

4.5 Discussion

In this cohort, observational study of 305 marathon runners, we examined the influence of psychological stress, anxiety, neuroticism, ELA, and sleep, on URTI in the lead up to and following a marathon event, during the common cold season, in the UK. In line with our hypothesis, we found that, after accounting for sex, age and prior illness, runners were more likely to report a URTI if they reported higher trait anxiety, greater perceived psychological stress, higher levels of neuroticism, early life adverse experience, or lower perceived sleep quality. Moreover, in line with our hypothesis, measurement timing influenced URTI risk; we found that runners who reported an ELA experience were over two

times more likely to report a pre-marathon URTI, and runners reporting lower perceived sleep quality were two times more likely to report a post-marathon URTI. Taken together, these novel findings support the role of psychological stress, anxiety, ELA and sleep in infection risk in athletic populations.

Marathon runners with higher trait anxiety or greater perceived psychological stress were more likely to report URTI; effects were largely driven by the pre-marathon period. Furthermore, runners with higher trait anxiety or greater perceived psychological stress reported longer URTI duration. Runners with higher state anxiety on the morning of the race, or who experienced life hassles in the 24 h before the marathon, were more likely to report a URTI in the post-marathon period. Our findings align with work showing that psychological stress increased URTI incidence following common cold exposure in non-athletic populations (Cohen *et al.*, 1991; Cohen *et al.*, 1995). Our research strengthens initial evidence showing that athletes reporting higher perceived psychological stress and more depression symptoms were more likely to report an illness (Drew *et al.*, 2017) because we reported a larger number of URTI (N = 81 vs. 16), our measures were prospective, and trait anxiety and neuroticism, which are stable characteristics (Spielberger, 1983; Costa *et al.*, 1986), predicted URTI prevalence. We also found that individuals with higher neuroticism were more likely to report a URTI, which aligns with previous work showing that higher neuroticism, or negative affect, increases URTI symptoms (Cohen *et al.*, 1993). Although it has previously been suggested that individuals with higher neuroticism are likely to over-report symptoms (Cohen *et al.*, 1995; Johnson, 2003), in the current study, we found no relationship between neuroticism and URTI duration and severity. Given that illness prior to competition has been shown to reduce success, and increase risk of injury and serious complication (Svendsen *et al.*, 2016; Van Tonder *et al.*, 2016; Timpka *et al.*, 2017), it is particularly important to note that higher trait

anxiety, greater perceived psychological stress and higher levels of neuroticism were stronger predictors of URTI in the pre-marathon period.

Early life adversity was the strongest predictor of pre-marathon URTI; marathon runners were 2.3 times more likely to have a URTI if they reported an adverse childhood experience, which is suggested to cause immune dysregulation in adulthood (Fagundes *et al.*, 2013a; Elwenspoek *et al.*, 2017). A number of mechanisms are proposed that may lead to increased URTI risk in individuals with ELA, including elevated inflammatory responses, a reduced ability to dampen this inflammation, accelerated immunoscenescence, and reduced physiological stress reactivity (Fagundes *et al.*, 2013a; Elwenspoek *et al.*, 2017). Indeed, our study is one of the first to show an empirical relationship between ELA and URTI incidence. Our findings add to initial evidence, which showed that the offspring of parents who were separated and not speaking to one another had reduced resistance to the common cold as adults (Murphy *et al.*, 2017). Of interest, ELA predicted URTI incidence in runners only during the two weeks before the marathon, when the stressor was impending. Although the reason for the timing of this effect needs further investigation, we suggest that this effect may be linked to individuals who have experienced ELA having lower emotional regulation capabilities (Poole *et al.*, 2017). Indeed, research has shown that challenging environments (such as those that involve risk taking, competitive sport, or heavy exercise) provide opportunities to experience clear and intense emotions, to master these intense emotions, and to subsequently experience improved emotion regulation, feelings of agency, and mental health in other life domains (Roberts and Woodman, 2015). Again, URTI in the pre-marathon period is particularly impactful for athletes, given reports of reduced success in competition following a URTI (Palmer-Green *et al.*, 2013; Svendsen *et al.*, 2016).

Lower perceived sleep quality, measured prospectively and when runners were healthy (not reporting a URTI), was one of the strongest predictors of URTI in marathon

runners, which was particularly evident in the post-marathon period, when runners with lower perceived sleep quality, during the two weeks before or after the marathon, were over two times more likely to report an URTI. Indeed, both acute and chronic sleep disturbance have been shown to reduce immune cell number and function, and increase levels of cytokines and markers of inflammation, which lead to reduced host immune defence (Besedovsky *et al.*, 2019). Our findings extend previous work in non-athletic populations; individuals with poorer sleep efficiency were 5.5 times more likely to develop a cold following experimental exposure (Cohen *et al.*, 2009). Further, our findings suggest than an individual's perception of sleep plays a particular role in URTI risk, adding to research reporting that women who perceived they had inadequate sleep were 1.5 times more likely to develop pneumonia (Patel *et al.*, 2012). In contrast to previous studies (Cohen *et al.*, 2009; Prather *et al.*, 2015), we did not see an influence of sleep duration on URTI risk, which may be because we measured self-reported sleep duration. Indeed, following common cold exposure, behaviourally-assessed sleep duration was predictive of cold development, whereas self-reported sleep duration was not predictive (Prather *et al.*, 2015).

Although training load was not a significant predictor of URTI in marathon runners, as in previous studies (Peters and Bateman, 1983; Nieman *et al.*, 1990), it is worth noting that we monitored training distance only in the two weeks before the marathon, which likely included training volume tapering, and in the two weeks following the marathon, when runners likely rested. Readers should also be aware that runners in this population had an average age that is higher than a typical athletic population (men 46 ± 9 years, women 43 ± 8 years). Therefore, future work should examine the influence of psychosocial and sleep factors on URTI risk in younger athletic and military populations. Due to the remote nature of the data collection, and to ease participant burden, we did not acquire pathological confirmation of URTI, which allowed for the recruitment of a large number of runners and enabled us to

carry out sufficiently powered regression analyses. However, it is worth noting that our study was conducted in the common cold season in the UK, when it would be expected that symptoms are due to respiratory infection rather than allergy. Furthermore, a previous study using the same monitoring method, at the same time of year, in the same region, showed that 82% of participants returned positive swab results for common cold viruses (Hanstock *et al.*, 2016). Finally, in a study of Olympic athletes taking part in the Winter Olympic Games, aetiology of common cold was detected in 75% of athletes who experienced common cold symptoms (Valtonen *et al.*, 2019). However, future work should examine pathology confirmed URTI, or training time lost due to URTI, as recommended in the recent International Olympic Committee (IOC) consensus (International Olympic Committee *et al.*, 2020).

Our novel findings illustrate that psychological stress, anxiety, ELA and sleep are important predictors of URTI risk in athletes. Given these findings, athletes with higher psychological stress, anxiety or neuroticism, who have experienced ELA, or who have poorer perceived sleep quality may benefit from being more vigilant in avoiding infection, for example by avoiding sick people and ensuring good hygiene (Walsh, 2018). Furthermore, it was interesting that runners who reported experiencing greater life hassles in the 24 h before the marathon were more likely to have a URTI post-marathon. As such, athletes should take steps to minimise disruptions, such as travelling well in advance of the event, allowing extra time for travel delays, and researching where accommodation and registration are in advance of the event, which aligns with previous recommendations (Svendsen *et al.*, 2016). Athletes may also wish to consider developing psychological stress management strategies. Indeed, individuals with higher trait anxiety, greater perceived psychological stress, higher state anxiety, or ELA experiences may particularly benefit from the use of such strategies. Additionally, athletes with poor perceived sleep quality may also consider using strategies to

improve sleep to reduce the risk of illness, such as optimising sleep hygiene routine by reducing psychological strain and going ‘screen-free’ an hour before bedtime (Walsh, 2018). However, future work should investigate whether interventions, which manage psychological stress and anxiety, or improve sleep, can reduce infection risk in athletes.

In conclusion, in a prospective, cohort study of 305 marathon runners, which used daily illness monitoring, we found that runners were at greater risk of URTI during the two weeks before or after a marathon if they reported higher psychological stress, anxiety, or neuroticism, early life adverse experience, or lower perceived sleep quality. Runners who had experienced early life adversity were over two times more likely to report a pre-marathon URTI. Runners with poorer perceived sleep quality were two times more likely to report a post-marathon URTI. Future work should investigate whether interventions to manage psychological stress and anxiety, or improve sleep perception, can reduce URTI risk in athletes and military personnel.

CHAPTER FIVE

Psychological Stress and Anxiety Play an Important Role in the Mucosal Immune Response to Exercise

5.1 Abstract

Objectives Psychological stress has recently been shown to play a role in the *in vivo* immune response to exercise and increased infection risk in athletes. However, the role of psychological stress in the mucosal immune response to exercise remains unknown. The aim of the current study was to examine the influence of psychological stress and anxiety on the mucosal immune response to exercise. **Methods** In Study 1, a repeated-measures, crossover study, 45 recreationally active participants (51% male) gave unstimulated saliva samples immediately before, immediately after and 30 minutes after one hour of treadmill running at 65% VO_{2peak} and one hour of seated rest, at the same time of day, separated by at least 48 h. Participants reported trait anxiety, perceived psychological stress during the month prior to, and state anxiety immediately before the exercise bout. In Study 2, a cohort observation study, 215 marathon runners (72% male) provided unstimulated saliva samples before and after a marathon. Further, they completed measures of trait anxiety, perceived stress (specifically in the two weeks before and two weeks after the marathon), and state anxiety on the morning of the race. Saliva SIgA concentrations were assessed by ELISA. Mean intra-assay CVs were 3.3 and 2.9% for Study 1 and 2, respectively. **Results** In Study 1, perceived psychological stress, trait anxiety and state anxiety negatively correlated with saliva SIgA secretion rate (SR) change pre to post exercise ($P < 0.05$; r = -0.68, -0.59, and -0.61, respectively). Aligning with Study 1, men with higher trait anxiety and perceived stress had a greater reduction in saliva SIgA SR pre to post marathon ($P < 0.05$; $R^2 = 0.020$ and 0.031;

unstandardized B (SE) = -0.394 (0.225) and -0.769 (0.361), respectively; Study 2). Psychological variables did not significantly influence the mucosal immune response to exercise in women ($P > 0.05$). **Conclusion** Psychological stress and anxiety influence the mucosal immune response to exercise in men.

5.2 Introduction

Exercise immunology has largely focused on the role of physical stress in immune modulation. Athletes experience increased URTI risk following strenuous exercise and during periods of heavy training, which has been linked to a transient suppression of immune function (Peters and Bateman, 1983; Nieman *et al.*, 1990; Walsh *et al.*, 2011b; Hellard *et al.*, 2015). Indeed, a transient depression of mucosal immunity following a prolonged bout of exercise has often been implicated in increased URTI susceptibility (Nieman, 1994; Walsh *et al.*, 2011b). However, the salivary SIgA response to acute exercise has yielded variable results, which may be influenced by exercise mode, intensity and duration, as well as other environmental, behavioural and psychosocial factors (Bishop and Gleeson, 2009; Walsh *et al.*, 2011b). Indeed, recent research has shown that several other factors play a role in increased URTI risk in athletes, such as prior infection, nutritional inadequacy, inadequate sleep, exposure to environmental extremes, and long haul travel (Ekblom *et al.*, 2006; Svendsen *et al.*, 2016; Walsh, 2018; Wentz *et al.*, 2018).

Psychological stress is well known to influence URTI susceptibility in non-exercising populations (Cohen *et al.*, 1991; Cohen, 1995). Psychological and physical stress share mechanisms by which they alter immune function, primarily through the hypothalamic-pituitary-adrenocortical (HPA) and sympathetic-adrenal-medullary (SAM) axes (Perna *et al.*, 1997; Kohut, 2019). Despite shared mechanisms between psychological and physical stress,

and multiple review articles highlighting the likely role of psychological factors in athlete URTI susceptibility (Clow and Hucklebridge, 2001; Dhabhar, 2014; Walsh, 2018), few empirical studies have examined the influence of psychological stress on the immune response to exercise. Providing initial evidence, state anxiety and perceived psychological stress measured before exercise were found to determine the strength of the *in vivo* immune response to exercise (Edwards *et al.*, 2018). Further, we found, in **Chapter 4**, that psychological stress and anxiety increased URTI risk in marathon runners. However, the role of psychological stress in the mucosal immune response to exercise remains unknown.

Sex differences are reported for the immune response to exercise and psychological stress (Gillum *et al.*, 2011; Gillum *et al.*, 2014; He *et al.*, 2014; Stephens *et al.*, 2016; Birkett *et al.*, 2017). Although heterogeneity in the literature, caused by a lack of control over menstrual cycle and contraceptive use, has led to mixed results. Salivary SIgA was found to be higher in women compared to men in two studies (Gomez *et al.*, 1993; Gillum *et al.*, 2014) but not different between men and women in another study (He *et al.*, 2014). However, the study reporting no difference made no account for menstrual phase (He *et al.*, 2014). Furthermore, women are more likely to report higher psychological stress and anxiety, and appear to be more vulnerable to increased depression associated with inflammation than men (Bekhbat and Neigh, 2018). However, well-controlled research examining sex differences in the immune response to exercise and psychological stress is lacking. Indeed, the only previous study to show an influence of psychological stress on the immune response to exercise included only males (Edwards *et al.*, 2018).

Therefore, the aim of the study was to examine the role of psychological stress and anxiety on the mucosal immune response to exercise in men and women, in response to a controlled, moderate, lab-based bout of exercise (Study 1) and a field-based marathon (Study

2). We hypothesised that perceived psychological stress, trait anxiety, and state anxiety would have a negative relationship with the mucosal immune response to exercise.

5.3 Methods

This study received local ethics approval and was conducted in accordance with the Declaration of Helsinki (2013).

5.3.1 Study one

5.3.2 Participants and study design

In a repeated-measures, crossover study, 45 participants (N = 26 male: age = 21 ± 3 years, height = 179 ± 7 cm, body mass = 76 ± 9 kg, BMI = 24 ± 3 kg·m^{-2}, $\dot{V}O_{2peak}$ = 50 ± 8 ml·kg·min^{-1}, trait anxiety = 36 ± 8 AU, N = 19 female: age = 23 ± 3 years, height = 167 ± 6 cm, body mass = 67 ± 10 kg, BMI = 24 ± 3 kg·m^{-2}, $\dot{V}O_{2peak}$ = 40 ± 5 ml·kg·min^{-1}, trait anxiety = 42 ± 11 AU) agreed to participant in the study and gave informed consent. Participants were recreationally active (3-9 h of aerobic exercise per week), non-smokers, who did not take prescription medication for the duration of the study or the preceding month. Participants were asked to refrain from alcohol, caffeine, over-the-counter medication and heavy exercise during the 24 h preceding all experimental trials and did not self-report URS in the 7 days preceding experimental trials. Female participants self-reported length and current day of menstrual cycle, participants who had irregular menstrual cycles, had cycles of <23 days or >32 days in line with Cole *et al.* (2009), or had changed their contraception within the last six months, were excluded. Ten females were normally menstruating and not using contraceptives, four were using oral contraceptives (OC) and five were using the implant. Exercise and seated rest trials were completed on the same day of two separate cycles for

female participants who were non-contraceptive users. Menstrual phase was estimated assuming follicular and luteal phases have a 50% split throughout the cycle for non-contraceptive users. OC users completed trials during days 2-7, which was considered the follicular phase (when not taking OC or taking sugar pill), or during days 9 – 28, which was considered the luteal phase (when taking OC). Implant users were considered to be in the follicular phase. Sixty-three percent of females completed their trials during the luteal phase.

5.3.3 Preliminary measures and familiarization

Anthropometric measures were recorded on arrival at the laboratory. After this, $\dot{V}O_{2peak}$ was estimated by means of a ramped exercise test on a treadmill (HP Cosmos Mercury 4.0; Nussdorf-Traunstein, Germany). After 3 min of walking at 5 $km \cdot h^{-1}$ with an incline of 1%, speed increased at a rate of 1$km \cdot h \cdot min^{-1}$ to a max of 18 $km \cdot h^{-1}$, after which the incline increased at a rate of 1%$\cdot min^{-1}$ until volitional exhaustion. Pulmonary gas exchange was measured breath-by-breath for the duration of the test (Cortex Metalyser 3B, Biophysik, Leipzig, Germany), as described previously (Diment *et al.*, 2015). Participants then rested for 30 min while completing questionnaires about their trait anxiety using the trait portion of the State Trait Anxiety Inventory (STAI) (Spielberger, 1983). The running speed that elicited 65% $\dot{V}O_{2peak}$ was then determined and verified using the interpolation of the running speed – $\dot{V}O_2$ relationship. All subjects were familiarized with laboratory equipment.

5.3.4 Experimental procedures

Participants completed two experimental trials in a randomized, crossover design, separated by at least 48 h. The exercise trial (EX) involved 1 h of continuous treadmill running at 65% $\dot{V}O2peak$ in temperate conditions (20 ± 1 °C, 47 ± 8% RH), whereas the control trial (REST) consisted of 1 h seated rest in a similar environment (20 ± 1 °C, 44 ± 8% RH). Participants arrived to the laboratory fasted, apart from two participants who arrived to

the laboratory at 12:00 after being provided with a standardised meal (2201 kilojoules, 71g carbohydrate, 17g protein, 18g fat) that morning. Nude body mass (NBM) was recorded before and after exercise on a digital platform scale to determine water allowance. Participants were provided with a standardised meal (2201 kilojoules, 71g carbohydrate, 17g protein, 18g fat) and received 5 $ml \cdot kg^{-1}$ NBM of water. To confirm euhydration, fifteen minutes after the standardised meal participants provided a urine sample and urine specific gravity (USG) was measured using a refractometer (Atago Uricon-Ne refractometer; NSG Precision cells, New York City, New York, USA). Participants with USG ≥1.025 were provided with a bolus of 500 ml of water and USG was re-measured after 15 minutes, this process was repeated until USG <1.025 was achieved (Armstrong et al., 2016). Participants with USG <1.025 on first measurement were provided with fluids proportional to 35$ml \cdot kg \cdot d^{-1}$ for the 90 minute pre-trial period. Twenty minutes before beginning either EX or REST participants completed questionnaires about their perceived psychological stress in the previous month and their state anxiety at that moment, using the perceived stress scale (PSS) (Cohen et al., 1983) and the state portion of the STAI (Spielberger, 1983), respectively. The 1 h exercise and seated rest bouts took place from 09:00 – 10:00, 10:30 – 11:30 or 13:30 – 14:30, participants completed both their EX and REST at the same time of day separated by at least 48h, in a randomised order. Saliva samples were collected immediately before (pre), immediately after (post) and 30 minutes after (30 min post) EX and at equivalent time points during REST. During EX, participants were provided with the equivalent 3$ml \cdot kg \cdot h^{-1}$ plain water at 15, 30 and 45 min of the exercise bout to partially offset fluid losses through sweating. During REST, participants were provided with the equivalent of 35$\cdot ml \cdot d^{-1}$ for the 60 min period at 15, 30 and 45 min. Heart rate (HR) was monitored continuously throughout the 1 h EX and REST periods. During EX only, Borg's rating of perceived exertion (RPE) was recorded at 15, 30, 45 and 60 min.

