FACTORS INFLUENCING INFECTION RISK IN ENDURANCE ATHLETES

By

Ida Siobhan Svendsen

A thesis submitted to Loughborough University in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

Loughborough University

August 2015

ABSTRACT

High training loads or prolonged bouts of acute exercise can increase susceptibility to opportunistic infections. Such infections, although generally medically innocuous, can have profound negative implications for athletic performance. This thesis presents a series of studies investigating which factors influence infection risk in athletes, as well as exploring potential strategies to maintain immunocompetence during heavy training.

In Chapters 2 and 3, a large cohort of elite winter endurance athletes were followed over a number of years to determine patterns and frequency of illness in this population, and to identify training- and competition-related predictors of infection. Incidence rates and seasonal patterns of illness were found to be broadly similar to elite athletes from summer sports, and to the general population. Competition, air travel, greater day-to-day fluctuations in training load and lower performance level were significant predictors of illness.

When high training loads are combined with insufficient recovery, athletes may become overreached or overtrained. Previous studies suggest that increasing carbohydrate intake can be an effective means of preventing overreaching during periods of intense training. In Chapter 4 we therefore investigated the efficacy of carbohydrate supplementation in reducing immune disturbances and symptoms of overreaching. The lower carbohydrate does (20 g/h during exercise) was found to be equally effective in preserving immunity and power output as the higher dose (60 g/h), with modest immune and performance changes observed in both groups following eight days of intensified training.

Many athletes fail to ingest sufficient fluid to maintain euhydration during exercise. However, Chapter 5 found that moderate hypohydration, elicited by a 24 h period of fluid restriction, had little effect on immune responses to prolonged exercise.

Altitude training is an important component of the training process of most of today's elite endurance athletes. Chapters 6 and 7 explored the effects of acute and prolonged hypoxic training on immunity. Despite a somewhat augmented stress hormone response to exercise in hypoxia, altitude training was found to have little negative effect on host defence, providing relative exercise intensity at altitude and sea-level was matched.

ACKNOWLEDGEMENTS

There are so many people to whom I owe thanks for making these past three years such a positive experience. Below are just a few who deserve a special mention.

Firstly, my supervisor, Mike Gleeson. For giving me guidance and support when I needed it, and a little free rein when I wanted it. I hugely appreciate everything you've taught me and your wisdom, excellent tan and interminable repertoire of innuendos are things I will always aspire to! You will be sorely missed when you retire from academia.

Sophie Killer. You are not only a great friend, but someone I very much respect and admire. I still count my lucky stars that I got to work with you on my first two studies. In the absence of immunology puns, an exceptionally mediocre rhyme remains the only appropriate way to fully express my feelings (I can only hope no one else will ever read this!):

Those long days in the lab could have been really drab,
But the good times with you are what got me through.
Lycra-clad men, Skyfall, and cakes. Tiny hamster pillows and sunny quarry breaks.
So much more fun than it had any right to be; thanks for brightening up my Ph.D.!

Espen Tønnessen. For everything you've done for me and all the opportunities you've given me since I first sent you that e-mail five years ago. Your passion is contagious, and your high expectations of me have pushed me to be better in the hope that I can live up to them. I look forward to many more years working together.

Rebecca Randell, James Carter, Carl Hulston, Nikki Gibson, Sam Poole and Gareth Sandford. For your invaluable help on the training study. It would not have been possible without you.

Erlend Hem. For the hours you spent regulating the recompression chamber; for the things you've taught me, and the things you've tried and failed to teach me (primarily, how to appreciate fine wine). Your hard-earned approval means an awful lot to me.

Jamie Pringle. For the bike rides that helped keep me sane during the write-up, and for giving up your valuable time to read and feedback on my thesis.

Elisabeth Solbakken. For your help on the altitude studies and, more importantly, for your excellent company and friendship.

And finally, my parents, Deirdre and Jo Inge, and my brother, Jonas. For making me the person I am today and for being there for me, always.

TABLE OF CONTENTS

LIST OF PUBLICATIONS

Published

- **Svendsen IS**, Taylor IM, Tønnessen E, Bahr R, & Gleeson M (2016). Training-related and competition-related risk factors for respiratory tract and gastrointestinal infections in elite cross-country skiers. *Brit J Sports Med.*

- **Svendsen IS**, Killer SC, Carter JM, Randell RK, & Gleeson M (2016). Impact of intensified training and carbohydrate supplementation on immunity and markers of overreaching in highly trained cyclists. *Eur J Appl Physiol.*

- **Svendsen IS**, Gleeson M., Haugen TA, & Tønnessen E. (2015). Effect of an intense period of competition on race performance and self-reported illness in elite cross-country skiers. *Scand J Med Sci Sports*.

- **Svendsen IS**, Killer SC, & Gleeson M. (2014). Influence of hydration status on changes in plasma cortisol, leukocytes, and antigen-stimulated cytokine production by whole blood culture following prolonged exercise. *ISRN Nutrition*.

- Tønnessen E, **Svendsen IS**, Olsen IC, Guttormsen A, & Haugen T. (2015). Performance Development in Adolescent Track and Field Athletes According to Age, Sex and Sport Discipline. *PloS one*.

- Killer SC, **Svendsen IS**, & Gleeson M. (2015). The influence of hydration status during prolonged endurance exercise on salivary antimicrobial proteins. *Eur J Appl Physiol.*

- Killer, SC, **Svendsen IS**, & Gleeson, M (2015). Evidence of disturbed sleep and mood state in well-trained athletes during short-term intensified training with and without a high carbohydrate nutritional intervention. *J Sports Sci.*

- Wadley AJ, Killer SC, **Svendsen IS**, & Gleeson M. (2015). The impact of intensified training with a high or moderate carbohydrate feeding strategy on resting and exercise-induced oxidative stress. *Eur J Appl Physiol*.

- Tønnessen E, **Svendsen IS**, Rønnestad BR, Hisdal J, Haugen TA, & Seiler S. (2015). The Annual Training Periodization of 8 World Champions in Orienteering. *Int J Sports Physiol Perform*.

- Heaney JLJ, Gleeson M, Phillips AC, Taylor IM, Drayson MT, Goodall M, He CS, **Svendsen IS**, Killer SC, & Campbell JP (2016). Salivary immunoglobulin free light

1

chains: reference ranges and responses to exercise in young and older adults. *Exer Immunol Rev*.

- Tønnessen E, Sylta Ø, Haugen TA, Hem E, **Svendsen IS**, & Seiler S. (2014). The road to gold: training and peaking characteristics in the year prior to a gold medal endurance performance. *PloS one*.

- Haugen TA, Tønnessen E, **Svendsen IS**, & Seiler S. (2014). Sprint time differences between single-and dual-beam timing systems. *J Strength Cond Res*.

- Tønnessen E, Rasdal V, **Svendsen IS,** Haugen TA, Hem E, Sandbakk Ø (2015). The Training Characteristics of World-Class Nordic Combined Athletes: Concurrent Optimization of Endurance Capacity and Explosiveness. *Int J Sports Physiol Perform.*

Accepted

- **Svendsen IS**, Hem E, & Gleeson M. Effect of acute exercise and hypoxia on markers of systemic and mucosal immunity. *Eur J Appl Physiol*.

Submitted

- Wadley AJ, **Svendsen IS**, & Gleeson M. Heightened exercise-induced oxidative stress at simulated training level altitude vs. sea level in trained cyclists. *Int J Sport Nutr & Exercise Metabolism.*

In Preparation

- Killer SC, **Svendsen IS,** & Gleeson M. Plasma and Urinary Metabolomics for Monitoring Training Stress in Well-Trained Athletes.

- Killer SC, **Svendsen IS**, Randell RK, Carter JM, Jeukendrup AE, & Gleeson M. The Physical, Mental and Hormonal Responses to Short-Term Intensified Training in Well-Trained Cyclists with a High Carbohydrate Nutritional Intervention.

- Heaney JLJ, Gleeson M, Drayson MT, **Svendsen IS**, Killer SC, & Campbell JP. Intensified training increases salivary free light chains in trained cyclists: periods of high training volume may increase oral inflammation

Conference proceedings

- **Svendsen IS**, Killer SC & Gleeson M. Influence of Prolonged Exercise and Hydration Status on Antigen-Stimulated Cytokine Production by Whole Blood Culture.

International Society of Exercise and Immunology Conference 2013, Newcastle, Australia. Oral Presentation.

- **Svendsen IS**, Killer SC, Carter J, Randell RK, Gibson NE, Jeukendrup AE, & Gleeson M. Effect of carbohydrate supplementation during intensified training on markers of immunity in highly trained cyclists. *American College of Sports Medicine Conference 2014, Orlando, USA. Poster Presentation.*

- **Svendsen IS**, Hem E, Tønnessen E, & Gleeson, M. Effect of altitude training on mucosal immunity and sleep in elite cross-country skiers. *European College of Sport Science Annual Congress 2015, Malmo, Sweden. Mini-oral Presentation.*

- Killer SC, **Svendsen IS**, Carter JM, Randell RK, Pool S, Sandford GN, Jeukendrup AE & Gleeson M. The Physical, Mental and Hormonal Responses to Short-Term Intensified Training in Well-Trained Cyclists with a High Carbohydrate Nutritional Intervention. *World Congress of Cycling Science 2014, Leeds, UK. Oral Presentation.*

- Killer SC, **Svendsen IS** & Gleeson M. The Influence of Hydration Status during Prolonged Endurance Exercise on Salivary Antimicrobial Proteins. *International Sport and Exercise Nutrition Conference 2013, Newcastle, UK. Poster Presentation.*

- Killer SC, **Svendsen IS**, Carter JM, Randell RK, Pool S, Sandford GN, Jeukendrup AE & Gleeson M. Impact of Short Term Intensified Training On Sleep Quality and Mood State in Well Trained Cyclists. *American College of Sports Medicine Conference 2014, Orlando, USA. Poster Presentation.*

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Meaning
ACSM	American College of Sports Medicine
ACTH	Adrenocorticotropic hormone
AMP	Antimicrobial protein
ANOVA	Analysis of variance
CHO	Carbohydrate
CI	Confidence interval
CON	Control group
DH	Dehydrated condition
DTaP/IPV/Hib	Vaccine containing diphtheria, tetanus, pertussis, polio, and Haemophilus influenzae type b antigens
EDTA	Ethylenediaminetetraacetic acid
EGF	Endothelial growth factor
ELISA	Enzyme-linked immunosorbent assay
ES	Effect Size
EU	Euhydrated condition
FIS	International Ski Federation
H	Hypoxic condition
Hb	Haemoglobin concentration
H-CHO	High carbohydrate condition
HCT	Haematocrit
HIT	High-intensity training
HR	Heart rate
IFN-γ	Interferon gamma
IOC	International Olympic Committee
IL	International level
IL-	Interleukin-
IT	Intensified training
kg bm	Kilograms body mass
L-CHO	Low carbohydrate condition
LL-37	Cathelicidin LL-37
LIT	Low-intensity training

LPS	Lipopolysaccharide
masl	Metres above sea level
MCP-1	Monocyte chemoattractant protein-1
N	Normoxic condition
NK	Natural killer cell
OR	Overreaching
OT	Overtraining
PBMCs	Peripheral blood mononuclear cells
PV	Plasma volume
RBC	Red blood cell concentration
RPE	Rating of perceived exertion
S	Antigen-stimulated
SD	Standard deviation
SE	Sleep efficiency
S-IgA	Salivary secretory immunoglobulin-A
SL	Sea level
TDS	Tour de Ski
TNF-α	Tumour necrosis factor alpha
Treg	T regulatory cell
TRIMP	Training impulse
URI	Upper-respiratory infection
URS	Upper-respiratory symptoms
US	Unstimulated
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Minute ventilation
VEGF	Vascular endothelial growth factor
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
WL	World-leading level
XC	Cross-country

CHAPTER 1: GENERAL INTRODUCTION

There is a general consensus that moderate levels of physical activity are beneficial for multiple aspects of health, including immune function (Nieman and Pedersen, 1999, Klentrou et al., 2002). However, high training loads or prolonged, intense bouts of acute exercise may actually increase susceptibility to opportunistic infections, particularly of the upper-respiratory tract (Nieman, 1994, Moreira et al., 2009). As such, the relationship between infection susceptibility and exercise load has often been described as a J-shaped curve, although this is not always supported in the literature (Fondell et al., 2011; Malm, 2006).