5.3.5 Study two

5.3.6 Participants and study design

In a cohort, observational study, 305 marathon runners (*N* = 154 men: age = 46 ± 10 years, height = 177 ± 16 cm, body mass = 78 ± 11 kg, BMI = 24 ± 3 kg·m^{-2}, trait anxiety = 37 ± 9 AU, *N* = 61 women: age = 44 ± 9 years, height = 166 ± 6 years, body mass = 62 ± 8 kg, BMI = 22 ± 2 kg·m^{-2}, trait anxiety = 40 ± 10 AU) agreed to participate in the study, gave informed consent and provided pre and post marathon saliva samples (Figure 5.1). Participants completed remote questionnaires at recruitment, on the morning of a marathon, and two weeks after a marathon in autumn, Wales, UK, which had a total climb of 939 m with mixed terrain (road and trail). Average finish times were 4.4 ± 0.7 h for men and 4.9 ± 0.7 h for women, respectively.

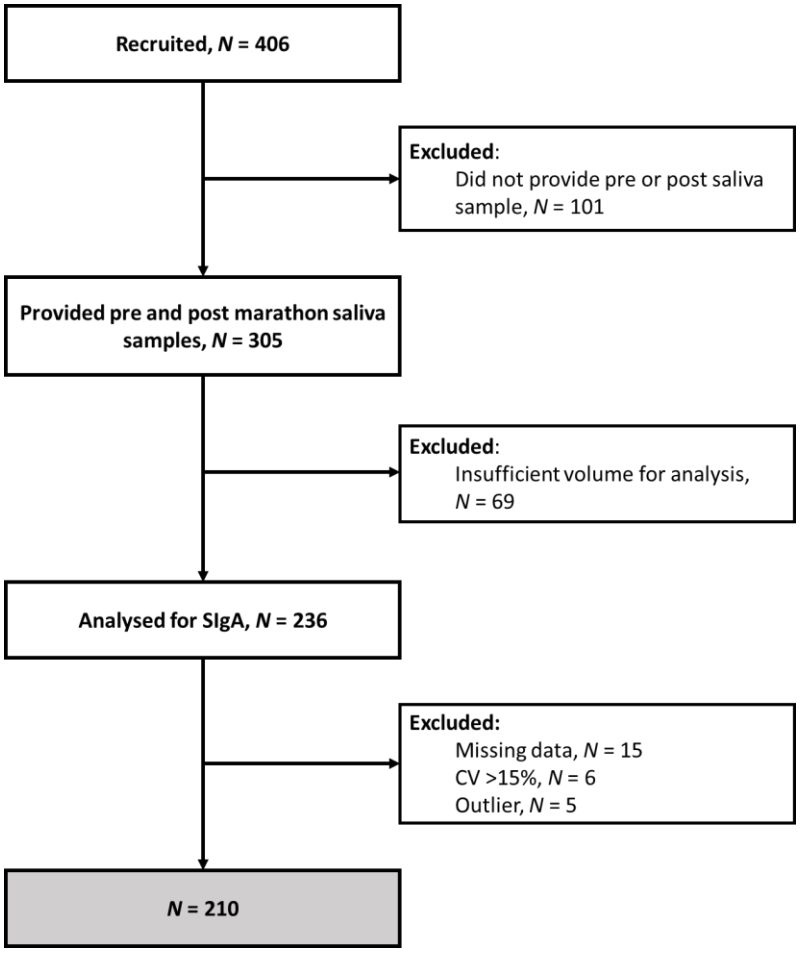


Figure 5.1. Flow chart of participants who were recruited, provided saliva samples, and were included in the final data analysis for Study 2.

5.3.7 Experimental procedures

Participants completed questionnaires remotely using Qualtrics online questionnaire software (Qualtrics, San Francisco, USA). At recruitment, participants reported demographic data (sex, age, height, body mass) and answered the trait portion of the STAI (Spielberger, 1983). On the morning of the marathon, participants reported their state anxiety using the state portion of the STAI (Spielberger, 1983), either remotely or in person at the race, before providing the first saliva sample. Saliva samples were collected between 07:30am and 10:15am on the morning of the marathon (race start time 10:30am) and immediately after the

marathon at the finish line. Finally, two weeks after the completion of the marathon, participants reported their perceived stress during the month prior using the perceived stress scale (Cohen *et al.*, 1983), measurement was taken at this time to allow for the measurement of psychological stress for another aspect of this large research project (**Chapter 4**).

5.3.8 Saliva collection and analysis

For Study 1, saliva samples were collected at the same time of day for the seated rest trial and the exercise trial within participants. Saliva samples were collected for 5 min, or up to 9 min if adequate volume was not achieved after 5 min, in a pre-weighed 30 ml tube using the passive dribble method (Strazdins *et al.*, 2005). Samples were weighed immediately after collection, centrifuged at 1500 g and 4°C for 10 minutes, aliquoted and then stored at -80 °C before analysis. For Study 2, saliva samples were collected for 2 min using Versi-Sal™ (Oasis Diagnostics, Vancouver, WA, USA) as described previously (Fortes *et al.*, 2012). Samples were stored at 4°C before being centrifuged at 1500g and 4°C for 10 minutes and then stored at -80°C until analysis. Samples were analysed for secretory immunoglobulin A (SIgA) using enzyme-linked immunosorbent assay (ELISA, Salimetrics, Pennsylvania, USA). Mean intra-assay CVs for sample duplicates were 2.9% and 3.3% for Study 1 and Study 2, respectively. Assuming the density to be 1.00 $g \cdot mL^{-1}$ for saliva, SIgA secretion rate (SR) was calculated by multiplying flow rate (FR) by IgA concentration, as described previously (Oliver *et al.*, 2007).

5.3.9 Statistical analysis

For Study 1, we estimated a minimum sample size of 37 participants based on a medium to large effect size based on previous data (Edwards *et al.*, 2018), to produce an 80% chance of obtaining statistical significance at the 0.05 level. In Study 1, we used repeated measures ANOVA (2 group (EX, REST) x 3 time points (pre, post, 30 min post)) to examine

differences in saliva FR, SIgA concentration and SIgA SR, for all participants, and when examining men and women separately. Correlation was used to examine the relationship between trait anxiety, perceived psychological stress and state anxiety with saliva FR, SIgA concentration and SIgA SR before (pre), after (post), 30 min post and the change between pre and post EX and REST in all participants, and males and females separately. To further illustrate the influence of perceived psychological stress on the mucosal immune response to exercise in men, we performed additional analyses by categorising the male population based on PSS scores. Firstly, we removed individuals defined as having high PSS scores (PSS ≥20 AU; $N = 2$) (Cohen and Williamson, 1988; Khalili *et al.*, 2017). Next, we categorised the population based on PSS scores using a median split; whereby, PSS was defined as low stress (LOW: PSS <12; mean) and moderate stress (MOD: PSS 12–19; mean). The PSS ranges for LOW and MOD are in line with those reported in the literature (Cohen and Williamson, 1988). A two-way, mixed model ANOVA (2 groups (LOW PSS, MOD PSS) x 2 time points (pre, post)) was used to examine the mucosal immune response to exercise between LOW and MOD perceived psychological stress categories. For study 2, we estimated we needed 157 participants based on a small to medium effect size, to produce an 80% chance of obtaining statistical significance at the 0.05 level. In study 2, one-tailed paired t-tests were performed to compare pre and post marathon saliva FR, SIgA concentration and SIgA SR, for all participants, and when comparing men and women separately. Linear regression was used to assess the relationship between trait anxiety and perceived psychological stress, and saliva FR, SIgA concentration and SIgA SR pre, post and the change pre to post marathon in males and females. As in study 1, to further illustrate the influence of perceived psychological stress on the mucosal immune response to exercise in men, we categorised the population as low stress (LOW: PSS < 12; mean), moderate stress (MOD: PSS 12–19; mean) and high stress (HIGH: PSS ≥20; mean). An independent-samples T test was used to compare the change in

saliva SIgA SR pre to post the marathon between LOW and HIGH perceived psychological stress categories. Statistical analyses were performed using a common statistical software package (SPSS 25, IBM, Chiciago, IL). Significance was accepted as $P < 0.05$. Data are presented as mean ± SD unless otherwise stated. Data points that were more than three-times the interquartile range were deemed as outliers and removed. Data were checked for normal distribution and, in cases where the assumption of normality was violated, were square-root transformed prior to analysis.

5.4 Results

5.4.1 Study one

Participants had lower saliva FR and SIgA SR overall during EX compared to REST ($P < 0.05$; FR 181 ± 151 vs 222 ± 181 μl·min^{-1}, SIgA SR 35 ± 28 vs 42 ± 31 μg·min^{-1}, EX vs. REST, respectively). When examining men and women separately, men had lower saliva FR and SIgA SR overall during EX compared to REST ($P < 0.05$; Figure 5.2A & C) and no differences between EX and REST for saliva SIgA concentration ($P > 0.05$; Figure 5.2B). There were no differences between EX and REST for saliva FR, SIgA concentration or SIgA SR for women (P > 0.05; Figure 5.2D, E & F, respectively). Average heart rate (HR) was higher during EX than REST ($P < 0.05$; 159 ± 12 vs 69 ± 16 bpm, respectively). During EX, RPE rose steadily from “light” to “somewhat hard” between 15 and 60 min ($P < 0.05$; 11 ± 2 vs 13 ± 3 AU, respectively).

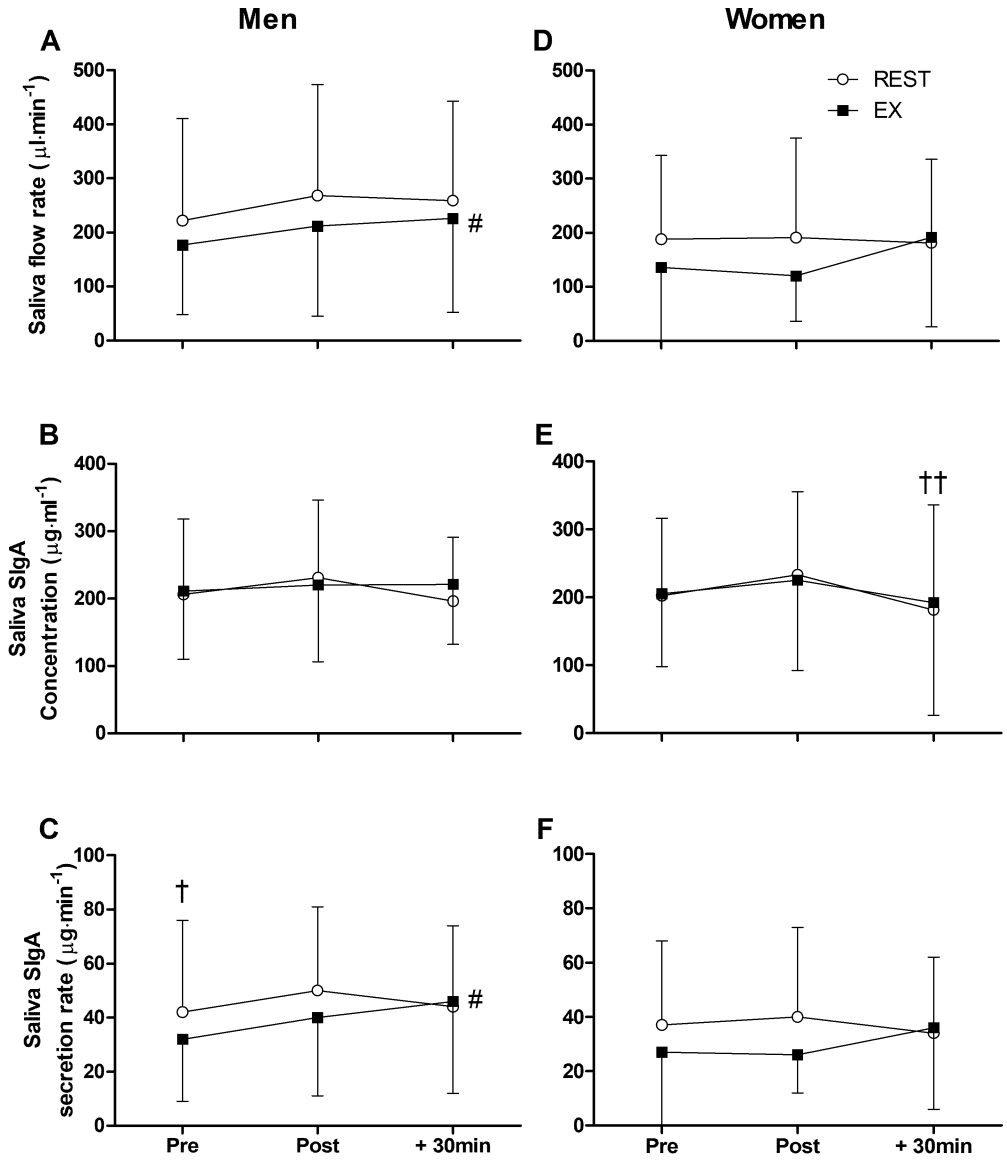


Figure 5.2. Saliva flow rate, SIgA concentration and SIgA SR before (pre), immediately after (post) and 30 min after (+ 30 min) 1 h of treadmill running at 65% $\dot{V}O_{2peak}$ (EX) or 1 h of seated rest (REST), for men (A, B and C, respectively) and women (D, E and F, respectively) (Study 1). # EX lower than REST, $P < 0.05$. † pre lower than post and + 30 min, $P < 0.05$. †† + 30 min lower than post, $P < 0.05$. Data are mean ± SD.

Perceived psychological stress was similar between EX and REST ($P > 0.05$; 14 ± 5 vs 15 ± 6 AU, respectively). State anxiety was higher during EX than REST (P < 0.05; 34 ±

11 vs 30 ± 9 AU, respectively). When examining men and women separately, women had higher trait anxiety, perceived psychological stress pre EX, perceived psychological stress pre REST, and state anxiety pre REST, compared to men ($P < 0.05$; trait anxiety 42 ± 11 vs 35 ± 9 AU, perceived psychological stress pre EX 16 ± 6 vs 13 ± 5 AU, perceived psychological stress pre REST 16 ± 6 vs 13 ± 5 AU, state anxiety pre REST 33 ± 11 vs 28 ± 7 AU, respectively). Men and women in Study 1 presented largely low-moderate levels of stress and anxiety, with few individuals reporting high perceived psychological stress or state anxiety (high perceived psychological stress (≥20 AU): men $N = 2$, women $N = 4$; high state anxiety (≥40 AU): men $N = 4$, women $N = 7$) (Spielberger, 1983; Cohen and Williamson, 1988; Khalili *et al.*, 2017).

5.4.2 The influence of psychological stress on the mucosal immune response to exercise

Participants with higher trait anxiety had lower saliva FR and SIgA SR during REST and following EX ($P < 0.05$; Table 5.1). Participants with higher perceived psychological stress had lower saliva FR during REST and EX, and lower SIgA SR during REST and following EX ($P < 0.05$; Table 5.1). Psychological stress and anxiety were not related to saliva SIgA concentration ($P > 0.05$).

Table 5.1. Pearson correlation coefficients (r) for the relationship between trait anxiety or perceived psychological stress and saliva flow rate (FR) and SIgA secretion rate (SR) pre, post, 30 min post and the change pre to post 1 h of treadmill running at 65% VO_{2peak} (EX) and 1 h of seated rest (REST) for all participants (Study 2). Significant interactions are in bold. *, $P < 0.05$; **, $P < 0.01$.

Variable	Trait anxiety		Perceived psychological stress	
	EX	REST	EX	REST
FR pre	-0.225	**-0.471****	**-0.281***	**-0.370****
FR post	**-0.375****	**-0.538****	**-0.316***	**-0.327***
FR 30 min post	**-0.416****	**-0.491****	**-0.373****	**-0.355***
FR change pre to post	-0.249	**-0.282***	-0.133	-0.045
SIgA SR pre	-0.182	**-0.326****	-0.230	**-0.272***
SIgA SR post	**-0.376****	**-0.450****	**-0.340***	**-0.284***
SIgA SR 30 min post	**-0.358***	**-0.392****	**-0.365***	**-0.294***
SIgA SR change pre to post	-0.199	-0.196	-0.173	-0.021

When examining men only, higher trait anxiety, perceived psychological stress and state anxiety were negatively correlated with the change in saliva SIgA SR pre to post EX ($P < 0.05$; Figure 5.3A, B & C) but not during REST ($P > 0.05$). Indeed, men with low psychological stress had an increase in saliva SIgA SR pre to post exercise whereas men with moderate perceived psychological stress saw no change ($P > 0.05$; Figure 5.4). Furthermore, higher perceived psychological stress, trait anxiety and state anxiety were negatively correlated with the change in saliva FR pre to post EX ($P < 0.05$; Figure 5.5A, B & C). Trait anxiety and state anxiety were inversely related to saliva FR change pre to post REST ($P < 0.05$; r = -0.44 & -0.38, respectively). Perceived psychological stress was not related to saliva FR change pre to post REST ($P > 0.05$). In women, there was no relationship between perceived psychological stress, trait anxiety or state anxiety and SIgA SR change pre to post EX ($P > 0.05$; Figure 5.3D, E & F, respectively) or REST ($P > 0.05$). Furthermore, in

women, no psychological variable was related to the saliva FR change pre to post EX (P > 0.05; Figure 5.5D, E & F) or REST (P > 0.05).