Whatever the influence on immunity, reaching an international level in any sport invevitably requires a huge amount of dedicated training. For elite endurance athletes, high training loads are therefore inevitable. Depending on the sport, these athletes typically train between 500 hours (e.g. long distance running) (Billat et al., 2001, Billat et al., 2003) to in excess of 1000 hours per year (e.g. rowing, cycling, triathlon) (Fiskerstrand and Seiler, 2004, Neal et al., 2011, Zapico et al., 2007).

With medals often being decided by seconds or fractions of seconds, even minor infections generally considered medically innocuous can meaningfully and deleteriously impact on performance, and on an athlete's career. This is compounded by the fact that the risk of such infections is typically highest during important periods of heavy training and competition (Hellard et al., 2015, Nieman et al., 1990).

1.1 INFECTION INCIDENCE IN ATHLETES

Upper respiratory infection (URI) is the most common medical complaint affecting athletes (Fricker et al., 2005, Reeser et al., 2003). Anecdotally, athletes and coaches report that elite

and highly trained endurance athletes experience a greater number of URIs than the general population, but there is currently little empirical evidence in the scientific literature to support this theory. Indeed, studies suggest that there is little difference in overall infection rates between athletes and the general population (Fricker et al., 2000, Hellard et al., 2015, Monto, 2002; Fondell et al., 2011), but that URI episodes in athletes typically cluster around competitions and intensified training periods rather than following normal seasonal trends (Walsh et al., 2011b). Most likely, athletes are not at substantially higher risk of infection compared to the general population, other than during the periods they are competing or undertaking particularly high training loads (Pyne and Gleeson, 1998). Malm (2006) suggested that the relationship between training load and infection susceptibility is more akin to an S-shape than a J-shape, proposing that, although high training loads typically suppress immunity, in order to be a successful elite athlete an individual must have an immune system able to withstand infections, even during severe physiological and psychological stress.

A number of studies have investigated sex-differences in infection susceptibility. In the general population, males appear to be somewhat more prone to respiratory infections (Falagas et al., 2007) likely due to a combination of anatomic, lifestyle, behavioural, and socioeconomic differences between males and females, and to the immunomodulatory effects of sex hormones (Verthelyi, 2001). Oestrogens generally enhance the immune response, while androgens suppress both cellular and humoral immunity. However, a number of recent studies on athletes have found that female athletes actually experience a higher frequency of URI than their male counterparts. Reports on medical services provided during recent summer and winter Olympic Games all indicate that female athletes may be more susceptible to this type of infection (Engebretsen et al., 2010, Engebretsen et al., 2013, Soligard et al., 2015). He et al. (2014) found no sex-differences in the incidence of URI, but the duration of symptoms was significantly longer for females. Increased URI incidence and/or severity in

female athletes might be explained by lower secretion rates of salivary amylase, lactoferrin and lysozyme (He et al., 2014) and salivary secretory immunoglobulin-A (S-IgA) (Evans et al., 2000).

Since studies on URI in athletes commonly report short duration of symptoms (1-3 days) it may be that many of these events are due to viral reactivation rather than a primary infection (Gleeson et al., 2002, Walsh et al., 2011b). Ekblom and colleagues (2006) found that only athletes who had recently experienced an infectious episode were at increased risk of illness following a race, suggesting that post-race increases in URI may be related to performing strenuous exercise too soon following a previous infection. It is also likely that a number of reported URI events are due to non-infectious causes of inflammation. Indeed, Spence et al. (2007) found that viral or bacterial pathogens were isolated in fewer than 30% of URI cases reported by competitive triathletes suggesting that, in a large number of cases, respiratory symptoms reported by athletes may be due to non-infectious causes. In general, it would appear that approximately one third of URIs reported by athletes are due to infection, one third are due to a non-infectious medical cause, and one third have an unknown aetiology (e.g. physical/mechanical damage such as drying of airways, or migration to airways of inflammatory cytokines produced as a result of muscle damage) (Cox et al., 2008, Walsh et al., 2011b). Irrespective of whether or not they are due to infection, symptomatic URI are associated with reduced athletic performance (Pyne et al., 2001). However, since differentiating between infection and other inflammatory stimuli which mimic URI is clearly problematic, the term upper-respiratory symptoms (URS) will be used in the place of URI for the remainder of this thesis.

The following sections introduce some of the key factors that might influence immune function and infection risk in athletic populations.

1.2 INFLUENCE OF TRAINING & COMPETITION ON IMMUNITY

1.2.1 Acute endurance exercise

Various aspects of host defence appear to be negatively affected during the hours immediately following an acute bout of prolonged exercise. This results in what has been termed an "open window" for infection, lasting from 3 to 72 hours post exercise depending on the marker measured (Nieman and Pedersen, 1999). Almost certainly, this immunosuppression is multifactorial in origin, since changes in a large number of immune variables are observed.

1.2.1.1 Leukocyte number

Prolonged exercise is associated with a pronounced leukocytosis that persists for several hours post-exercise, the magnitude of which is dependent on both the intensity and duration of the exercise (Nieman et al., 1993, Robson et al., 1999). This is principally driven by an increase in circulating neutrophils, while monocyte and lymphocyte numbers increase to a lesser extent. However, during the recovery period following exercise, the number of lymphocytes falls below baseline, primarily due to a reduction in NK cells and type 1 (pro-inflammatory) T cells in the circulation (Gleeson and Bishop, 2005). This biphasic response appears to be due to the initial actions of elevated plasma catecholamines and shear stress which cause demargination of cells into the circulation (Shephard, 2003), followed by the more delayed action of elevated plasma cortisol causing an exodus of lymphocytes from the bloodstream during recovery from exercise (Walsh et al., 2011b). The reduction in the number of circulating lymphocytes during recovery is transient, typically lasting no more than a few hours, and likely represents a temporary redistribution of cells to reservoir sites.

1.2.1.2 Leukocyte function

In addition to changes in the absolute and relative number of leukocytes in the circulation, the ability of some cell types to mount an appropriate response to an immune challenge appears to be compromised following prolonged exercise. Nieman et al. (1995) investigated the effect of 2.5 hours of intensive running on lymphocyte number and function. Serum cortisol was elevated post-exercise, while the mitogen-induced lymphocyte proliferative response was decreased relative to controls for more than 3 h post-exercise. A number of studies have also reported reductions in mitogen-stimulated T cell production of TNF-α, IFN-γ, IL-2 and IL-1β following prolonged exercise (Starkie et al., 2001, Lancaster et al., 2005b, Lancaster et al., 2004, Lewicki et al., 1988, Baum et al., 1997), resulting in a shift in the type 1/type 2 T lymphocyte cytokine balance toward a more type 2 (anti-inflammatory) response. Since reductions in T cell function, and particularly type 1 responses, are associated with increased susceptibility to viral infections (Fabbri et al., 2003), this may be a mechanism through which acute, prolonged exercise increases the risk of URS. However, changes in *in vitro* mitogen-stimulated T cell proliferation and cytokine production should be interpreted with caution, since results may be confounded by a number of factors. Differences in the number of responder cells in blood samples collected before and after exercise, increased cell apoptosis in cell culture (Green and Rowbottom, 2003) and differences in immune responses to powerful T cell mitogens as opposed to specific antigens (Bishop et al., 2005) may all influence total cytokine production and proliferative responses.

NK cell cytotoxic capacity is augmented during exercise and reduced post-exercise, primarily due to changes in absolute numbers of NK cells. However, intense, prolonged exercise also appears to result in reduced cytotoxic capacity per cell (Gleeson and Bishop, 2005). Similarly, there is a reduction in neutrophil phagocytic function and degranulation in response to bacterial challenge which persists for several hours post-exercise (Robson et al.,

1999, Peake, 2002, Muns, 1994, Bishop et al., 2001b, Bishop et al., 2001a). Although the number of monocytes in the circulation increases following exercise, toll-like receptor expression decreases (Simpson et al., 2009, Lancaster et al., 2005a). During exercise there is a preferential mobilization of CD14+CD16+ pro-inflammatory monocytes, but the number of these is subsequently reduced in the circulation during recovery (Simpson et al., 2009) which might explain the concurrent reduction observed for monocyte production of TNF-α and IL-6 in response to LPS-stimulation (Starkie et al., 2005, Smits et al., 1998, Kvernmo et al., 1992). A single bout of prolonged exercise also significantly reduces both the induction and elicitation of in vivo T-cell-mediated immunity to a novel antigen (diphenylcyclopropenone), as measured via experimental contact-hypersensitivity (Harper Smith et al., 2011, Davison et al., 2015), which may be indicative of increased infection susceptibility.

1.2.1.3 Mucosal immunity

Studies on the effect of acute endurance exercise on mucosal immunity have yielded somewhat inconsistent results. Generally, it would appear that S-IgA secretion is reduced or unchanged following exercise of moderate intensity (Laing et al., 2005b, Walsh et al., 2002) and increased following high-intensity exercise (Blannin et al., 1998). Saliva flow rate is reduced following strenuous exercise (Blannin et al., 1998), but this is typically compensated for, at least to some degree, by increases in S-IgA concentration. A smaller number of studies have explored changes in other salivary anti-microbial proteins, and the available data indicate that concentrations and secretion rates of salivary lactoferrin, lysozyme LL-37 and α-amylase all increase following exercise (Killer et al., 2015, Kunz et al., 2015, West et al., 2010, Gillum et al., 2015). Changes in mucosal immunity with acute exercise are likely linked to the effect of sympathetic nervous system activation on transcytosis and exocytosis of salivary proteins (Walsh et al., 2011b), likely explaining why these responses are dependent on exercise intensity.

1.2.2 Endurance training

In addition to transient changes in immune variables following an acute bout of exercise, chronic exercise training appears to result in more prolonged changes to some aspects of immune function. Although this remains somewhat contested, a number of studies have observed that athletes with higher training loads experience a greater rate of URS, and high running mileage appears to be positively related to URS risk (Nieman et al., 1990, Heath et al., 1991), as does training volume in triathletes (Spence et al., 2007).

1.2.2.1 Leukocyte number

Regular endurance training does not appear to notably alter resting blood leukocyte counts, providing blood samples are collected when athletes are fully recovered from (i.e. at least 24 hours after) the previous exercise bout (Walsh et al., 2011b).

1.2.2.2 Leukocyte function

Gleeson et al. (2011a) found that athletes with a high training load (≥11 h moderate- high intensity training per week) experienced more than twice as many URS episodes and had approximately threefold higher resting IL-2, IL-4, and IL-10 production by antigen-stimulated whole blood culture than athletes with a low (3–6 h per week) training volume. An elevated anti-inflammatory cytokine response to immune challenge could be one mechanism through which heavy training loads might increase susceptibility to infection. Increased antigen-stimulated IL-10 production, in particular, appears to be a risk factor for URS (Gleeson et al., 2012). Although also released by monocytes, IL-10 is primarily produced by lymphocytes and, in particular, regulatory T (Treg) cells. Endurance trained athletes with a high training load have a significantly higher Treg percentage of total lymphocytes and antigen-stimulated IL-10 production compared to sedentary individuals

(Handzlik et al., 2013). Other aspects of leukocyte function do not appear to differ markedly between endurance athletes and the general population (Walsh et al., 2011b).