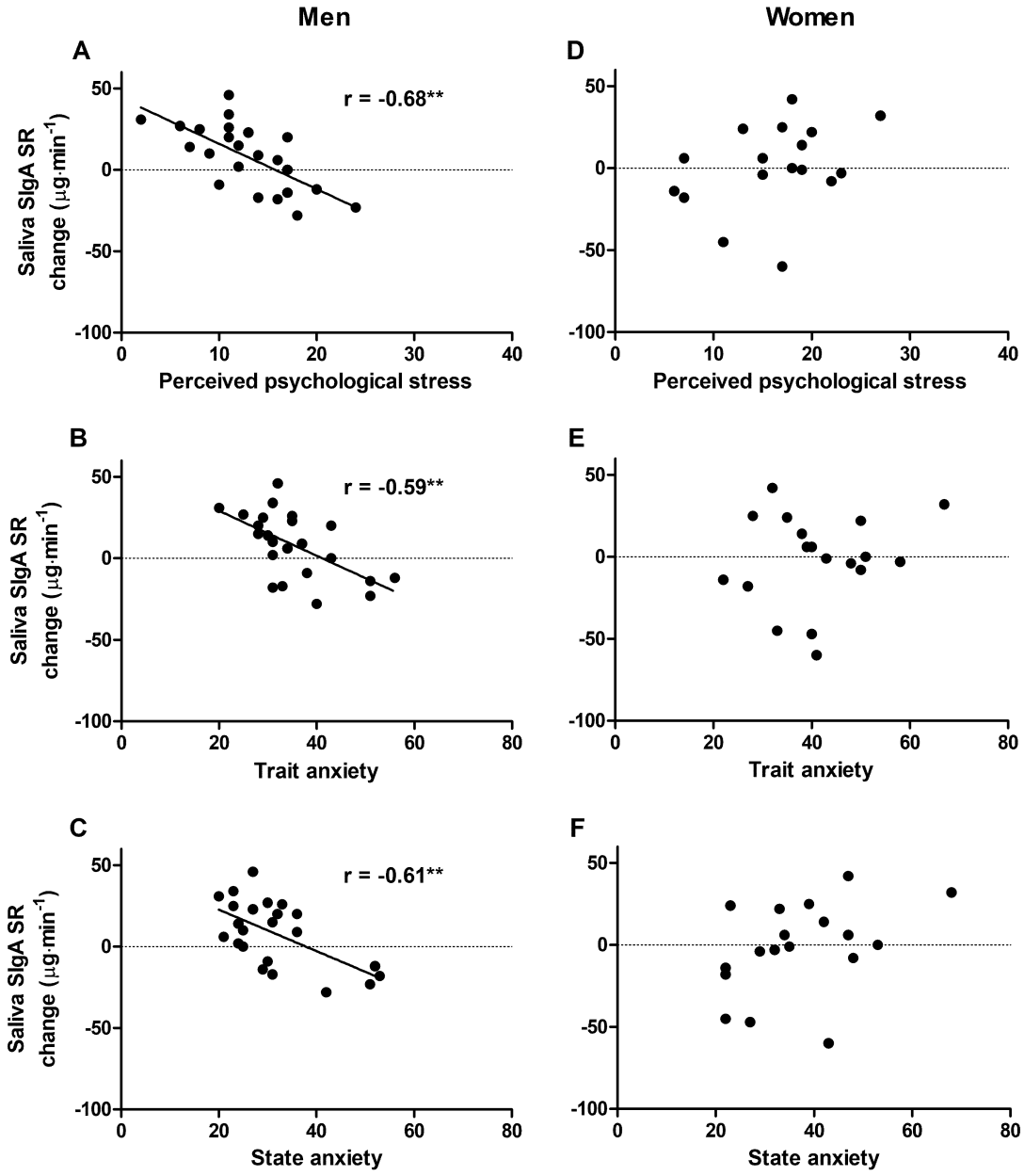


Figure 5.3. The relationship between saliva SIgA secretion rate (SR) change pre to post 1 h of treadmill running at 65% VO_{2peak} (EX) and perceived psychological stress, trait anxiety and state anxiety in men (A, B & C, respectively) and women (D, E & F, respectively) (Study 1). *, P < 0.05; **, P < 0.01.

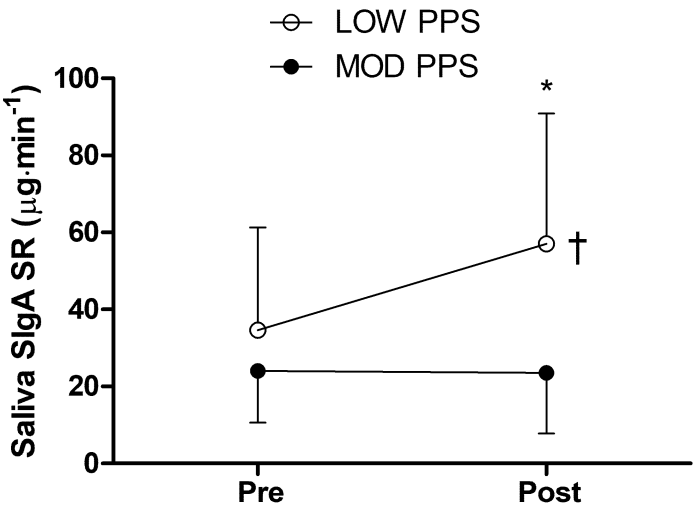


Figure 5.4. Saliva SIgA SR before (pre) and after (post) 1 h of treadmill running at 65% VO_{2peak} (EX) in men with low PPS (LOW; <12 AU) and moderate PPS (MOD; 12-19 AU) (Study 1). † higher at post than pre in the LOW PPS group, $P < 0.05$. * LOW PPS higher than MOD PPS at post, $P < 0.05$. Data are mean ± SD.

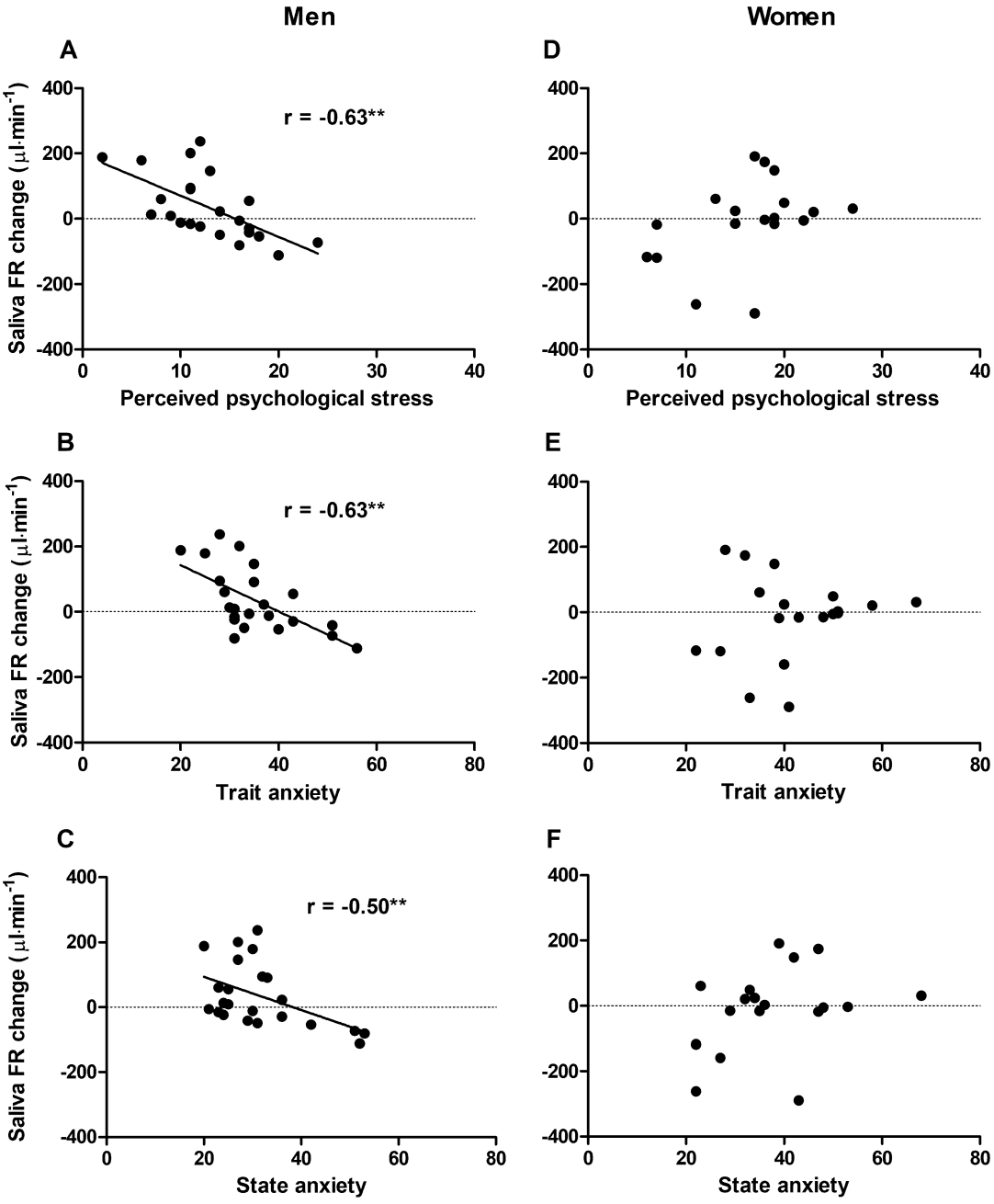


Figure 5.5. The relationship between **s**aliva flow rate (FR) change pre to post 1 h of treadmill running at 65% VO_{2peak} (EX) and perceived psychological stress, trait anxiety and state anxiety in men (A, B & C, respectively) and women (D, E & F, respectively) (Study 1). **, $P < 0.01$.

5.4.3 Study two

Saliva FR and SIgA SR decreased and SIgA concentration increased following the marathon ($P < 0.05$; FR 266 ± 208 vs 91 ± 65 μl·min^{-1}, SIgA SR 33 ± 24 vs 19 ± 14 μg·min^{-1}, SIgA concentration 165 ± 101 vs 247 ± 156 μg·min^{-1}, pre *vs*. post, respectively). Given that we found sex differences in Study 1, we then examined men and women separately and found that salivary SIgA SR decreased in men but not women ($P < 0.05$ & $P > 0.05$, Figure 5.6C & F, respectively). Saliva FR increased and SIgA concentration decreased in both men and women ($P < 0.05$; Figure 5.6A, B, D & E).

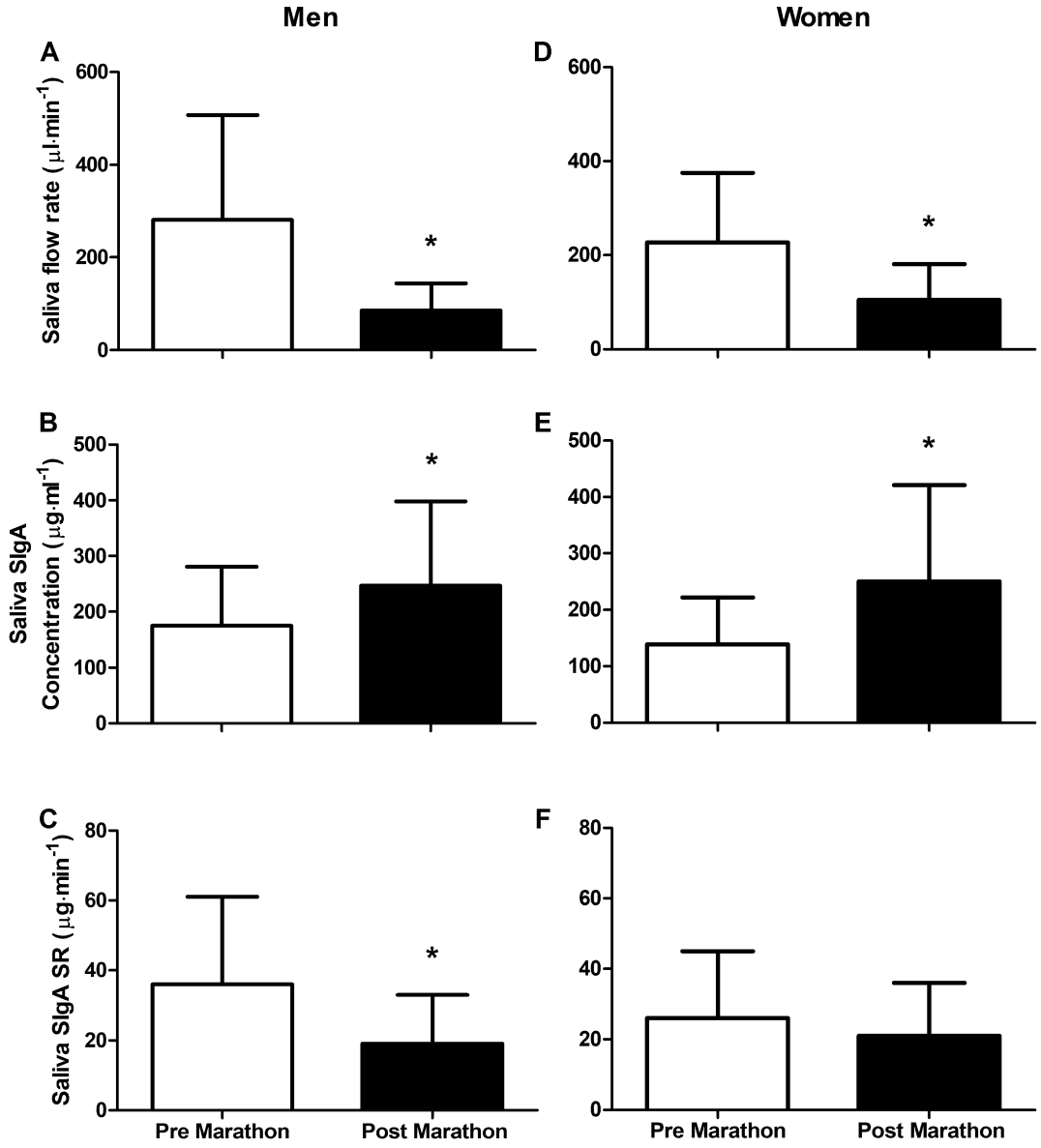


Figure 5.6. Saliva flow rate, SIgA concentration and SIgA secretion rate (SR) before (pre) and after (post) the marathon for men (A, B & C, respectively) and women (D, E & F, respectively) (Study 2). * different to pre marathon, $P < 0.05$. Data are mean ± SD.

Average perceived psychological stress was 14 ± 6 AU, which is similar to norms for adults of this age (Cohen and Williamson, 1988). Average state anxiety on the morning of the race was 40 ± 11 AU, which is similar to pre competition levels reported in collegiate athletes

(Wilson *et al.*, 2000) and higher than norms reported for working adults (Spielberger, 1983), and likely reflects the significance of the marathon event. As in Study 1, women had higher trait anxiety, perceived psychological stress and state anxiety compared to men ($P < 0.05$; trait anxiety 40 ± 9 vs 37 ± 9 AU, perceived psychological stress 17 ± 7 vs 13 ± 6 AU, state anxiety 44 ± 11 vs 38 ± 10 AU, respectively).

5.4.4 The influence of psychological stress on the mucosal immune response to exercise

When examining men and women collectively, psychological stress was not related to any salivary variable pre, post or the change pre to post marathon. However, aligning with Study 1, men with higher trait anxiety and perceived psychological stress had a greater reduction in saliva SIgA SR pre to post marathon ($P < 0.05$; $R^2 = 0.020$ and 0.031; unstandardized B (SE) = -0.394 (0.225) and -0.769 (0.361), respectively). Indeed, the reduction in saliva SIgA SR was significantly greater in high perceived psychological stress men compared to low perceived psychological stress men ($P < 0.05$; Figure 5.7). Furthermore, men with higher trait anxiety had a greater reduction in saliva FR pre to post marathon ($P < 0.05$; $R^2 = 0.028$; B (SE) = -3.924 (1.922)), which again aligns with Study 1. Perceived psychological stress was not related to salivary flow rate in men ($P > 0.05$). No psychological variables were related to saliva SIgA concentration in men ($P > 0.05$). State anxiety was not related to any salivary variable in men ($P > 0.05$). As in study 1, we saw no relationship between any psychological and salivary variable in women ($P > 0.05$).

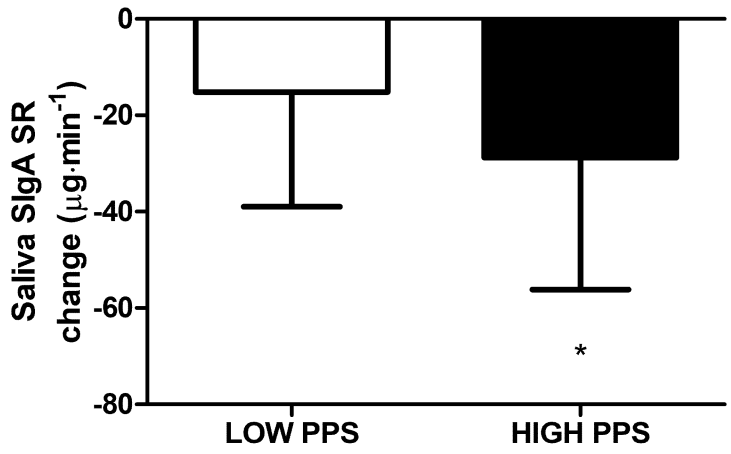


Figure 5.7. Saliva SIgA secretion rate (SR) change pre to post marathon in low perceived psychological stress (PPS; <12 AU) vs high PPS (≥20 AU), in men only (Study 2). * greater reduction compared to low PSS, $P < 0.05$. Data are mean ± SD.