1.2.2.3 Mucosal immunity

A number of studies have investigated differences in markers of mucosal immunity between athletes and non-athletes, as well as investigating longitudinal changes in salivary antimicrobial proteins (AMPs) following heavy periods of training. Salivary levels of both S-IgA and lactoferrin have been found to be lower in elite endurance athletes compared to age-matched controls (West et al., 2010, Tomasi et al., 1982a). Gleeson et al. (1999) found that salivary S-IgA levels in swimmers were 4.1% lower for each additional month of training, and that S-IgA levels were inversely correlated with the number of infections in both elite swimmers and moderately exercising controls. Similarly, Neville et al. (2008) found that, although S-IgA was highly variable both within and between athletes, a decline in an individual's relative S-IgA was predictive of URS risk. An S-IgA concentration less than 40% of an individual's mean healthy value was associated with a one in two chance of contracting a URS within three weeks. Indeed, S-IgA secretion has been suggested to be one of the most useful clinical biomarkers to predict URS incidence in athletes (Fahlman and Engels, 2005) and is the only immune marker to date to show a strong and consistent relationship with URS risk. The underlying mechanism for depressed S-IgA secretion following training may be related to altered hypothalamic-pituitary-adrenal axis activity inhibiting S-IgA synthesis and/or transcytosis (Walsh et al., 2011b).

1.2.3 Intensified training & overreaching

As well as differences in habitual training, studies indicate that short-term increases in training load can influence infection risk. Overload is a key training principle used by athletes and coaches in order to improve physical performance, and periods of intensified training are

commonly incorporated over the course of a training season. The aim of such periods, although initially resulting in acute fatigue and a short-term decrement in performance, is to achieve an eventual supercompensation in performance capacity following tapering/recovery (Mujika et al., 1995). However, when excessive physical overload is combined with insufficient recovery and/or other life stressors such as lack of sleep, emotional or psychological stress, inadequate diet/energy intake, or environmental stress (heat, cold, altitude etc.), the total load placed on the athlete may become too great and lead to a state of overreaching or overtraining. Overreaching (OR) is defined as an accumulation of training and/or non-training stress resulting in a short-term decline in performance capacity, from which recovery may take from several days to several weeks (Meeusen et al., 2013). This can be further differentiated into Functional OR (where recovery takes from two days up to one week, and which can be incorporated into a training programme to facilitate long-term performance development) or Non-functional OR (where recovery can take several weeks) (**Figure 1.1**). Overtraining (OT) is defined as an accumulation of training and/or non-training stress resulting in a long-term decline in performance capacity, from which recovery can take several months. Hence, the main distinguishing difference between OT and OR is the time required to restore performance capacity. Functional OR, where performance recovers within a few days, may be considered a routine aspect of an athlete's training. An athlete suffering from OT, however, may take up to several years to recover.

Training (overload)	Intensified Training		
Acute Fatigue *(hours - day)*	**Functional Overreaching** *(days - week)*	**Non-Functional Overreaching** *(weeks - months)*	**Overtraining Syndrome** *(months - years)*
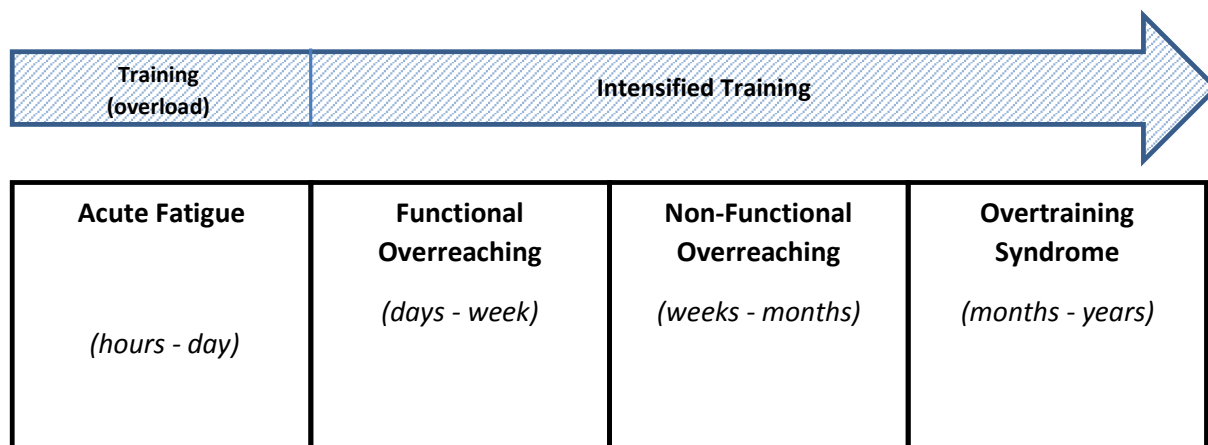
Figure 1.1 The overtraining continuum. Brackets indicate typical timescales required for recovery (adapted from (Meeusen et al., 2013))

Because a certain level of functional OR is required in order to improve athletic performance, athletes and coaches continually tread a fine line between insufficient training stimulus and excessive overload. As a result, imbalances between training and recovery, particularly in the presence of other life stressors, lead to a relatively high incidence of non-functional OR/OT amongst elite athletes, with the career prevalence reported to be 50% or higher (Morgan et al., 1987, Matos et al., 2011).

Much anecdotal evidence suggests that athletes suffering from non-functional OR or OT experience increased URS incidence and symptom duration/severity. Indeed, an increased susceptibility to infection is one of the most common symptoms of OT (McKenzie, 1999). As well as transient changes associated with a single bout of strenuous and/or prolonged exercise, studies suggest that periods of particularly intense training may lead to a more chronic immune dysfunction in OR or OT athletes, and could explain a concurrent increase in URS susceptibility. Immune disturbances associated with intensified training include reduced salivary S-IgA levels (Gleeson et al., 1995, Mackinnon and Hooper, 1994), reduced numbers of NK cells (Gleeson et al., 1995, Meyer et al., 2004) and absolute numbers of CD3+ and CD4+ cells (Baj et al., 1994a), decreased exercise-induced mobilization of leukocytes

(Lancaster et al., 2004, Witard et al., 2012) impaired neutrophil function (oxidative burst and degranulation) (Baj et al., 1994a, Robson-Ansley et al., 2007), reduced Glutamine/Glutamate ratio (Halson et al., 2003, Rowbottom et al., 1995, Coutts et al., 2007, Parrybillings et al., 1992), and elevated plasma levels of IL-6 and CK at rest (Robson-Ansley et al., 2007). In addition, some studies suggest an impaired capacity of immune cells to produce cytokines in response to an immune challenge following a period of IT (Baj et al., 1994a, Lancaster et al., 2004, Morgado et al., 2012).

Hormonal changes appear to play a key role in the pathogenesis of OT (Urhausen et al., 1995), and many of the changes in immune variables in response to intensified training have been attributed to the immunomodulatory influence of exercise-induced elevations in circulating stress hormones, and to changes in the sensitivity of the Hypothalamic-Pituitary-Adrenal axis (Meeusen et al., 2013). A glucocorticoid released from the adrenal cortex in response to stress, cortisol has immunosuppressive and anti-inflammatory effects (Auphan et al., 1995), acting to mediate the recovery from immune activation early in the stress response, thus preventing an "overshoot" of the immune reaction (Sorrells and Sapolsky, 2007). Cortisol acts to suppress the production of pro-inflammatory cytokines (Petrovsky et al., 1998), as well as up-regulate the expression of certain anti-inflammatory cytokines (Elenkov and Chrousos, 2002), thus functioning as a negative feedback mechanism and protecting against excessive inflammation. The Testosterone:cortisol ratio has been stipulated as a useful diagnostic tool for OT, with some studies reporting an increase in resting plasma cortisol concentration, often alongside a concurrent decrease in testosterone, following intensified training (Slivka et al., 2010, Coutts et al., 2007), suggesting a shift towards a more catabolic state. However, it would appear that a decrease in this ratio in actual fact only indicates the physiological stress of the training and cannot distinguish between normal and OR/OT athletes (Meeusen et al., 2013) and several other studies have found no change in

plasma cortisol concentrations at rest following a short-term period of IT (Urhausen et al., 1998, Mackinnon et al., 1997, Hooper et al., 1993). However, there is some evidence available that OR athletes have a diminished rise in cortisol with acute exercise (Snyder et al., 1995, Lancaster et al., 2004, Urhausen et al., 1998, Witard et al., 2012, Verde et al., 1992). This appears to be due, at least in part, to diminished adrenal responsiveness to adrenocorticotropic hormone (ACTH) (Lehmann et al., 1998).

1.3 INFLUENCE OF NUTRITIONAL STATUS ON IMMUNITY

1.3.1 Macronutrient intake

It is well known that malnutrition impairs immune function (Calder and Jackson, 2000) but studies suggest that endurance athletes frequently undertake periods of training during which energy intake is not sufficient to match energy expenditure (Edwards et al., 1993, Venkatraman and Pendergast, 2002). Overall energy balance is clearly an important factor in maintaining immunocompetence in athletes, but equally important is that this energy is provided in the form of a well-balanced diet, in order to avoid deficiencies in those nutrients that play a central part in immune cell triggering, interaction, differentiation and functional expression (Gleeson et al., 2004).

1.3.1.1 Protein

Not surprisingly, since the immune system relies on fast cell replication and generation of proteins such as cytokines and immunoglobulins, inadequate protein intake is associated with impaired host defence – particularly T-lymphocyte function - and increased infection susceptibility (Gleeson et al., 2004, Bishop et al., 1999). Although protein intake in athletes generally appears to be sufficient (van Erp-Baart et al., 1989, Venkatraman and Pendergast,

2002), some athletes may be at risk of protein deficiency, particularly during periods of heavy training and/or weight loss.

Glutamine is the most abundant, nonessential amino acid in human muscle and plasma, and is an important fuel for a number of immune cells, particularly lymphocytes and macrophages (Venkatraman and Pendergast, 2002, Walsh et al., 1998). Prolonged exercise is associated with reduced plasma glutamine (Castell and Newsholme, 1997, Gleeson et al., 1998), and it has been suggested that oral glutamine supplementation may ameliorate some aspects of the post-exercise immunosuppression and reduce infection risk (Castell and Newsholme, 1997). However, although an attractive hypothesis, the majority of studies do not support that reduced plasma glutamine meaningfully contributes to exercise-induced immunosuppression (Rohde et al., 1998, Gleeson, 2008, Walsh et al., 2000).

1.3.1.2 Carbohydrate (CHO)

A number of studies have explored the impact of dietary CHO or acute CHO supplementation during exercise on immune responses to physical activity. Consuming a high CHO diet for 2-3 days has been associated with an attenuated rise in plasma cortisol during exercise, better maintained plasma glutamine concentration post-exercise, and smaller exercise-induced perturbations in the number of circulating leukocytes, neutrophils and lymphocytes during the post-exercise period (Gleeson et al., 1998, Mitchell et al., 1998, Bishop et al., 2001b). A high CHO diet has also been found to have a favourable effect on post-exercise S-IgA levels in competitive endurance athletes (Costa et al., 2005) as well as ameliorating exercise-induced increases in plasma cytokines (Bishop et al., 2001c). However, it does not appear to better maintain lymphocyte proliferative responses (Mitchell et al., 1998) or neutrophil degranulation (Bishop et al., 2001b).