5.5 Discussion

The aim of **Chapter 5** was to examine the role of psychological stress and anxiety on the mucosal immune response to exercise, in both a repeated-measures, crossover study, involving a moderate bout of lab-based exercise (Study 1), and a cohort observational study, involving a real-world marathon (Study 2). In support of our hypothesis, we found that psychological stress, trait anxiety and state anxiety influenced the mucosal immune response to exercise in men. In men, perceived psychological stress, trait anxiety, and state anxiety were negatively correlated with the mucosal immune response to exercise (Study 1 and 2). In response to a controlled, moderate bout of exercise, in individuals with predominantly low-moderate psychological stress and anxiety, men with lower psychological stress and anxiety experienced an increase in saliva SIgA SR, whereas those with moderate stress and anxiety saw little change (Study 1). Then, in a real world, high stress environment, men with higher

perceived psychological stress and trait anxiety had a greater reduction in mucosal immunity in response to a marathon (Study 2). Our novel findings support the recommendation that exercise physiologists should account for psychological stress and anxiety when examining the immune response to exercise.

These studies are the first to empirically show that psychological stress and anxiety influence the mucosal immune response to exercise. Specifically, we found that, in men, perceived psychological stress, trait anxiety, and state anxiety were negatively correlated with the mucosal immune response to exercise. Our findings align with recent work showing that *a priori* perceived psychological stress and state anxiety accounted for considerable variation in the *in vivo* immune response to a single bout of exercise, over and above the influence of exercise (R^2 = 0.13 and 0.20, respectively; $P < 0.05$) (Edwards *et al.*, 2018). Previously, exercise immunologists have presented mixed results when examining the influence of acute exercise bouts on mucosal immunity, reporting increases, decreases and no change in SIgA concentration and SIgA SR (Bishop *et al.*, 2000; Nieman *et al.*, 2002; Laing *et al.*, 2005; Bishop *et al.*, 2006; Nieman *et al.*, 2006; Oliver *et al.*, 2007; Davison *et al.*, 2009; Costa *et al.*, 2012; Killer *et al.*, 2015). A number of factors have previously been suggested to play a role in these mixed results, including exercise intensity and duration, varied saliva collection methods, and a lack of account of circadian variations (Bishop and Gleeson, 2009). However, our work indicates that psychological stress likely played a key role in previously reported mixed findings of the mucosal immune response to exercise. To date, no other studies examining the mucosal immune response to exercise have measured or accounted for psychological stress. Furthermore, numerous previous studies report blood sampling alongside saliva sampling (Bishop *et al.*, 2000; Nieman *et al.*, 2002; Laing *et al.*, 2005; Bishop *et al.*, 2006; Killer *et al.*, 2015), which would likely influence psychological stress and anxiety, and hence mucosal immune measures. Therefore, our findings may explain

previous mixed findings within the literature, given the marked influence of psychological stress and anxiety on the mucosal immune response to exercise shown here. Indeed, we show that individuals with low compared to moderate perceived psychological stress and anxiety have strikingly different immune responses to a moderate bout of exercise; men with low perceived psychological stress had an increase in saliva SIgA SR whereas men with moderate psychological stress saw no change.

Chronic stress, such as perceived psychological stress, leads to prolonged SNS activity and elevated cortisol, which downregulates SIgA synthesis and secretion (Walsh *et al.*, 2011b). However, acute stress can cause increased transocytosis of SIgA, through acute increases in SNS activity and catecholamines (Walsh *et al.*, 2011b). We found that, following a moderate bout of exercise (Study 1), individuals with lower perceived psychological stress had increased SIgA SR but those with moderate perceived psychological stress saw little change. Furthermore, when examining a longer exercise bout, we found that men with higher perceived psychological stress and trait anxiety had a greater reduction in SIgA SR following a marathon. Individuals performing a prolonged bout of exercise during periods of higher chronic psychological stress may exacerbate the immunosuppressive effects of chronic psychological stress, leading to greater reductions in mucosal immunity after exercise. Whereas, men with low psychological stress appear to experience a benefit from a moderate acute bout of exercise on mucosal immunity. Indeed, given that psychological and physical stress have the same effector limbs in the body, namely the HPA and SAM axes (Walsh, 2018), it is likely that the combined influence of both physical and psychological stressors dictates the direction and magnitude of the mucosal immune response to exercise.

In both Study 1 and 2, we found that psychological stress and anxiety played an important role in the mucosal immune response to exercise in men, but not women. Although there is relatively less research examining mucosal immune response to physical and

psychological stress in women, differences between men and women have been reported. Of the studies which account for menstrual phase and contraceptive use, males and females display subtle differences in the immune response to exercise, and women are reported to be at increased risk of URTI, but findings are inconsistent (Gillum *et al.*, 2011; Gillum *et al.*, 2014; He *et al.*, 2014; Drew *et al.*, 2017; Rutherfurd-Markwick *et al.*, 2017). Women have been suggested to experience exaggerated immunosuppression in response to chronic stress and may respond in a more pro-inflammatory fashion to an acute stressor (Bekhbat and Neigh, 2018). Furthermore, women and men display differences in the immune response to different types of stressors; men saw an increase whereas women saw a decrease in SIgA SR in response to an active stressor (mental arithmetic), but there were no differences in the mucosal immune response to a passive stressor (cold exposure) between men and women, where both sexes saw a reduction in SIgA SR (Willemsen *et al.*, 2002). Unfortunately, much of the existing literature on stress and immunity examines only male participants. Currently, the only study examining the influence of psychological stress on the immune response to exercise includes only men (Edwards *et al.*, 2018). It seems unlikely that psychological and physical stress do not play some role in immune modulation in women. Therefore, more work is required to determine differences between men and women in the immune consequences of physical and psychological stress.

Taken together, the findings of Study 1 and 2, which incorporate both field and lab-based exercise, accompanied by previous work in *in vivo* immunity (Edwards *et al.*, 2018), support the recommendation that exercise physiologists should account for psychological stress and anxiety when examining the immune response to exercise. Indeed, our findings highlight that psychological stress and anxiety have a marked influence on the mucosal immune response to exercise, which is of particular importance because mucosal immunity is the first line of defence against infection, and has been linked to increased URTI risk (Neville

et al., 2008; Hanstock *et al.*, 2016). As such, individuals with higher psychological stress and anxiety, particularly males, may be at increased risk of URTI following prolonged exercise. Therefore, future work should also examine whether interventions to manage psychological stress and anxiety can minimise the reduction in mucosal immunity following prolonged exercise, and subsequently reduce URTI incidence.

In conclusion, these findings show that perceived psychological stress, trait anxiety and state anxiety influence the mucosal immune response to exercise in men. In men, perceived psychological stress, trait anxiety, and state anxiety had a negative correlation with the mucosal immune response to both a moderate, controlled, lab-based exercise bout and a prolonged, field-based, marathon. Our novel findings support recommendations that exercise scientists should account for psychological stress and anxiety when examining the immune response to exercise.

CHAPTER SIX

General Discussion

6.1 Summary of main findings

The aims for this PhD were to understand: 1) the influence of vitamin D supplementation by oral vitamin D_3 or safe, low-level, simulated sunlight on URTI and mucosal immunity (**Chapter 3**); 2) the role of psychosocial factors, and sleep, on URTI risk in marathon runners (**Chapter 4**); and 3) the influence of psychological stress and anxiety on the mucosal immune response to exercise (**Chapter 5**). We found, in both a cohort, observational study and a randomised placebo-controlled trial (RCT), that vitamin D sufficiency reduced URTI burden in military recruits, but did not influence mucosal immunity (**Chapter 3**). In the second empirical chapter, a large, prospective, observational study in marathon runners, we found that runners were more likely to report a URTI if they reported greater psychological stress, anxiety, or neuroticism, adverse early life experience, or poorer perceived sleep quality (**Chapter 4**). Finally, we found that psychological stress and anxiety influenced the mucosal immune response to exercise; perceived psychological stress, trait anxiety and state anxiety had a negative relationship with the mucosal immune response to exercise in men (**Chapter 5**). The remainder of this chapter covers the main implications that arise from each study, the application of the findings to the broader literature, methodological limitations apparent in the thesis chapters, and suggests possible directions for future work.

6.2 Vitamin D, upper respiratory tract infection and mucosal immunity

6.2.1 Vitamin D sufficiency reduced URTI burden

The key finding from **Chapter 3** was that vitamin D sufficiency reduced URTI burden, which aligns with the recent proposal that vitamin D may improve immune tolerance of infectious pathogens through its anti-inflammatory properties (Walsh, 2019). For example, by limiting an over exuberant immune response, which can be wasteful and cause damage (Ayres and Schneider, 2012). Vitamin D's anti-inflammatory effects have already been shown to be beneficial in the treatment of multiple sclerosis, cancer and tuberculosis (Smolders *et al.*, 2010; Krishnan and Feldman, 2011; Coussens *et al.*, 2012). In agreement, vitamin D deficient endurance athletes were found to have longer URTI duration and severity compared to those with higher vitamin D status' (25(OH)D 30–50, 50–120, >120 nmol/L) (He *et al.*, 2013). Given the contemporary view, that evidence supporting immunosuppression in athletes is lacking, nutritional supplementation to improve immune tolerance may be a more appropriate goal in young, otherwise healthy, athletes and military personnel (Walsh, 2019). Recently, vitamin D supplementation has been suggested to have the potential to influence the novel coronavirus disease 2019 (COVID-19), which is currently causing a global pandemic. Acute respiratory distress syndrome (ARDS) is reported to occur secondary to COVID-19, and is caused by a number of mechanisms associated with inflammation, including cytokine storm and neutrophil activation (Quesada-Gomez *et al.*, 2020). Therefore, vitamin D supplementation has been proposed to be beneficial in reducing the severity of COVID-19, due to its anti-inflammatory properties (Laird *et al.*, 2020). Indeed, our findings of reduced duration and severity of URTI with vitamin D supplementation corroborate the suggestion that vitamin D has the potential to reduce the severity of respiratory infections. However, RCT's to confirm any benefits of vitamin D in treating or preventing COVID-19 are sorely needed (Lanham-New *et al.*, 2020).

During a secondary analysis, we found that vitamin D supplementation had greater beneficial effects in recruits with baseline 25(OH)D <50 nmol·L^{-1}: reduced URTI duration, as well as greater reductions in total days with URTI and URTI severity. Our findings are consistent with a recent meta-analysis, which found that vitamin D supplementation had strongest protective effects in those who began supplementation with poorer vitamin D status (Martineau *et al.*, 2017). However, it is worth noting that supplementation began in January and February in our study, when vitamin D status is at its annual nadir. Indeed, the majority (79%) of our participants had 25(OH)D <50 nmol·L^{-1} at baseline. Therefore, it is yet to be elucidated whether vitamin D supplementation would also reduce URTI burden in individuals who began vitamin D sufficient.

6.2.2 Vitamin D supplementation did not influence mucosal immunity

Vitamin D supplementation, which achieved vitamin D sufficiency in almost all participants, did not influence mucosal immunity, specifically salivary SIgA or cathelicidin. Our finding was in contrast to previous work showing that individuals with optimal vitamin D status (25(OH)D >120 nmol·L^{-1}) had higher saliva SIgA SR compared to those with 25(OH)D 12–30 nmol·L^{-1}, 30–50 nmol·L^{-1} or 50–120 nmol·L^{-1} (He *et al.*, 2013). However, 25(OH)D >120 nmol·L^{-1} is higher than the Institute of Medicine (IOM) and European Food Safety Association (EFSA) recommendation for vitamin D sufficiency of 25(OH)D ≥50 nmol·L^{-1} (Institute of Medicine, 2011; European Food Safety Authority, 2016), which was targeted in our study. Indeed, the same group then supplemented individuals with 5000 IU·day^{-1} of oral D_3, above the government guidelines for tolerable upper intake (4000 IU·d$_{-1}$) (European Food Safety Authority, 2016), and found significant increases in saliva SIgA and cathelicidin. Furthermore, a recent paper supplemented oral vitamin D_3 (1000 IU·day^{-1}) and calcium (2000 mg·day^{-1}) for 12 weeks and found only a modest increase in saliva SIgA SR at

week 4, and no influence of supplementation on SIgA at latter time points (Scott *et al.*, 2019). Vitamin D has been suggested to play a vital role in the upregulating AMP production through the active form $1,25(OH)_2D$ (He *et al.*, 2016a). However, our null findings, taken together with the ambiguous findings of the previous literature, suggest that vitamin D supplementation may not provide meaningful beneficial effects for mucosal immune function in otherwise healthy individuals. Indeed, the lack of influence of vitamin D supplementation on mucosal immunity aligns with our finding that vitamin D supplementation did not reduced URTI prevalence but instead reduced URTI burden, which may be due to increased immune tolerance rather than improved host defence.

6.2.3 No additional benefits of simulated-sunlight over oral D_3 supplementation

Despite the proposal that vitamin D supplementation by UVB radiation may convey additional benefits, the results of **Chapter 3** showed that there were no additional benefits of vitamin D supplementation by UVB irradiation compared to oral vitamin D_3 for URTI prevalence, duration or severity, or mucosal immunity. As we did not see any additional benefits of simulated sunlight exposure compared to oral D_3, and because UVB exposure increases time burden, increases the risk of erythema, and requires bulky irradiation cabinets, we recommend oral vitamin D_3 supplementation. Oral D_3 supplementation is simple and safe, has no risk of erythema, is effective regardless of sun-reactive skin type, and aligns with current IOM and EFSA recommendations. However, future work examining the long-term effects of simulated sunlight radiation for immune health, inflammation and chronic disease is warranted.

6.3 Psychological stress and anxiety influence upper respiratory tract infection and mucosal immunity

In **Chapters 4 and 5**, we found that psychological stress and anxiety influenced URTI risk and the mucosal immune response to exercise. First, we found that marathon runners were more likely to report an URTI if they reported higher trait anxiety, perceived psychological stress, neuroticism, state anxiety on the morning of the race, or greater perceived life hassles in the 24h leading up to the race (**Chapter 4**). Then, we found that perceived psychological stress, trait anxiety and state anxiety were negatively correlated with the mucosal immune response to exercise (**Chapter 5**). Our findings align with those examining the influence of psychological stress on URTI susceptibility in the general population, which show that psychological stress and anxiety play a key role in URTI susceptibility (Cohen *et al.*, 1991; Cohen, 1995; Cohen *et al.*, 1995). Indeed, our work provides empirical support for propositions made in recent review articles regarding the influence of psychological stress on immune modulation in exercising individuals (Walsh, 2018; Simpson *et al.*, 2020). The findings further recent research in Olympic athletes showing that those with higher perceived stress and more depression symptoms were more likely to self-report missing training due to medical illness during the month prior to completing the questionnaire (Drew *et al.*, 2017).

We found that higher trait anxiety, greater perceived psychological stress, and greater levels of neuroticism, were stronger predictors of URTI during the pre-marathon period, than the post-marathon period. Previous research has shown that athletes often experience increased anxiety and stress in the lead up to sporting competition (Ford *et al.*, 2017). Indeed, it may be that increased anxiety prior to competition, in individuals with higher trait anxiety and perceived psychological stress, plays a significant role in URTI susceptibility prior to

competition. Importantly, pre-competition illness has been shown to reduce competition performance (Svendsen *et al.*, 2016), increase the likelihood of not finishing a race (Van Tonder *et al.*, 2016; Gordon *et al.*, 2017), increase the risk of injury (Timpka *et al.*, 2017), and can lead to increased risk of serious complications (e.g. myositis and myopericarditis) (Van Tonder *et al.*, 2016). Therefore, pre-competition is one of the most important times to monitor and manage illness symptoms in athletes, and **section 6.7.4** will discuss future directions in this regard.

We also found that athletes reporting higher levels of neuroticism were at greater risk of reporting an URTI. Our findings are in agreement with other studies showing that individuals with higher negative affect were more likely to have a common cold following experimental exposure (Cohen *et al.*, 1993). Despite previous work suggesting that individuals higher in neuroticism may over-report symptoms (Cohen *et al.*, 1995; Johnson, 2003), we found that neuroticism was not related to URTI duration or severity. However, future work should use pathology confirmation of URTI to explore the influence of neuroticism on over-reporting URTI symptoms. Other personality traits, such as perfectionism, optimism, and alexithymia, have also received attention for their role in health and immune function (Guilbaud *et al.*, 2003; Segerstrom, 2003; Rasmussen *et al.*, 2009), indicating a route for future research to examine personality traits and infection risk.

Following on from our finding that URTI risk was influenced by psychological stress and anxiety in marathon runners, we examined the role of psychological stress and anxiety on the mucosal immune response to exercise (**Chapter 5**). We found that psychological stress and anxiety influenced the mucosal immune response to exercise. In men, perceived psychological stress, trait anxiety and state anxiety were negatively correlated with the mucosal immune response to exercise in both a controlled, moderate, lab-based bout of exercise and in a field-based marathon. Our findings may explain some of the mixed results

reported when examining the mucosal immune response to exercise; papers report increases, decreases and no change in SIgA concentration and SR (Bishop *et al.*, 2000; Nieman *et al.*, 2002; Laing *et al.*, 2005; Bishop *et al.*, 2006; Nieman *et al.*, 2006; Oliver *et al.*, 2007; Davison *et al.*, 2009; Costa *et al.*, 2012; Killer *et al.*, 2015). Indeed, no previous study has accounted for psychological stress when examining the mucosal immune response to exercise, and many include blood sampling, which is likely to influence psychological stress and anxiety (Bishop *et al.*, 2000; Nieman *et al.*, 2002; Laing *et al.*, 2005; Bishop *et al.*, 2006; Killer *et al.*, 2015). We saw markedly different mucosal immune responses to exercise between individuals with different levels of psychological stress and anxiety; individuals with low stress had increased SIgA SR in response to a moderate bout of exercise, whereas those with moderate stress saw no change. It is therefore unsurprising that previous work, which did not account for psychological stress when examining the mucosal immune response to exercise, saw contrasting results. Our findings add to recent literature, which showed that psychological stress and anxiety played a key role in the *in vivo* immune response to exercise (Edwards *et al.*, 2018). Taken together, our novel findings, and previous *in vivo* research (Edwards *et al.*, 2018), support the recommendation that exercise physiologists should account for psychological stress and anxiety when examining the immune response to exercise.