Of studies investigating the effects of acute CHO supplementation during exercise on immune function, the vast majority have reported an attenuated cortisol response due to better maintenance of plasma glucose (Gunzer et al., 2012, Davison et al., 2015). However, data on whether this translates into better maintained immune function are somewhat inconsistent. CHO supplementation has been consistently found to attenuate the exercise-induced increase in circulating leukocytes, neutrophils and monocytes (Nieman et al., 2006b, Davison et al., 2015) and diminish post-exercise reductions in LPS-stimulated neutrophil degranulation (Bishop et al., 2003) but does not appear to ameliorate exercise-induced changes in NK cell activity (Nieman et al., 1997b), mitogen-stimulated cytokine production (Henson et al., 2004), granulocyte and monocyte phagocytosis or oxidative burst (Nieman et al., 1997a), *in vivo* cell-mediated immunity (Davison et al., 2015), or S-IgA (Henson et al., 1998b, Li and Gleeson, 2005). Henson et al. (1998) found that CHO supplementation before, during, and after 2.5 hours of intensive running had a significant effect in maintaining lymphocyte proliferative responses, although subsequent studies have not been able to replicate this (Henson et al., 2004). Nieman et al. (2003) found that ingestion of a 6% CHO beverage during prolonged, strenuous running attenuated the exercise-induced increase in plasma IL-6, IL-10, and IL-1ra in overnight fasted marathon runners, while Peake and colleagues (Peake et al., 2008) found no effect of CHO supplementation on either plasma cytokines or neutrophil degranulation responses to cycling when exercise was performed 2 h after a meal. Hence, it appears that the efficacy of acute CHO supplementation in alleviating exercise-associated immune disturbances depends not only on the immune marker measured, but also on the exercise intensity/duration and the pre-exercise CHO status of participants. It is also worth noting that CHO ingestion markedly improves endurance exercise performance. As a result, studies investigating immune responses to an exercise bout of fixed duration and intensity with and without CHO may not reflect the real-world situation in which an athlete will likely

be capable of exercising harder and/or for longer when ingesting CHO. Indeed, when exercise is performed to exhaustion, CHO ingestion increases time to fatigue but appears to have little effect on immunity (Bishop et al., 2001a). As well as supplementation strategies during exercise, some studies have found that CHO feeding, with or without additional protein, immediately after a bout of prolonged exercise may be effective in improving bacterially stimulated neutrophil degranulation (Costa et al., 2011) and salivary lysozyme (Costa et al., 2012) during the recovery period.

As well as potentially alleviating some of the immunological disturbances associated with acute exercise, studies suggest that energy, and specifically CHO, intake may play a role in the prevention of OR and OT. Achten et al. (2004) found that an increase in dietary CHO from 5.4 to 8.5 g/kg bm/day better maintained physical performance and mood state in runners undergoing a period of intensified training, reducing the symptoms of OR. Similarly, Halson and colleagues (2004) found that CHO supplementation during an intensified training period alleviated the performance decrement and symptoms of OR in cyclists. Costa et al. (2005) found that consumption of a high CHO diet (12 g/kg bm/day) during a period of intensified training had a favourable effect on markers of immunity, attenuating cortisol release and increasing salivary S-IgA concentration.

The mechanism through which increased CHO intake tempers immune disturbances associated with acute, prolonged exercise and intensified training may be via an attenuation of the stress response to exercise. During exercise, plasma cortisol concentration has been found to be inversely related to plasma glucose availability (Tsintzas et al., 1996), with hypoglycaemia stimulating an increase in cortisol release (Gleeson et al., 1998). Maintaining plasma glucose also provides a direct energy substrate for immune cells, which have very high metabolic rates (Gunzer et al., 2012).

It is, however, worth noting that the majority of studies that have investigated the influence of CHO supplementation on immune responses to exercise and training have studied responses to very high doses (e.g. 1L of 6% CHO per hour of exercise) in fasted participants. As such, these studies may do not always reflect typical real-world practices of athletes.

1.3.1.4 Fat

To date, relatively little is known about the role of dietary fat in modifying the immune response to exercise. Pedersen et al. (2000) found that that ingestion of a fat-rich diet during training was detrimental to the immune system, specifically NK-cell activity, compared to a CHO-rich diet. However, it is not clear whether this negative effect is due to excess fat intake or inadequate CHO intake. On the other hand, diets very low in fat may also compromise immunity in athletes, likely primarily due to negative energy balance (Venkatraman et al., 2001).

1.3.2 Micronutrient intake

A growing body of literature suggests that vitamin D status is an important predictor of infection risk in athletes. Although some foods contain vitamin, the principle natural source is dermal synthesis from cholesterol, with this being dependent on sunlight (specifically UVB) exposure. As such, vitamin D levels vare typically lower during winter and early spring (Klingberg et al., 2015). He et al. (2013) found that athletes deficient in vitamin D (plasma 25(OH)D < 30 nmol/L) reported increased frequency, duration and severity of URS compared to athletes with optimal vitamin D status (>120 nmol/L). Vitamin D deficiency was also associated with lower S-IgA secretion rate and diminished pro-inflammatory cytokine production by monocytes and lymphocytes. In a subsequent study, the authors found that two weeks of daily vitamin D supplementation (5000 IU/day) had a beneficial effect in up-regulating the expression of both S-IgA and cathelicidin in athletes during a winter training

period, suggesting that this might improve resistance to respiratory infections (He et al., 2015).

Some studies also suggest that megadoses of vitamin C may be effective in reducing infection risk in athletes. Peters et al. (1993) found that ingesting 600 mg of vitamin C for 3 weeks prior to an ultramarathon significantly reduced the incidence of post-race URS. However, the effects of vitamin C supplementation on exercise-induced immune perturbations and infection incidence in athletes are inconsistent, and a number of other studies have found no positive effect (Nieman et al., 2002).

There is little evidence that supplementation with other vitamins and minerals is effective in maintaining immunocompetence in athletes who consume an otherwise balanced diet (Walsh et al., 2011a), and overconsumption of certain vitamins, notably vitamin E and vitamin A, may in fact impair immunity (Gleeson et al., 2004). It is also possible to question to what extent it is desirable to block the immune and inflammatory responses and oxidative stress associated with prolonged exercise, since doing so may interfere with signalling mechanisms for endurance adaptation (Gomez-Cabrera et al., 2008, Walsh et al., 2011a).