Our findings in **Chapter 5** provide some mechanistic explanation for the finding of increased URTI risk in **Chapter 4**. Indeed, we found that individuals reporting higher psychological stress and anxiety had lower saliva FR and SIgA SR at rest and following exercise (**Chapter 5**). Taken together with the finding that psychological stress had an influence on the mucosal immune response to exercise, our findings suggest that poorer mucosal immune defence in those with higher psychological stress and anxiety may be implicated in the increase URTI risk in those with higher psychological stress and anxiety.

6.3.1 Psychological stress and anxiety play a role in the mucosal immune response to exercise in men but not women

While there was a clear influence of psychological stress and anxiety on the mucosal immune response to exercise in men, we saw no influence in women (**Chapter 5**). Previous literature examining the immune response to exercise and psychological stress in women is sparse, and lacks rigour, as studies largely overlook menstrual phase and contraceptive use. Differences have been reported between men and women for their immune response to both exercise and psychological stress (Gomez *et al.*, 1993; Gillum *et al.*, 2014; He *et al.*, 2014; Stephens *et al.*, 2016; Birkett *et al.*, 2017). However, previous research has not examined the combined influence of psychological and physical stress on immunity in women. Indeed, one of the few papers that has confirmed an influence of psychological stress on the immune response to exercise included only male participants (Edwards *et al.*, 2018).

Women are more likely to report higher psychological stress and anxiety, and appear to be more vulnerable to the depressive effects of inflammation than men (Bekhbat and Neigh, 2018). Women more often suffer with autoimmune diseases, anxiety, depression, phobias, or panic disorders (Kudielka and Kirschbaum, 2005). Furthermore, women have been reported to be more susceptible to URTI (Drew *et al.*, 2017). Therefore, it seems likely that psychological factors play some role in immune modulation in women. In actuality, we are likely missing the bigger picture, due to heterogeneity in the literature caused by a lack of research accounting for menstrual phase and contraceptive use, as well as less research including female participants compared to male participants.

6.4 Early life adversity increases the risk of URTI in marathon runners

Another novel finding from **Chapter 4** was that runners who experienced an early life adverse experience (before the age of 18 years) were over two times more likely to have a URTI during the two weeks before a marathon. Although a number of review articles have hypothesised a relationship between ELA and immune dysregulation in adulthood (Fagundes *et al.*, 2013a; Elwenspoek *et al.*, 2017), our study is one of the first showing an empirical relationship between ELA and URTI. Indeed, the findings further initial work showing that ELA influences URTI risk; offspring of parents who were separated and not speaking were more susceptible to experimental common cold exposure during adulthood, compared to those whose parents were intact, or separated but still communicated (Murphy *et al.*, 2017). Early life adversity is thought to influence immune function in adulthood through elevated inflammatory responses, reduced ability to dampen inflammation, accelerated immunosenescence, reduced physiological stress reactivity and overly exaggerated psychological stress reactivity (Fagundes *et al.*, 2013a; Elwenspoek *et al.*, 2017). However, further research is required to elucidate the role of ELA in URTI risk and immune function.

We found that early life adversity predicted pre-marathon URTI, but there was no influence of ELA on post-marathon URTI. As mentioned in **section 6.3**, illness before competition has important implications for athletes. A potential explanation for the influence of ELA on pre but not post marathon URTI risk can be linked to research in emotional regulation. Individuals who have experienced early life adversity have lower emotional regulation capacity and are higher in alexithymia (a personality trait reflecting an inability to identify or describe one's own emotions) (Berenbaum, 1996; Poole *et al.*, 2017). Furthermore, research has shown that particularly challenging environments, such as events involving prolonged endurance exercise, provide opportunities for individuals to experience clear and intense emotions, which may be easier to understand than the emotions experienced

in everyday life. The experience and subsequent mastery of these emotions in the particular environment leads to improved emotional regulation, feelings of agency and mental health in other life domains; only individuals high in alexithymia experienced a significant reduction in anxiety following a marathon or ultramarathon (Woodman *et al.*, 2010; Roberts and Woodman, 2015; Woodman and Welch, 2020). Therefore, runners may not experience the same negative influence of ELA on URTI risk following the marathon, as a result of the emotional experience of the event itself. Although this hypothesis is reasonable, it remains largely speculative. Therefore, future work should investigate the relationship between ELA, emotional regulation, and illness incidence in athletes and around sporting events.

Our findings indicate a potential beneficial effect of the marathon on URTI risk in those with ELA, but longer-term, it is conceivable that individuals may also experience deleterious effects as a result of this coping mechanism. Individuals who seek out these types of events, in order to experience intense emotions, are likely to become acclimatised to these intense emotions over time, and consequently will receive diminished emotional regulation benefit from the same activity (Roberts and Woodman, 2015). Such individuals are then likely to seek out more extreme forms of exercise, in order to gain the emotional regulation benefits (Barlow *et al.*, 2015). While our work indicated that the increased risk of URTI in those who had early life adverse experiences was no longer present following the marathon event, it may be that increased prolonged activity may also bring about adverse outcomes, such as risks associated with extreme prolonged exercise and exercising when ill. Therefore, future work examining the long-term influence of exercise as a coping mechanism in individuals who have experienced ELA is warranted.

6.5 Poorer perceived sleep quality increases URTI risk in marathon runners

The strongest predictor of post-marathon URTI in **Chapter 4** was poorer perceived sleep quality; runners were two times more likely to have a post-marathon URTI if they reported poorer perceived sleep quality during the two weeks before or after the marathon. Although previous work has documented the influence of sleep duration and efficiency on URTI susceptibility (Cohen *et al.*, 2009; Prather *et al.*, 2015), we found that it was, in fact, an individual's perception of sleep quality that was the strongest predictor of URTI. Interestingly, this finding is consistent with research showing that women who perceived they had inadequate sleep were at 50% greater risk of pneumonia diagnosis (Patel *et al.*, 2012). Therefore, it seems that an individual's perception of their sleep is as important as, if not more important than, sleep duration or quality. Indeed, it may be that optimal sleep varies between individuals. For example, individuals may report a longer sleep duration but perceive it to be poor sleep because of a low perceived quality. Alternately, others may sleep for a relatively short length of time but perceive a higher sleep quality.

We did not find any influence of self-reported sleep duration on URTI risk. Such an effect is not wholly unsurprising as the current literature is mixed. Some studies have shown self-reported sleep to predict susceptibility to URTI (Cohen *et al.*, 2009). Indeed, a recent study found that military recruits self-reporting sleeping less than 6 h per night were four times more likely to be diagnosed with an URTI and lost more training days due to URTI compared to recruits reporting 7-9 h sleep per night (Wentz *et al.*, 2018). However, a study showed that behavioural-assessed, but not self-reported, sleep duration, predicted URTI risk (Prather *et al.*, 2015). Therefore, how researchers measure URTI duration may play a key role in whether they report a relationship to URTI susceptibility. Indeed, sleep measurement that

accounts for an individual's perception of combined sleep duration and quality may tell us more about an individual's URTI risk than behaviourally assessed sleep duration and efficiency.

6.6 Methodological limitations

One limitation of our research is that we do not have pathology confirmation of URTI. However, **Chapters 3 and 4** were conducted during winter and the common cold season, respectively, when one would expect most symptoms to be due to respiratory infection rather than allergy. Furthermore, a previous study using the same monitoring method showed that 82% of participants returned positive swab results for common cold viruses (Hanstock *et al.*, 2016). Additionally, in a study of Olympic athletes taking part in the Winter Olympic Games, aetiology of common cold was detected in 75% of athletes who experienced common cold symptoms (Valtonen *et al.*, 2019). However, future work should conduct pathological confirmation where possible, or look at training time lost to indicate meaningfulness of URTIs, in line with recent IOC consensus recommendations (International Olympic Committee *et al.*, 2020).

We did not find any influence of training distance on URTI incidence in **Chapter 4**. This finding was in contrast to previous marathon research, which found that runners with higher training load were more likely to have an infectious episode during the two month period before the LA marathon (Nieman *et al.*, 1990) and were more likely to report upper respiratory symptoms following an ultramarathon (Peters and Bateman, 1983). However, training load influence was not a key outcome of the study, as we only monitored self-reported training distance during the two weeks before the marathon, when runners were likely tapering, and during the two weeks after the marathon, when runners were likely

resting after the marathon. Instead, the key aim was to understand how psychosocial and behavioural factors, which are often overlooked in exercise immunology, play a role in URTI risk in exercising individuals. However, future work using prolonged and more rigorous training load monitoring, for example by calculating training impulse (TRIMP), may shed further light on the role of training load in URTI susceptibility, alongside factors such as psychological stress and sleep.

Although we saw differences between men and women for the influence of psychological stress on the mucosal immune response to exercise (**Chapter 5**), we could not examine the influence of psychosocial factors on URTI in females only in **Chapter 4**, due to a low proportion of female participants (36%), and insufficient information regarding menstrual phase and contraceptive use. Therefore, future work should examine the influence of psychological stress on URTI risk in women, when accounting for menstrual phase and contraceptive use.

6.7 Future directions

6.7.1 Vitamin D supplementation to maintain vitamin D sufficiency

In **Chapter 3**, we identified that vitamin D supplementation led to reduced URTI burden. We targeted vitamin D supplementation during the annual nadir, when 75% of participants had vitamin D status below government recommended sufficiency. Indeed, we saw greater effects of vitamin D supplementation for reducing URTI burden when examining recruits who began with poorer vitamin D status (25(OH)D <50 nmol·L^{-1}). A possible future direction would be to supplement oral vitamin D_3 throughout the summer, rather than to restore vitamin D status at the annual nadir. Doing so would allow us to examine whether

vitamin D supplementation reduces URTI burden regardless of vitamin D status, for example in vitamin D sufficient individuals. Furthermore, it would be interesting to explore whether vitamin D supplementation at URTI onset could reduce inflammation and therefore reduce URTI burden.

6.7.2 Anti-inflammatory effects of vitamin D supplementation

The key finding from **Chapter 3** was that vitamin D supplementation reduced URTI burden, but did not reduce URTI prevalence. Taken together with our finding that vitamin D supplementation did not influence mucosal immunity, it is plausible that the effects of vitamin D supplementation may be tolerogenic in nature, due to vitamin D's anti-inflammatory properties. Indeed, vitamin D supplementation has been shown to have anti-inflammatory effects in other research areas, such as the treatment of cancer, multiple sclerosis and tuberculosis (Smolders *et al.*, 2010; Krishnan and Feldman, 2011; Coussens *et al.*, 2012) and has been proposed as a potential aid in the current fight against the COVID-19 pandemic (Laird *et al.*, 2020). Unfortunately, we did not measure markers of inflammation. Therefore, future work should examine inflammatory markers in response to vitamin D supplementation, and whether individuals who are supplemented with vitamin D, and subsequently have an URTI, have lower inflammatory responses compared to those receiving placebo supplementation. Indeed, inflammatory markers may be lower both generally and during an infectious episode in those supplemented with vitamin D, indicating that vitamin D's anti-inflammatory properties play a role in immune tolerance.

6.7.3 Elite athlete and military populations

In **Chapter 4**, we found that runners reporting higher psychological stress and anxiety, early life adversity, or poorer perceived sleep quality, were at greater risk of reporting a URTI. Furthermore, in **Chapter 5**, we found that psychological stress and anxiety

influence the mucosal immune response to exercise. However, these individuals could largely be described as well-trained/recreationally active, as opposed to elite. Initial evidence suggests that there may indeed be differences between athletic populations; two papers from the same team found that there was an influence of psychological stress on URTI risk before but not after final Olympic team selection, despite no difference in psychological stress and anxiety, suggesting differences between more and less successful athletes (Drew *et al.*, 2017; Drew *et al.*, 2018). Indeed, these differences may suggest some survival bias in higher-level athletes, in line with the proposed "S-shaped curve" of infection risk (Malm, 2006; Drew *et al.*, 2018). Another interesting population to study would be military personnel, given that they experience multiple stressors during training and work; high physical demands, disrupted sleep, increased psychological stress, extreme environmental conditions, and nutritional deficits. Therefore, future work should explore the influence of psychosocial and sleep factors on infection risk and immunity in athletic and military populations.

6.7.4 Interventions to reduce psychological stress and anxiety, and improve sleep

In **Chapters 4 and 5**, we found that psychological stress and anxiety, and poorer perceived sleep quality, increased URTI risk. An interesting next step would be to examine whether interventions, which manage psychological stress and anxiety, provide individuals with coping mechanisms, and/or improve sleep, can reduce illness incidence and mitigate the reduction in mucosal immunity following a prolonged exercise bout. Cognitive, behavioural and mindfulness based approaches have been shown to be effective in reducing stress (Regehr *et al.*, 2013). Recent research has shown promising effects of mindfulness interventions for specific markers of inflammation, cell-mediated immunity, and biological aging (Black and Slavich, 2016). Indeed, eight weeks of mindfulness meditation increased antibody titers to influenza vaccination (Davidson *et al.*, 2003). Furthermore, cognitive behavioural therapy has shown promise in improving markers of immune function following

coronary artery bypass, in early-stage breast cancer, and in women with chronic insomnia following breast cancer (McGregor *et al.*, 2004; Savard *et al.*, 2005; Doering *et al.*, 2007).

Interventions to improve sleep may also prove beneficial in reducing URTI risk. Such as aiming for >7 h sleep per night, avoiding restricting sleep over many days and 'catching up', daytime napping, optimising sleep hygiene routines, ensuring darkness at bedtimes, managing caffeine intake, and encouraging good sleep/wake routines (Simpson *et al.*, 2017; Walsh, 2018). Although little empirical research has studied the influence of interventions to improve sleep on immunity and URTI risk, initial research shows some promise; sleep extension in professional rugby decreased cortisol and increased reaction times (Swinbourne *et al.*, 2018). Indeed, future research should examine whether interventions to manage psychological stress and anxiety, or improve sleep, reduce URTI risk or modulate immune function in active populations.

6.7.5 Sex differences in exercise immunology

As mentioned in **section 6.3.1**, we found a significant influence of psychological stress on the mucosal immune response to exercise in men. However, we did not see any influence of psychological stress on the mucosal immune response to exercise in women. Indeed, there is a distinct underrepresentation of women in numerous areas of exercise physiological research; a recent review of sports and exercise medicine research in three major Sport and Exercise Medicine journals demonstrated that 39% of participants were women compared to 61% men (Costello *et al.*, 2014). Where women are included in exercise immunological research, there is often a lack of control around menstrual phase and contraceptive use, or testing occurs when female sex hormone levels are low, to mimic male physiology, rather than truly studying the physiological effects of female sex hormones (de Jonge *et al.*, 2019). Studies examining the influence of sex on immunity have reported some

differences between men and women for URTI incidence and mucosal immune markers, although findings are inconsistent (Gillum *et al.*, 2014; He *et al.*, 2014; Drew *et al.*, 2017). Exercise scientists must make more effort to study the influence of exercise and other factors on immunity in women. Furthermore, research should study the influence of menstrual phases, rather than trying to minimise the effects of sex hormones, or seeing them as a confounding factor, given the likely important physiological implications of female hormones.

6.8 Conclusions

The major conclusion from **Chapter 3** was that vitamin D sufficiency, in line with government guidelines, reduced URTI burden. Specifically, vitamin D sufficient recruits were more likely to report a physician-diagnosed URTI compared to those with 25(OH)D <50 nmol·L^{-1}. Then, vitamin D supplementation, which achieved sufficiency in almost all (95%), reduced URTI burden, specifically total days with URTI and peak URTI severity. Interestingly, we found that recruits who began vitamin D supplementation with 25(OH)D <50 nmol·L^{-1} saw greater benefits; reduced URTI duration, and greater reductions in total days with URTI and peak URTI severity. Vitamin D supplementation did not reduce URTI incidence, or influence mucosal immunity, which suggests that vitamin D may improve immune tolerance, rather than host defence. We also examined the influence of vitamin D supplementation by simulated sunlight compared to oral vitamin D_3 and found no additional beneficial effects of simulated sunlight. Therefore, oral vitamin D_3 supplementation is an easy and effective supplementation strategy, which does not require significant time burden or bulky irradiation cabinets, is effective regardless of sun-reactive skin type, and has no risk of erythema.

In **Chapters 4 and 5**, we found that psychological stress and anxiety influence URTI susceptibility and the mucosal immune response to exercise. Marathon runners with higher psychological stress and anxiety were more likely to suffer with a URTI, and perceived psychological stress, trait anxiety and state anxiety had a negative relationship to the mucosal immune response to both a moderate, lab-based exercise bout and a field-based marathon. Taken together, **Chapters 4 and 5** indicate that psychological stress and anxiety play a key role in URTI risk and mucosal immunity in exercising individuals. Furthermore, our findings support the recommendation that exercise physiologists should account for psychological stress and anxiety when examining the immune response to exercise. We also found that runners reporting ELA were over two times more likely to report a pre-marathon URTI and runners reporting poorer perceived sleep quality were two times more likely to report a post-marathon URTI.

The findings of this thesis show that vitamin D supplementation reduces URTI burden in young, and otherwise healthy individuals. Our research also highlights the key role of psychological stress and anxiety in URTI risk and mucosal immunity, and the need for future research in exercise immunology to account for psychological stress and anxiety. Furthermore, we have highlighted the importance of an individual's perception of their sleep, and the role of new and novel factor, early life adversity, in URTI risk.

References

Advisory Group on Non-ionising Radiation (2017). Ultraviolet radiation, vitamin D and health. London, Public Health England**:** 7.

Allgrove J. E., Gomes E., Hough J. and Gleeson M. (2008). Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. *J Sports Sci* 26(6): 653-661.

Armstrong L. E., Kavouras S. A., Walsh N. P. and Roberts W. O. (2016). Diagnosing dehydration? Blend evidence with clinical observations. *Curr Opin Clin Nutr Metab Care* 19(6): 434-438.

Ayres J. S. and Schneider D. S. (2012). Tolerance of infections. *Annu Rev Immunol* 30: 271-294.

Baeke F., Takiishi T., Korf H., Gysemans C. and Mathieu C. (2010). Vitamin D: modulator of the immune system. *Curr Opin Pharmacol* 10(4): 482-496.

Barlow M., Woodman T., Chapman C., Milton M., Stone D., Dodds T. and Allen B. (2015). Who takes risks in high-risk sport?: the role of alexithymia. *J Sport Exerc Psychol* 37(1): 83-96.

Barrett B., Locken K., Maberry R., Schwamman J., Brown R., Bobula J. and Stauffacher E. A. (2002). The Wisconsin Upper Respiratory Symptom Survey (WURSS): a new research instrument for assessing the common cold. *J Fam Pract* 51(3): 265.

Baumeister D., Akhtar R., Ciufolini S., Pariante C. M. and Mondelli V. (2016). Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-alpha. *Mol Psychiatry* 21(5): 642-649.

Bekhbat M. and Neigh G. N. (2018). Sex differences in the neuro-immune consequences of stress: Focus on depression and anxiety. *Brain Behav Immun* 67: 1-12.

Berenbaum H. (1996). Childhood abuse, alexithymia and personality disorder. *J Psychosom Res* 41(6): 585-595.

Bergman P., Lindh A. U., Bjorkhem-Bergman L. and Lindh J. D. (2013). Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *PLoS One* 8(6): e65835.

Bermon S., Castell L. M., Calder P. C., Bishop N. C., Blomstrand E., Mooren F. C., Kruger K., Kavazis A. N., Quindry J. C., Senchina D. S., Nieman D. C., Gleeson M., Pyne D. B., Kitic C. M., Close G. L., Larson-Meyer D. E., Marcos A., Meydani S. N., Wu D., Walsh N. P. and Nagatomi R. (2017). Consensus Statement Immunonutrition and Exercise. *Exerc Immunol Rev* 23: 8-50.

Berry D. J., Hesketh K., Power C. and Hyppönen E. (2011). Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br J Nutr* 106(09): 1433-1440.

Besedovsky L., Lange T. and Haack M. (2019). The Sleep-Immune Crosstalk in Health and Disease. *Physiol Rev* 99(3): 1325-1380.

Bibbey A., Carroll D., Roseboom T. J., Phillips A. C. and de Rooij S. R. (2013). Personality and physiological reactions to acute psychological stress. *International Journal of Psychophysiology* 90(1): 28-36.

Bikle D. (2009). Nonclassic actions of vitamin D. *J Clin Endocrinol Metab* 94(1): 26-34.

Birkett M., Johnson L. and Gelety C. (2017). Investigation of Sex Differences In sIgA Response to the Trier Social Stress Test. *Stress Health* 33(2): 158-163.

Bishop N. C., Blannin A. K., Armstrong E., Rickman M. and Gleeson M. (2000). Carbohydrate and fluid intake affect the saliva flow rate and IgA response to cycling. *Med Sci Sports Exerc* 32(12): 2046-2051.

Bishop N. C. and Gleeson M. (2009). Acute and chronic effects of exercise on markers of mucosal immunity. *Front Biosci (Landmark Ed)* 14: 4444-4456.
Bishop N. C., Walker G. J., Scanlon G. A., Richards S. and Rogers E. (2006). Salivary IgA responses to prolonged intensive exercise following caffeine ingestion. *Med Sci Sports Exerc* 38(3): 513-519.
Black D. S. and Slavich G. M. (2016). Mindfulness meditation and the immune system: a systematic review of randomized controlled trials. *Ann N Y Acad Sci* 1373(1): 13-24.
Bosch J. A., Brand H. S., Ligtenberg A. J. M., Bermond B., Hoogstraten J. and Nieuw Amerongen A. V. (1998). The response of salivary protein levels and SIgA to an academic examination are associated with daily stress. *Journal of Psychophysiology* 12: 384-391.
Bosch J. A., Ring C., de Geus E. J., Veerman E. C. and Amerongen A. V. (2002). Stress and secretory immunity. *Int Rev Neurobiol* 52: 213-253.
Brandtzaeg P. (1981). Transport models for secretory IgA and secretory IgM. *Clin Exp Immunol* 44(2): 221-232.
Campbell J. P. and Turner J. E. (2018). Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan. *Front Immunol* 9: 648.
Cannon W. (1932). Wisdom of the Body. United States, W.W. Norton & Company.
Carins J. and Booth C. (2002). Salivary immunoglobulin-A as a marker of stress during strenuous physical training. *Aviat Space Environ Med* 73(12): 1203-1207.
Carroll D., Phillips A. C. and Der G. (2008). Body mass index, abdominal adiposity, obesity, and cardiovascular reactions to psychological stress in a large community sample. *Psychosom Med* 70(6): 653-660.
Carswell A. T., Oliver S. J., Wentz L. M., Kashi D. S., Roberts R., Tang J. C. Y., Izard R. M., Jackson S., Allan D., Rhodes L. E., Fraser W. D., Greeves J. P. and Walsh N. P. (2018). Influence of Vitamin D Supplementation by Sunlight or Oral D3 on Exercise Performance. *Med Sci Sports Exerc*.
Cashman K. D., Hill T. R., Lucey A. J., Taylor N., Seamans K. M., Muldowney S., Fitzgerald A. P., Flynn A., Barnes M. S., Horigan G., Bonham M. P., Duffy E. M., Strain J. J., Wallace J. M. and Kiely M. (2008). Estimation of the dietary requirement for vitamin D in healthy adults. *Am J Clin Nutr* 88(6): 1535-1542.
Charan J., Goyal J. P., Saxena D. and Yadav P. (2012). Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis. *J Pharmacol Pharmacother* 3(4): 300-303.
Chun R. F., Liu P. T., Modlin R. L., Adams J. S. and Hewison M. (2014). Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Front Physiol* 5: 151.
Clow A. and Hucklebridge F. (2001). The impact of psychological stress on immune function in the athletic population. *Exerc Immunol Rev* 7: 5-17.
Cobb J. M. and Steptoe A. (1996). Psychosocial stress and susceptibility to upper respiratory tract illness in an adult population sample. *Psychosom Med* 58(5): 404-412.
Cohen J. (1988). Statistical power analysis for the behavioral sciences. Hillsdale, NJ, Erlbaum.
Cohen S. (1995). Psychological stress and susceptibility to upper respiratory infections. *Am J Respir Crit Care Med* 152(4 Pt 2): S53-58.
Cohen S., Doyle W. J., Alper C. M., Janicki-Deverts D. and Turner R. B. (2009). Sleep habits and susceptibility to the common cold. *Arch Intern Med* 169(1): 62-67.

Cohen S., Doyle W. J., Skoner D. P., Fireman P., Gwaltney J. M., Jr. and Newsom J. T. (1995). State and trait negative affect as predictors of objective and subjective symptoms of respiratory viral infections. *J Pers Soc Psychol* 68(1): 159-169.

Cohen S., Kamarck T. and Mermelstein R. (1983). A global measure of perceived stress. *J Health Soc Behav* 24(4): 385-396.

Cohen S., Tyrrell D. A. and Smith A. P. (1991). Psychological stress and susceptibility to the common cold. *N Engl J Med* 325(9): 606-612.

Cohen S., Tyrrell D. A. and Smith A. P. (1993). Negative life events, perceived stress, negative affect, and susceptibility to the common cold. *J Pers Soc Psychol* 64(1): 131-140.

Cohen S. and Williamson G. M. (1988). Perceived Stress in a Probablity Sample of the United States. The social psychology of health. a. O. S. Spacapan S. Newbury Park, CA, Sage**:** 31-67.

Cole L. A., Ladner D. G. and Byrn F. W. (2009). The normal variabilities of the menstrual cycle. *Fertil Steril* 91(2): 522-527.

Costa P. T., Jr., McCrae R. R., Zonderman A. B., Barbano H. E., Lebowitz B. and Larson D. M. (1986). Cross-sectional studies of personality in a national sample: 2. Stability in neuroticism, extraversion, and openness. *Psychol Aging* 1(2): 144-149.

Costa R. J., Fortes M. B., Richardson K., Bilzon J. L. and Walsh N. P. (2012). The effects of postexercise feeding on saliva antimicrobial proteins. *Int J Sport Nutr Exerc Metab* 22(3): 184-191.

Costello J. T., Bieuzen F. and Bleakley C. M. (2014). Where are all the female participants in Sports and Exercise Medicine research? *Eur J Sport Sci* 14(8): 847-851.

Coussens A. K., Wilkinson R. J., Hanifa Y., Nikolayevskyy V., Elkington P. T., Islam K., Timms P. M., Venton T. R., Bothamley G. H., Packe G. E., Darmalingam M., Davidson R. N., Milburn H. J., Baker L. V., Barker R. D., Mein C. A., Bhaw-Rosun L., Nuamah R., Young D. B., Drobniewski F. A., Griffiths C. J. and Martineau A. R. (2012). Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. *Proc Natl Acad Sci U S A* 109(38): 15449-15454.

Davidson R. J., Kabat-Zinn J., Schumacher J., Rosenkranz M., Muller D., Santorelli S. F., Urbanowski F., Harrington A., Bonus K. and Sheridan J. F. (2003). Alterations in brain and immune function produced by mindfulness meditation. *Psychosom Med* 65(4): 564-570.

Davies J. H. and Shaw N. J. (2011). Preventable but no strategy: vitamin D deficiency in the UK. *Arch Dis Child* 96(7): 614-615.

Davison G., Allgrove J. and Gleeson M. (2009). Salivary antimicrobial peptides (LL-37 and alpha-defensins HNP1-3), antimicrobial and IgA responses to prolonged exercise. *Eur J Appl Physiol* 106(2): 277-284.

Davison G., Perkins E., Jones A. W., Swart G. M., Jenkins A. R., Robinson H. and Dargan K. (2020). ColdZyme(R) Mouth Spray reduces duration of upper respiratory tract infection symptoms in endurance athletes under free living conditions. *Eur J Sport Sci*: 1-23.

de Jonge X. J., Thompson B. and Han A. (2019). Methodological Recommendations for Menstrual Cycle Research in Sports and Exercise. *Med Sci Sports Exerc* 51(12): 2610-2617.

Dhabhar F. S. (2014). Effects of stress on immune function: the good, the bad, and the beautiful. *Immunol Res* 58(2-3): 193-210.

Dhabhar F. S. and McEwen B. S. (1997). Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. *Brain Behav Immun* 11(4): 286-306.

Diment B. C., Fortes M. B., Edwards J. P., Hanstock H. G., Ward M. D., Dunstall H. M., Friedmann P. S. and Walsh N. P. (2015). Exercise Intensity and Duration Effects on In Vivo Immunity. *Med Sci Sports Exerc* 47(7): 1390-1398.

Doering L. V., Cross R., Vredevoe D., Martinez-Maza O. and Cowan M. J. (2007). Infection, depression, and immunity in women after coronary artery bypass: a pilot study of cognitive behavioral therapy. *Altern Ther Health Med* 13(3): 18-21.

Dougherty L. R., Klein D. N. and Davila J. (2004). A growth curve analysis of the course of dysthymic disorder: the effects of chronic stress and moderation by adverse parent-child relationships and family history. *J Consult Clin Psychol* 72(6): 1012-1021.

Drew M., Vlahovich N., Hughes D., Appaneal R., Burke L. M., Lundy B., Rogers M., Toomey M., Watts D., Lovell G., Praet S., Halson S. L., Colbey C., Manzanero S., Welvaert M., West N. P., Pyne D. B. and Waddington G. (2018). Prevalence of illness, poor mental health and sleep quality and low energy availability prior to the 2016 Summer Olympic Games. *Br J Sports Med* 52(1): 47-53.

Drew M. K., Vlahovich N., Hughes D., Appaneal R., Peterson K., Burke L., Lundy B., Toomey M., Watts D., Lovell G., Praet S., Halson S., Colbey C., Manzanero S., Welvaert M., West N., Pyne D. B. and Waddington G. (2017). A multifactorial evaluation of illness risk factors in athletes preparing for the Summer Olympic Games. *J Sci Med Sport* 20(8): 745-750.

Dulson D. K. G., A. G.; Kilding, A. E.; Lu, J.; Pook, C. (2019). Does caffeine exert dose-response effects on salivary secretory IgA following prolonged submaximal running? *Translational Sports Medicine* 2(4): 178-185.

Edwards J. P., Walsh N. P., Diment P. C. and Roberts R. (2018). Anxiety and perceived psychological stress play an important role in the immune response after exercise. *Exerc Immunol Rev* 24: 26-34.

Ekblom B., Ekblom O. and Malm C. (2006). Infectious episodes before and after a marathon race. *Scand J Med Sci Sports* 16(4): 287-293.

Elwenspoek M. M. C., Kuehn A., Muller C. P. and Turner J. D. (2017). The effects of early life adversity on the immune system. *Psychoneuroendocrinology* 82: 140-154.

European Food Safety Authority (2016). Scientific opinion on dietary reference values for vitamin D, ESFA J. **14(10):** 1-145.

Fagundes C. P., Bennett J. M., Derry H. M. and Kiecolt-Glaser J. K. (2011). Relationships and Inflammation across the Lifespan: Social Developmental Pathways to Disease. *Soc Personal Psychol Compass* 5(11): 891-903.

Fagundes C. P., Glaser R. and Kiecolt-Glaser J. K. (2013a). Stressful early life experiences and immune dysregulation across the lifespan. *Brain Behav Immun* 27(1): 8-12.

Fagundes C. P., Glaser R., Malarkey W. B. and Kiecolt-Glaser J. K. (2013b). Childhood adversity and herpesvirus latency in breast cancer survivors. *Health Psychol* 32(3): 337-344.

Fahlman M. M. and Engels H. J. (2005). Mucosal IgA and URTI in American college football players: a year longitudinal study. *Med Sci Sports Exerc* 37(3): 374-380.

Fitzgerald D., Beckmans C., Joyce D. and Mills K. (2019). The influence of sleep and training load on illness in nationally competitive male Australian Football athletes: A cohort study over one season. *J Sci Med Sport* 22(2): 130-134.

Fitzpatrick T. B. (1988). The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 124(6): 869-871.

Ford J. L., Ildefonso K., Jones M. L. and Arvinen-Barrow M. (2017). Sport-related anxiety: current insights. *Open Access J Sports Med* 8: 205-212.

Fortes M. B., Diment B. C., Di Felice U. and Walsh N. P. (2012). Dehydration decreases saliva antimicrobial proteins important for mucosal immunity. *Appl Physiol Nutr Metab* 37(5): 850-859.
Gallagher S., Phillips A. C., Evans P., Der G., Hunt K. and Carroll D. (2008). Caregiving is associated with low secretion rates of immunoglobulin A in saliva. *Brain Behav Immun* 22(4): 565-572.
Ghilotti F., Pesonen A. S., Raposo S. E., Winell H., Nyren O., Trolle Lagerros Y. and Plymoth A. (2018). Physical activity, sleep and risk of respiratory infections: A Swedish cohort study. *PLoS One* 13(1): e0190270.
Gill S. K., Teixeira A. M., Rosado F., Hankey J., Wright A., Marczak S., Murray A. and Costa R. J. (2014). The impact of a 24-h ultra-marathon on salivary antimicrobial protein responses. *Int J Sports Med* 35(11): 966-971.
Gillum T., Kuennen M., Miller T. and Riley L. (2014). The effects of exercise, sex, and menstrual phase on salivary antimicrobial proteins. *Exerc Immunol Rev* 20: 23-38.
Gillum T. L., Kuennen M., Gourley C., Schneider S., Dokladny K. and Moseley P. (2013). Salivary antimicrobial protein response to prolonged running. *Biol Sport* 30(1): 3-8.
Gillum T. L., Kuennen M. R., Castillo M. N., Williams N. L. and Jordan-Patterson A. T. (2015). Exercise, but not acute sleep loss, increases salivary antimicrobial protein secretion. *J Strength Cond Res* 29(5): 1359-1366.
Gillum T. L., Kuennen M. R., Schneider S. and Moseley P. (2011). A review of sex differences in immune function after aerobic exercise. *Exerc Immunol Rev* 17: 104-121.
Ginde A. A., Mansbach J. M. and Camargo C. A., Jr. (2009). Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch.Intern.Med.* 169(4): 384-390.
Gleeson M., Bishop N., Oliveira M. and Tauler P. (2013). Influence of training load on upper respiratory tract infection incidence and antigen-stimulated cytokine production. *Scand J Med Sci Sports* 23(4): 451-457.
Gleeson M., Cripps A. W. and Clancy R. L. (1995a). Modifiers of the human mucosal immune system. *Immunol Cell Biol* 73(5): 397-404.
Gleeson M., McDonald W. A., Cripps A. W., Pyne D. B., Clancy R. L. and Fricker P. A. (1995b). The effect on immunity of long-term intensive training in elite swimmers. *Clin Exp Immunol* 102(1): 210-216.
Gleeson M., Nieman D. C. and Pedersen B. K. (2004a). Exercise, nutrition and immune function. *J Sports Sci* 22(1): 115-125.
Gleeson M. and Pyne D. B. (2000). Special feature for the Olympics: effects of exercise on the immune system: exercise effects on mucosal immunity. *Immunol Cell Biol* 78(5): 536-544.
Gleeson M., Pyne D. B. and Callister R. (2004b). The missing links in exercise effects on mucosal immunity. *Exerc Immunol Rev* 10: 107-128.
Gomez E., Ortiz V., Saint-Martin B., Boeck L., Diaz-Sanchez V. and Bourges H. (1993). Hormonal regulation of the secretory IgA (sIgA) system: estradiol- and progesterone-induced changes in sIgA in parotid saliva along the menstrual cycle. *Am J Reprod Immunol* 29(4): 219-223.
Gordon L., Schwellnus M., Swanevelder S., Jordaan E. and Derman W. (2017). Recent acute prerace systemic illness in runners increases the risk of not finishing the race: SAFER study V. *Br J Sports Med* 51(17): 1295-1300.
Gosling S. D. R., P. J.; Swann, W. B. Jr. (2003). A Very Brief Measure of the Big Five Personality Domains. *Journal of Research in Personality* 37: 504-528.

Guilbaud O., Corcos M., Hjalmarsson L., Loas G. and Jeammet P. (2003). Is there a psychoneuroimmunological pathway between alexithymia and immunity? Immune and physiological correlates of alexithymia. *Biomed Pharmacother* 57(7): 292-295.

Halliday T. M., Peterson N. J., Thomas J. J., Kleppinger K., Hollis B. W. and Larson-Meyer D. E. (2011). Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. *Med Sci Sports Exerc* 43(2): 335-343.

Hanstock H. G., Walsh N. P., Edwards J. P., Fortes M. B., Cosby S. L., Nugent A., Curran T., Coyle P. V., Ward M. D. and Yong X. H. (2016). Tear Fluid SIgA as a Noninvasive Biomarker of Mucosal Immunity and Common Cold Risk. *Med Sci Sports Exerc* 48(3): 569-577.

Hart P. H., Gorman S. and Finlay-Jones J. J. (2011). Modulation of the immune system by UV radiation: more than just the effects of vitamin D? *Nat Rev Immunol* 11(9): 584-596.

Hart P. H., Norval M., Byrne S. N. and Rhodes L. E. (2019). Exposure to Ultraviolet Radiation in the Modulation of Human Diseases. *Annu Rev Pathol* 14: 55-81.

Hausswirth C., Louis J., Aubry A., Bonnet G., Duffield R. and Y L. E. M. (2014). Evidence of disturbed sleep and increased illness in overreached endurance athletes. *Med Sci Sports Exerc* 46(5): 1036-1045.

He C. S., Aw Yong X. H., Walsh N. P. and Gleeson M. (2016a). Is there an optimal vitamin D status for immunity in athletes and military personnel? *Exerc Immunol Rev* 22: 42-64.

He C. S., Bishop N. C., Handzlik M. K., Muhamad A. S. and Gleeson M. (2014). Sex differences in upper respiratory symptoms prevalence and oral-respiratory mucosal immunity in endurance athletes. *Exerc Immunol Rev* 20: 8-22.

He C. S., Fraser W. D., Tang J., Brown K., Renwick S., Rudland-Thomas J., Teah J., Tanqueray E. and Gleeson M. (2016b). The effect of 14 weeks of vitamin D3 supplementation on antimicrobial peptides and proteins in athletes. *J Sports Sci* 34(1): 67-74.

He C. S., Handzlik M., Fraser W. D., Muhamad A., Preston H., Richardson A. and Gleeson M. (2013). Influence of vitamin D status on respiratory infection incidence and immune function during 4 months of winter training in endurance sport athletes. *Exerc Immunol Rev* 19: 86-101.

Hellard P., Avalos M., Guimaraes F., Toussaint J. F. and Pyne D. B. (2015). Training-related risk of common illnesses in elite swimmers over a 4-yr period. *Med Sci Sports Exerc* 47(4): 698-707.

Hewison M. (2012). Vitamin D and immune function: an overview. *P Nutr Soc* 71(01): 50-61.

Holick M. F. (2007). Vitamin D deficiency. *N Engl J Med* 357(3): 266-281.

Holick M. F. (2009). Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 19(2): 73-78.

Holick M. F., Binkley N. C., Bischoff-Ferrari H. A., Gordon C. M., Hanley D. A., Heaney R. P., Murad M. H., Weaver C. M. and Endocrine S. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 96(7): 1911-1930.

Ihalainen J. K., Schumann M., Hakkinen K. and Mero A. A. (2016). Mucosal immunity and upper respiratory tract symptoms in recreational endurance runners. *Appl Physiol Nutr Metab* 41(1): 96-102.

Ingram L. A., Simpson R. J., Malone E. and Florida-James G. D. (2015). Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise. *Brain Behav Immun* 47: 100-108.

Institute of Medicine (2011). Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC, The National Academies Press.

International Olympic Committee I., Illness Epidemiology Consensus G., Bahr R., Clarsen B., Derman W., Dvorak J., Emery C. A., Finch C. F., Hagglund M., Junge A., Kemp S., Khan K. M., Marshall S. W., Meeuwisse W., Mountjoy M., Orchard J. W., Pluim B., Quarrie K. L., Reider B., Schwellnus M., Soligard T., Stokes K. A., Timpka T., Verhagen E., Bindra A., Budgett R., Engebretsen L., Erdener U. and Chamari K. (2020). International Olympic Committee Consensus Statement: Methods for Recording and Reporting of Epidemiological Data on Injury and Illness in Sports 2020 (Including the STROBE Extension for Sports Injury and Illness Surveillance (STROBE-SIIS)). *Orthop J Sports Med* 8(2): 2325967120902908.

Jackson G. G., Dowling H. F., Spiesman I. G. and Boand A. V. (1958). Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. *AMA Arch Intern Med* 101(2): 267-278.

Johnson M. (2003). The vulnerability status of neuroticism: over-reporting or geuine complaints? *Personality and individual differences* 35: 877-887.

Juliff L. E., Halson S. L. and Peiffer J. J. (2015). Understanding sleep disturbance in athletes prior to important competitions. *J Sci Med Sport* 18(1): 13-18.

Jung H. C., Seo M. W., Lee S., Kim S. W. and Song J. K. (2018). Vitamin D(3) Supplementation Reduces the Symptoms of Upper Respiratory Tract Infection during Winter Training in Vitamin D-Insufficient Taekwondo Athletes: A Randomized Controlled Trial. *Int J Environ Res Public Health* 15(9).

Juzeniene A. and Moan J. (2012). Beneficial effects of UV radiation other than via vitamin D production. *Dermatoendocrinol* 4(2): 109-117.

Kananen L., Surakka I., Pirkola S., Suvisaari J., Lonnqvist J., Peltonen L., Ripatti S. and Hovatta I. (2010). Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. *PLoS One* 5(5): e10826.

Kashi D. S., Oliver S. J., Wentz L. M., Roberts R., Carswell A. T., Tang J. C. Y., Jackson S., Izard R. M., Allan D., Rhodes L. E., Fraser W. D., Greeves J. P. and Walsh N. P. (2020). Vitamin D and the hepatitis B vaccine response: a prospective cohort study and a randomized, placebo-controlled oral vitamin D3 and simulated sunlight supplementation trial in healthy adults. *Eur J Nutr*.

Keaney L. C., Kilding A. E., Merien F. and Dulson D. K. (2018). The impact of sport related stressors on immunity and illness risk in team-sport athletes. *J Sci Med Sport* 21(12): 1192-1199.

Kessler R. C., McLaughlin K. A., Green J. G., Gruber M. J., Sampson N. A., Zaslavsky A. M., Aguilar-Gaxiola S., Alhamzawi A. O., Alonso J., Angermeyer M., Benjet C., Bromet E., Chatterji S., de Girolamo G., Demyttenaere K., Fayyad J., Florescu S., Gal G., Gureje O., Haro J. M., Hu C. Y., Karam E. G., Kawakami N., Lee S., Lepine J. P., Ormel J., Posada-Villa J., Sagar R., Tsang A., Ustun T. B., Vassilev S., Viana M. C. and Williams D. R. (2010). Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br J Psychiatry* 197(5): 378-385.

Khalili R., Sirati Nir M., Ebadi A., Tavallai A. and Habibi M. (2017). Validity and reliability of the Cohen 10-item Perceived Stress Scale in patients with chronic headache: Persian version. *Asian J Psychiatr* 26: 136-140.

Kiecolt-Glaser J. K., Glaser R., Gravenstein S., Malarkey W. B. and Sheridan J. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proc Natl Acad Sci U S A* 93(7): 3043-3047.

Kiecolt-Glaser J. K., Gouin J. P., Weng N. P., Malarkey W. B., Beversdorf D. Q. and Glaser R. (2011). Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. *Psychosom Med* 73(1): 16-22.

Killer S. C., Svendsen I. S. and Gleeson M. (2015). The influence of hydration status during prolonged endurance exercise on salivary antimicrobial proteins. *Eur J Appl Physiol* 115(9): 1887-1895.

Kohut M. (2019). Exercise and psychneuroimmunology. *Current Opinion in Behavioral Sciences* 28: 152-162.

Kongsbak M., Levring T. B., Geisler C. and Von Essen M. R. (2013). The vitamin D receptor and T cell function. *Front Immunol* 4: 148.

Krishnan A. V. and Feldman D. (2011). Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. *Annu Rev Pharmacol Toxicol* 51: 311-336.

Kudielka B. M. and Kirschbaum C. (2005). Sex differences in HPA axis responses to stress: a review. *Biol Psychol* 69(1): 113-132.

Laaksi I., Ruohola J. P., Mattila V., Auvinen A., Ylikomi T. and Pihlajamaki H. (2010). Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. *J Infect Dis* 202(5): 809-814.

Laaksi I., Ruohola J. P., Tuohimaa P., Auvinen A., Haataja R., Pihlajamäki H. and Ylikomi T. (2007). An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. *Am J Clin Nutr* 86(3): 714-717.

Laing S. J., Gwynne D., Blackwell J., Williams M., Walters R. and Walsh N. P. (2005). Salivary IgA response to prolonged exercise in a hot environment in trained cyclists. *Eur J Appl Physiol* 93(5-6): 665-671.

Laird E., Rhodes J. and Kenny R. A. (2020). Vitamin D and Inflammation: Potential Implications for Severity of Covid-19. *Ir Med J* 113(5): 81.

Lamm M. E. (1998). Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense. *Am J Physiol* 274(4): G614-617.

Lanham-New S. A., Webb A. R., Cashman K. D., Buttriss J. L., Fallowfield J. L., Masud T., Hewison M., Mathers J. C., Kiely M., Welch A. A., Ward K. A., Magee P., Darling A. L., Hill T. R., Greig C., Smith C. P., Murphy R., Leyland S., Bouillon R., Ray S. and Kohlmeier M. (2020). Vitamin D and SARS-CoV-2 virus/COVID-19 disease. *BMJ Nutrition, Prevention & Health* 3(1): 106-110.

Lastella M., Roach G. D., Halson S. L., Martin D. T., West N. P. and Sargent C. (2015a). Sleep/wake behaviour of endurance cyclists before and during competition. *J Sports Sci* 33(3): 293-299.

Lastella M., Roach G. D., Halson S. L. and Sargent C. (2015b). Sleep/wake behaviours of elite athletes from individual and team sports. *Eur J Sport Sci* 15(2): 94-100.

Leeder J., Glaister M., Pizzoferro K., Dawson J. and Pedlar C. (2012). Sleep duration and quality in elite athletes measured using wristwatch actigraphy. *J Sports Sci* 30(6): 541-545.

Leicht C. A., Goosey-Tolfrey V. L. and Bishop N. C. (2018). Exercise intensity and its impact on relationships between salivary immunoglobulin A, saliva flow rate and plasma cortisol concentration. *Eur J Appl Physiol* 118(6): 1179-1187.

Lovallo W. R. (2013). Early life adversity reduces stress reactivity and enhances impulsive behavior: implications for health behaviors. *Int J Psychophysiol* 90(1): 8-16.

Lovallo W. R., Farag N. H., Sorocco K. H., Cohoon A. J. and Vincent A. S. (2012). Lifetime adversity leads to blunted stress axis reactivity: studies from the Oklahoma Family Health Patterns Project. *Biol Psychiatry* 71(4): 344-349.

Malm C. (2006). Susceptibility to infections in elite athletes: the S-curve. *Scand J Med Sci Sports* 16(1): 4-6.
Mao S. and Huang S. (2013). Vitamin D supplementation and risk of respiratory tract infections: a meta-analysis of randomized controlled trials. *Scand J Infect Dis* 45(9): 696-702.
Marsland A. L., Cohen S., Rabin B. S. and Manuck S. B. (2001). Associations Between Stress, Trait Negative Affect, Acute Immune Reactivity, and Antibody Response to Hepatitis B Injection in Healthy Young Adults. *Health Psychology* 20(1): 4-11.
Martensson S., Nordebo K. and Malm C. (2014). High Training Volumes are Associated with a Low Number of Self-Reported Sick Days in Elite Endurance Athletes. *J Sports Sci Med* 13(4): 929-933.
Martineau A. R., Jolliffe D. A., Hooper R. L., Greenberg L., Aloia J. F., Bergman P., Dubnov-Raz G., Esposito S., Ganmaa D., Ginde A. A., Goodall E. C., Grant C. C., Griffiths C. J., Janssens W., Laaksi I., Manaseki-Holland S., Mauger D., Murdoch D. R., Neale R., Rees J. R., Simpson S., Jr., Stelmach I., Kumar G. T., Urashima M. and Camargo C. A., Jr. (2017). Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *Br Med J* 356: i6583.
Matthews C. E., Ockene I. S., Freedson P. S., Rosal M. C., Merriam P. A. and Hebert J. R. (2002). Moderate to vigorous physical activity and risk of upper-respiratory tract infection. *Med Sci Sports Exerc* 34(8): 1242-1248.
Maughan R. J., Burke L. M., Dvorak J., Larson-Meyer D. E., Peeling P., Phillips S. M., Rawson E. S., Walsh N. P., Garthe I., Geyer H., Meeusen R., van Loon L. J. C., Shirreffs S. M., Spriet L. L., Stuart M., Vernec A., Currell K., Ali V. M., Budgett R. G., Ljungqvist A., Mountjoy M., Pitsiladis Y. P., Soligard T., Erdener U. and Engebretsen L. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *Br J Sports Med* 52(7): 439-455.
McDowell S. L., Chaloa K., Housh T. J., Tharp G. D. and Johnson G. O. (1991). The effect of exercise intensity and duration on salivary immunoglobulin A. *Eur J Appl Physiol Occup Physiol* 63(2): 108-111.
McGregor B. A., Antoni M. H., Boyers A., Alferi S. M., Blomberg B. B. and Carver C. S. (2004). Cognitive-behavioral stress management increases benefit finding and immune function among women with early-stage breast cancer. *J Psychosom Res* 56(1): 1-8.
Mersky J. P., Janczewski C. E. and Topitzes J. (2017). Rethinking the Measurement of Adversity. *Child Maltreat* 22(1): 58-68.
Miller G. E., Chen E. and Parker K. J. (2011). Psychological stress in childhood and susceptibility to the chronic diseases of aging: moving toward a model of behavioral and biological mechanisms. *Psychol Bull* 137(6): 959-997.
Mohren D. C., Swaen G. M., Borm P. J., Bast A. and Galama J. M. (2001). Psychological job demands as a risk factor for common cold in a Dutch working population. *J Psychosom Res* 50(1): 21-27.
Monlezun D. J., Bittner E. A., Christopher K. B., Camargo C. A. and Quraishi S. A. (2015). Vitamin D status and acute respiratory infection: cross sectional results from the United States National Health and Nutrition Examination Survey, 2001-2006. *Nutrients* 7(3): 1933-1944.
Murdoch D. R., Slow S., Chambers S. T., Jennings L. C., Stewart A. W., Priest P. C., Florkowski C. M., Livesey J. H., Camargo C. A. and Scragg R. (2012). Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. *JAMA* 308(13): 1333-1339.

Murphy M. L. M., Cohen S., Janicki-Deverts D. and Doyle W. J. (2017). Offspring of parents who were separated and not speaking to one another have reduced resistance to the common cold as adults. *Proc Natl Acad Sci U S A* 114(25): 6515-6520.
Neville V., Gleeson M. and Folland J. P. (2008). Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Med Sci Sports Exerc* 40(7): 1228-1236.
Nieman D. C. (1994). Exercise, infection, and immunity. *Int J Sports Med* 15 Suppl 3: S131-141.
Nieman D. C., Henson D. A., Dumke C. L., Lind R. H., Shooter L. R. and Gross S. J. (2006). Relationship between salivary IgA secretion and upper respiratory tract infection following a 160-km race. *J Sports Med Phys Fitness* 46(1): 158-162.
Nieman D. C., Henson D. A., Fagoaga O. R., Utter A. C., Vinci D. M., Davis J. M. and Nehlsen-Cannarella S. L. (2002). Change in salivary IgA following a competitive marathon race. *Int J Sports Med* 23(1): 69-75.
Nieman D. C., Johanssen L. M., Lee J. W. and Arabatzis K. (1990). Infectious episodes in runners before and after the Los Angeles Marathon. *J Sports Med Phys Fitness* 30(3): 316-328.
Office for National Statistics (2019). Sickness absence in the UK labour market: 2018.
Oliver S. J., Costa R. J., Laing S. J., Bilzon J. L. and Walsh N. P. (2009). One night of sleep deprivation decreases treadmill endurance performance. *Eur J Appl Physiol* 107(2): 155-161.
Oliver S. J., Laing S. J., Wilson S., Bilzon J. L., Walters R. and Walsh N. P. (2007). Salivary immunoglobulin A response at rest and after exercise following a 48 h period of fluid and/or energy restriction. *Br J Nutr* 97(6): 1109-1116.
Orzech K. M., Acebo C., Seifer R., Barker D. and Carskadon M. A. (2014). Sleep patterns are associated with common illness in adolescents. *J Sleep Res* 23(2): 133-142.
Ouellet-Morin I., Odgers C. L., Danese A., Bowes L., Shakoor S., Papadopoulos A. S., Caspi A., Moffitt T. E. and Arseneault L. (2011). Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children. *Biol Psychiatry* 70(11): 1016-1023.
Palmer-Green D., Fuller C., Jaques R. and Hunter G. (2013). The Injury/Illness Performance Project (IIPP): A Novel Epidemiological Approach for Recording the Consequences of Sports Injuries and Illnesses. *J Sports Med (Hindawi Publ Corp)* 2013: 523974.
Patel S. R., Malhotra A., Gao X., Hu F. B., Neuman M. I. and Fawzi W. W. (2012). A prospective study of sleep duration and pneumonia risk in women. *Sleep* 35(1): 97-101.
Pedersen A., Zachariae R. and Bovbjerg D. H. (2010). Influence of psychological stress on upper respiratory infection--a meta-analysis of prospective studies. *Psychosom Med* 72(8): 823-832.
Perera S., Uddin M. and Hayes J. A. (1997). Salivary lysozyme: a noninvasive marker for the study of the effects of stress of natural immunity. *Int J Behav Med* 4(2): 170-178.
Perna F. M., Schneiderman N. and LaPerriere A. (1997). Psychological stress, exercise and immunity. *Int J Sports Med* 18 Suppl 1: S78-83.
Peters E. M. and Bateman E. D. (1983). Ultramarathon running and upper respiratory tract infections. An epidemiological survey. *S Afr Med J* 64(15): 582-584.
Phillips A. C., Carroll D., Evans P., Bosch J. A., Clow A., Hucklebridge F. and Der G. (2006). Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. *Brain Behav Immun* 20(2): 191-197.

Poole J. C., Dobson K. S. and Pusch D. (2017). Anxiety among adults with a history of childhood adversity: Psychological resilience moderates the indirect effect of emotion dysregulation. *J Affect Disord* 217: 144-152.
Prather A. A., Janicki-Deverts D., Hall M. H. and Cohen S. (2015). Behaviorally Assessed Sleep and Susceptibility to the Common Cold. *Sleep* 38(9): 1353-1359.
Prather A. A. and Leung C. W. (2016). Association of Insufficient Sleep With Respiratory Infection Among Adults in the United States. *JAMA Intern Med* 176(6): 850-852.
Puterman E., Gemmill A., Karasek D., Weir D., Adler N. E., Prather A. A. and Epel E. S. (2016). Lifespan adversity and later adulthood telomere length in the nationally representative US Health and Retirement Study. *Proc Natl Acad Sci U S A* 113(42): E6335-E6342.
Quesada-Gomez J. M., Entrenas-Castillo M. and Bouillon R. (2020). Vitamin D receptor stimulation to reduce acute respiratory distress syndrome (ARDS) in patients with coronavirus SARS-CoV-2 infections: Revised Ms SBMB 2020_166. *J Steroid Biochem Mol Biol* 202: 105719.
Ramiro L. S., Madrid B. J. and Brown D. W. (2010). Adverse childhood experiences (ACE) and health-risk behaviors among adults in a developing country setting. *Child Abuse Negl* 34(11): 842-855.
Rasmussen H. N., Scheier M. F. and Greenhouse J. B. (2009). Optimism and physical health: a meta-analytic review. *Ann Behav Med* 37(3): 239-256.
Regehr C., Glancy D. and Pitts A. (2013). Interventions to reduce stress in university students: a review and meta-analysis. *J Affect Disord* 148(1): 1-11.
Rhodes L. E., Webb A. R., Fraser H. I., Kift R., Durkin M. T., Allan D., O'Brien S. J., Vail A. and Berry J. L. (2010). Recommended summer sunlight exposure levels can produce sufficient (> or =20 ng ml(-1)) but not the proposed optimal (> or =32 ng ml(-1)) 25(OH)D levels at UK latitudes. *J Invest Dermatol* 130(5): 1411-1418.
Ricardo J. S., Cartner L., Oliver S. J., Laing S. J., Walters R., Bilzon J. L. and Walsh N. P. (2009). No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise. *Eur J Appl Physiol* 105(3): 499-504.
Roberts R. and Woodman T. (2015). Contemporary personality perspectives in sport psychology. In S. Hanton and S. Mellalieu (Eds). . Contemporary advances in sport psychology: A review. Oxford, UK, Routledge**:** 1-27.
Rutherfurd-Markwick K., Starck C., Dulson D. K. and Ali A. (2017). Salivary diagnostic markers in males and females during rest and exercise. *J Int Soc Sports Nutr* 14: 27.
Sabetta J. R., DePetrillo P., Cipriani R. J., Smardin J., Burns L. A. and Landry M. L. (2010). Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. *PLoS One* 5(6): e11088.
Sargent C., Lastella M., Halson S. L. and Roach G. D. (2014). The impact of training schedules on the sleep and fatigue of elite athletes. *Chronobiol Int* 31(10): 1160-1168.
Sargent C. and Roach G. D. (2016). Sleep duration is reduced in elite athletes following night-time competition. *Chronobiol Int* 33(6): 667-670.
Savard J., Simard S., Ivers H. and Morin C. M. (2005). Randomized study on the efficacy of cognitive-behavioral therapy for insomnia secondary to breast cancer, part II: Immunologic effects. *J Clin Oncol* 23(25): 6097-6106.
Schwaiger M., Grinberg M., Moser D., Zang J. C., Heinrichs M., Hengstler J. G., Rahnenfuhrer J., Cole S. and Kumsta R. (2016). Altered Stress-Induced Regulation of Genes in Monocytes in Adults with a History of Childhood Adversity. *Neuropsychopharmacology* 41(10): 2530-2540.

Schwellnus M. P., Derman W. E., Jordaan E., Page T., Lambert M. I., Readhead C., Roberts C., Kohler R., Collins R., Kara S., Morris M. I., Strauss O. and Webb S. (2012). Elite athletes travelling to international destinations >5 time zone differences from their home country have a 2-3-fold increased risk of illness. *Br J Sports Med* 46(11): 816-821.

Scientific Advisory Committee on Nutrition (2016). Vitamin D and health. P. H. England. London.

Scott J. M., Kazman J. B., Palmer J., McClung J. P., Gaffney-Stomberg E. and Gasier H. G. (2019). Effects of vitamin D supplementation on salivary immune responses during Marine Corps basic training. *Scand J Med Sci Sports*.

Segerstrom S. C. (2003). Individual differences, immunity, and cancer: lessons from personality psychology. *Brain Behav Immun* 17 Suppl 1: S92-97.

Segerstrom S. C. and Miller G. E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol Bull* 130(4): 601-630.

Simpson N. S., Gibbs E. L. and Matheson G. O. (2017). Optimizing sleep to maximize performance: implications and recommendations for elite athletes. *Scand J Med Sci Sports* 27(3): 266-274.

Simpson R. J., Campbell J. P., Gleeson M., Kruger K., Nieman D. C., Pyne D. B., Turner J. E. and Walsh N. P. (2020). Can exercise affect immune function to increase susceptibility to infection? *Exerc Immunol Rev* 26: 8-22.

Skein M., Duffield R., Edge J., Short M. J. and Mundel T. (2011). Intermittent-sprint performance and muscle glycogen after 30 h of sleep deprivation. *Med Sci Sports Exerc* 43(7): 1301-1311.

Smolders J., Peelen E., Thewissen M., Cohen Tervaert J. W., Menheere P., Hupperts R. and Damoiseaux J. (2010). Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. *PLoS One* 5(12): e15235.

Spence L., Brown W. J., Pyne D. B., Nissen M. D., Sloots T. P., McCormack J. G., Locke A. S. and Fricker P. A. (2007). Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. *Med Sci Sports Exerc* 39(4): 577-586.

Spielberger C. D. (1983). Manual for the State-Trait Anxiety Inventory. Palo Alto, CA, Consulting Psychologists Press.

Steerenberg P. A., van Asperen I. A., van Nieuw Amerongen A., Biewenga A., Mol D. and Medema G. J. (1997). Salivary levels of immunoglobulin A in triathletes. *Eur J Oral Sci* 105(4): 305-309.

Stephens M. A., Mahon P. B., McCaul M. E. and Wand G. S. (2016). Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones. *Psychoneuroendocrinology* 66: 47-55.

Stephenson B. T., Hynes E., Leicht C. A., Tolfrey K. and Goosey-Tolfrey V. L. (2019). Brief Report: Training Load, Salivary Immunoglobulin A, and Illness Incidence in Elite Paratriathletes. *Int J Sports Physiol Perform* 14(4): 536-539.

Strazdins L., Meyerkort S., Brent V., D'Souza R. M., Broom D. H. and Kyd J. M. (2005). Impact of saliva collection methods on sIgA and cortisol assays and acceptability to participants. *J Immunol Methods* 307(1-2): 167-171.

Surtees P., Wainwright N., Day N., Brayne C., Luben R. and Khaw K. T. (2003). Adverse experience in childhood as a developmental risk factor for altered immune status in adulthood. *Int J Behav Med* 10(3): 251-268.

Surtees P. G., Wainwright N. W., Pooley K. A., Luben R. N., Khaw K. T., Easton D. F. and Dunning A. M. (2011). Life stress, emotional health, and mean telomere length in the European Prospective Investigation into Cancer (EPIC)-Norfolk population study. *J Gerontol A Biol Sci Med Sci* 66(11): 1152-1162.

Svendsen I. S., Taylor I. M., Tonnessen E., Bahr R. and Gleeson M. (2016). Training-related and competition-related risk factors for respiratory tract and gastrointestinal infections in elite cross-country skiers. *Br J Sports Med* 50(13): 809-815.
Swinbourne R., Miller J., Smart D., Dulson D. K. and Gill N. (2018). The Effects of Sleep Extension on Sleep, Performance, Immunity and Physical Stress in Rugby Players. *Sports (Basel)* 6(2).
Takatsuji K., Sugimoto Y., Ishizaki S., Ozaki Y., Matsuyama E. and Yamaguchi Y. (2008). The effects of examination stress on salivary cortisol, immunoglobulin A, and chromogranin A in nursing students. *Biomed Res* 29(4): 221-224.
Tang J. C. Y., Nicholls H., Piec I., Washbourne C. J., Dutton J. J., Jackson S., Greeves J. and Fraser W. D. (2017). Reference intervals for serum 24,25-dihydroxyvitamin D and the ratio with 25-hydroxyvitamin D established using a newly developed LC-MS/MS method. *J Nutr Biochem* 46: 21-29.
Tanner A. and Day S. (2017). The Effects of a 4-Week, Intensified Training, and Competition Period on Salivary Hormones, Immunoglobulin A, Illness Symptoms, and Mood State in Elite Synchronised Swimmers. *Sports (Basel)* 5(3).
Tharp G. D. and Barnes M. W. (1990). Reduction of saliva immunoglobulin levels by swim training. *Eur J Appl Physiol Occup Physiol* 60(1): 61-64.
Thomas D. T., Erdman K. A. and Burke L. M. (2016). American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc* 48(3): 543-568.
Timpka T., Jacobsson J., Bargoria V., Periard J. D., Racinais S., Ronsen O., Halje K., Andersson C., Dahlstrom O., Spreco A., Edouard P. and Alonso J. M. (2017). Preparticipation predictors for championship injury and illness: cohort study at the Beijing 2015 International Association of Athletics Federations World Championships. *Br J Sports Med* 51(4): 271-276.
Tiollier E., Gomez-Merino D., Burnat P., Jouanin J. C., Bourrilhon C., Filaire E., Guezennec C. Y. and Chennaoui M. (2005). Intense training: mucosal immunity and incidence of respiratory infections. *Eur J Appl Physiol* 93(4): 421-428.
Tomasi T. B., Trudeau F. B., Czerwinski D. and Erredge S. (1982). Immune parameters in athletes before and after strenuous exercise. *J Clin Immunol* 2(3): 173-178.
Tyrka A. R., Parade S. H., Price L. H., Kao H. T., Porton B., Philip N. S., Welch E. S. and Carpenter L. L. (2016). Alterations of Mitochondrial DNA Copy Number and Telomere Length With Early Adversity and Psychopathology. *Biol Psychiatry* 79(2): 78-86.
Tyrka A. R., Price L. H., Kao H. T., Porton B., Marsella S. A. and Carpenter L. L. (2010). Childhood maltreatment and telomere shortening: preliminary support for an effect of early stress on cellular aging. *Biol Psychiatry* 67(6): 531-534.
Usui T., Yoshikawa T., Orita K., Ueda S. Y., Katsura Y., Fujimoto S. and Yoshimura M. (2011). Changes in salivary antimicrobial peptides, immunoglobulin A and cortisol after prolonged strenuous exercise. *Eur J Appl Physiol* 111(9): 2005-2014.
Valtonen M., Waris M., Vuorinen T., Eerola E., Hakanen A. J., Mjosund K., Gronroos W., Heinonen O. J. and Ruuskanen O. (2019). Common cold in Team Finland during 2018 Winter Olympic Games (PyeongChang): epidemiology, diagnosis including molecular point-of-care testing (POCT) and treatment. *Br J Sports Med* 53(17): 1093-1098.
Van Tonder A., Schwellnus M., Swanevelder S., Jordaan E., Derman W. and Janse van Rensburg D. C. (2016). A prospective cohort study of 7031 distance runners shows that 1 in 13 report systemic symptoms of an acute illness in the 8-12 day period before

a race, increasing their risk of not finishing the race 1.9 times for those runners who started the race: SAFER study IV. *Br J Sports Med* 50(15): 939-945.
Vuichard Gysin D., Dao D., Gysin C. M., Lytvyn L. and Loeb M. (2016). Effect of Vitamin D3 Supplementation on Respiratory Tract Infections in Healthy Individuals: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *PLoS One* 11(9): e0162996.
Waldron J. C., Scarpa A., Kim-Spoon J. and Coe C. L. (2016). Adult Sexual Experiences as a Mediator Between Child Abuse and Current Secretory Immunoglobulin A Levels. *J Interpers Violence* 31(5): 942-960.
Walsh N. P. (2018). Recommendations to maintain immune health in athletes. *Eur J Sport Sci* 18(6): 820-831.
Walsh N. P. (2019). Nutrition and Athlete Immune Health: New Perspectives on an Old Paradigm. *Sports Med*.
Walsh N. P., Bishop N. C., Blackwell J., Wierzbicki S. G. and Montague J. C. (2002). Salivary IgA response to prolonged exercise in a cold environment in trained cyclists. *Med Sci Sports Exerc* 34(10): 1632-1637.
Walsh N. P., Blannin A. K., Clark A. M., Cook L., Robson P. J. and Gleeson M. (1999). The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. *J Sports Sci* 17(2): 129-134.
Walsh N. P., Gleeson M., Pyne D. B., Nieman D. C., Dhabhar F. S., Shephard R. J., Oliver S. J., Bermon S. and Kajeniene A. (2011a). Position statement part two: maintaining immune health. *Exerc Immunol Rev* 17: 64-103.
Walsh N. P., Gleeson M., Shephard R. J., Gleeson M., Woods J. A., Bishop N. C., Fleshner M., Green C., Pedersen B. K., Hoffman-Goetz L., Rogers C. J., Northoff H., Abbasi A. and Simon P. (2011b). Position statement. Part one: Immune function and exercise. *Exerc Immunol Rev* 17: 6-63.
Walsh N. P., Halson S. L., Sargent C., Roach G. D., Nedelec M., Gupta L., Leeder J., Fullagar H. H., Coutts A. J., Edwards B. J., Pullinger S. A., Robertson C. M., Burniston J. G., Lastella M., Le Meur Y., Hausswirth C., Bender A. M., Grandner M. A. and Samuels C. H. (2020). Sleep and the athlete: narrative review and 2021 expert consensus recommendations. *Br J Sports Med*.
Webb A. R., Kift R., Berry J. L. and Rhodes L. E. (2011). The vitamin D debate: translating controlled experiments into reality for human sun exposure times. *Photochem Photobiol* 87(3): 741-745.
Webb A. R., Kift R., Durkin M. T., O'Brien S. J., Vail A., Berry J. L. and Rhodes L. E. (2010). The role of sunlight exposure in determining the vitamin D status of the U.K. white adult population. *Br J Dermatol* 163(5): 1050-1055.
Wentz L. M., Ward M. D., Potter C., Oliver S. J., Jackson S., Izard R. M., Greeves J. P. and Walsh N. P. (2018). Increased Risk of Upper Respiratory Infection in Military Recruits Who Report Sleeping Less Than 6 h per night. *Mil Med* 183(11-12): e699-e704.
Whitham M., Laing S. J., Dorrington M., Walters R., Dunklin S., Bland D., Bilzon J. L. and Walsh N. P. (2006). The influence of an arduous military training program on immune function and upper respiratory tract infection incidence. *Mil Med* 171(8): 703-709.
Willemsen G., Carroll D., Ring C. and Drayson M. (2002). Cellular and mucosal immune reactions to mental and cold stress: associations with gender and cardiovascular reactivity. *Psychophysiology* 39(2): 222-228.

Wilson G. S., Raglin J. S. and Harger G. J. (2000). A comparison of the STAI and CSAI-2 in five-day recalls of precompetition anxiety in collegiate track and field athletes. *Scand J Med Sci Sports* 10(1): 51-54.

Woodman T., Hardy L., Barlow M. and Le Scanff C. (2010). Motives for participation in prolonged engagement high-risk sports: An agentic emotion regulation perspective. *Psychology of Sport and Exercise* 11: 345-352.

Woodman T. and Welch C. (2020). No pain no gain: Alexithymia and the anxiolytic effect of endurance running. *Manuscript submitted for publication*.

APPENDIX A

Example Informed Consent

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

1	Title of project	
2	Name and e-mail address(es) of all researcher(s)	Sophie Harrison (s.harrison@bangor.ac.uk) Prof. Neil Walsh (n.walsh@bangor.ac.uk)

Please provide two copies and tick boxes accordingly, if you agree:

1 I confirm that I have read and understand the Information Sheet dated ……. for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 **Please delete as appropriate**

(i) Patients:

I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected. ☐

(ii) Students:

I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive, the outcome of my period of study, or my standing with my supervisor or with other staff members of the School. ☐

(iii) General members of the public:

I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. ☐

3 (If appropriate) I consent to the information collected about me being used to support other research in the future, and may be shared anonymously with other researchers. ☐

4 I understand that I may register any complaint I might have about this experiment with Dr. Jamie MacDonald, Head of School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

5 I agree to take part in the above study. ☐

Name of Participant …….
Signature …… Date ……..
Name of Person taking consent…….
Signature …… Date ……..

APPENDIX B

Example Medical Questionnaire

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

Name of Participant ……..……..
Age ……
Are you in good health? ☐ YES ☐ NO

If no, please explain

How would you describe your present level of activity?
Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (minutes per session) ……

How often?

< Once per month		2-3 times per week	
Once per month		4-5 times per week	
Once per week		> 5 times per week	

Have you suffered from a serious illness or accident? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer from allergies? ☐ YES ☐ NO
If yes, please give particulars:

Do you suffer, or have you ever suffered from:

	YES	NO		YES	NO
Asthma			Epilepsy		
Diabetes			High blood pressure		
Bronchitis					

Are you currently taking medication? ☐ YES ☐ NO

If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? ☐ YES ☐ NO

If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment?

☐ YES ☐ NO

If yes, please give particulars:

Do you have a history of immune-related illness or cardiovascular disease?

☐ YES ☐ NO

If yes, please give particulars:

PLEASE READ THE FOLLOWING CAREFULLY
Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

PLEASE COMPLETE AND SIGN THE DECLARATION BELOW
DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of _____.

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature *(participant)* …….. Date ……..
Print name ……
Signature *(experimenter)* ……. Date ……..
Print name ……

APPENDIX C

Jackson Common Cold Questionnaire

For each sign of illness, please cross **one** box that describes how you feel **today**:

	Not at all	**Mild**	**Moderate**	**Severe**
Sneezing				
Headache				
Feeling generally unwell				
Runny nose				
Blocked nose				
Sore throat				
Cough				
Chilliness				