1.4 INFLUENCE OF HYDRATION STATUS ON IMMUNITY

Athletes frequently commence training or competition in a mildly or moderately hypohydrated state (Da Silva et al., 2012, Fernando Aragon-Vargas et al., 2009, Volpe et al., 2009, Maughan et al., 2005) and typically fail to ingest sufficient fluid to offset sweat loss during exercise (Da Silva et al., 2012, Maughan et al., 2005, Maughan et al., 2004, Beis et al., 2012). Compared with exercise in a euhydrated state, hypohydration results in an augmented stress response and, thus, potentially greater disturbances to immune function. Maresh et al. (2006) found that athletes hypohydrated to ∼5% body mass loss had significantly higher plasma cortisol concentrations both before and after acute submaximal exercise. Similarly,

24

Bishop and colleagues (2004) found that fluid ingestion during prolonged exercise attenuated the cortisol response, although there was no effect on the neutrophil response. Mitchell et al. (2002), on the other hand, found that although hypohydration resulted in elevated plasma cortisol when exercise was performed in the heat, there was no effect of hydration status on cortisol concentration, total or differential leukocyte numbers, or lymphocyte function when exercising in temperate ambient conditions. As well as possibly attenuating the stress response to exercise, regular fluid intake appears to maintain saliva flow rate (Bishop et al., 2000, Fortes et al., 2012) and secretion rates of salivary lysozyme and α-amylase during prolonged exercise, although it does not appear to significantly alter S-IgA secretion rate (Fortes et al., 2012). As such, undertaking exercise in a hypohydrated state, within the range applicable to athletes, appears to have some implications for mucosal immunity. However, despite some studies reporting an augmented cortisol response, there is to date little evidence that systemic immunity is significantly affected, although a number of markers have not yet been investigated.

1.5 INFLUENCE OF ENVIRONMENTAL FACTORS ON IMMUNITY

In addition to high training loads, endurance athletes are commonly exposed to environmental stressors, such as hypoxia and extremes of temperature, which may impact on physiological and metabolic function, and compound exercise and training-induced immune disturbances.

1.5.1 Altitude

Hypoxic training is routinely used by competitive endurance athletes, both to improve exercise performance at sea level (Levine and StrayGundersen, 1997, Stray-Gundersen et al., 2001) and to acclimatise prior to competition at altitude (Fulco et al., 2000). However, there

is some evidence that athletes experience an increased incidence of opportunistic infectious illness during and/or immediately following a period of altitude training (Bailey et al., 1998). Exercise in hypoxic conditions typically causes greater physiological stress than exercise at sea level (Bailey and Davies, 1997). Taking into account this augmented stress response to exercise, coupled with reports of increased resting levels of adrenaline, cortisol and IL-6 at high altitude (Mazzeo, 2005), it is not altogether surprising that both acute and chronic hypoxic training have been found to impact negatively on mucosal (Tiollier et al., 2005) and cell-mediated immunity (Facco et al., 2005, Oliver et al., 2013, Pyne et al., 2000). However, it is still unclear how many aspects of immune function respond to hypoxic exercise, and particularly to the types of exercise and the relatively modest levels of hypoxia to which athletes are typically exposed during training or competition. Furthermore, it is well known that $\dot{V}O_{2max}$ is reduced at altitude, with the magnitude of this reduction being proportional to the level of hypoxia (Ferretti et al., 1997). Hence, it is important to differentiate between the effects of hypoxia *per se*, and the influence of increased relative exercise intensity at the same absolute workload. Indeed, Blegen et al. (2008) found that when cycling at the same relative exercise intensity (60% of altitude-specific $\dot{V}O_{2max}$), exercising in hypoxia equivalent to an altitude of ~2,500 m above sea-level did not result in greater elevations in cortisol, adrenaline, noradrenaline or plasma cytokines compared to normoxic exercise. Hence, it is possible to speculate that the increased infection risk reported during altitude training may be due, at least in part, to inadequate control of training load while at altitude.

1.5.2 Heat

Similar to the effects of hypoxia, exercise in the heat is associated with an augmented stress hormone response compared to exercise in thermoneutral conditions (Mitchell et al., 2002, Laing et al., 2005a). Indeed, studies that have used a thermal clamp to maintain rectal

temperature at <38ºC during exercise demonstrate that a substantial portion of the leukocytosis and cytokinaemia associated with prolonged exercise is due to elevated core temperature (Cross et al., 1996, Rhind et al., 2004, Rhind et al., 1999). However, this augmented stress response does not appear to manifest as impairments to immune function, with studies showing a limited effect of exercising in the heat on neutrophil degranulation (Laing et al., 2005a, Laing et al., 2008), salivary S-IgA responses (Laing et al., 2005b) NK cell activity (McFarlin and Mitchell, 2003) or LPS-stimulated cytokine production (Starkie et al., 2005) compared to an equivalent exercise bout in thermoneutral conditions. Hence, it does not appear that exercising in the heat poses any additional threat to host defence compared to exercise in thermoneutral conditions. This may be partly due to the fact that exposure to exercise-heat stress tends to be self-limiting, with individuals typically fatiguing more quickly than in thermoneutral conditions (Walsh et al., 2011a). It is worth noting, however, that laboratory studies have generally evoked only modest increases in core temperature of ≤2.5°C. The effect of more severe heat stress encountered in the field, where core temperatures of athletes can exceed 40°C, has yet to be elucidated.

1.5.3 Cold

The effect of cold exposure on infection susceptibility in humans remains somewhat controversial. Although the popular belief is that low temperatures increase the risk of respiratory infections, exercising in cold (-6ºC) ambient conditions is associated with similar saliva flow rate and S-IgA responses in trained cyclists as exercise in temperate (20ºC) conditions (Walsh et al., 2002). Indeed, although there are currently only a small number of published studies that have examined the effect of exercising in cold environments on immune function, the available data do not support the idea that this poses any additional threat to immunity compared with exercise in thermoneutral conditions (Walsh and Whitham,

2006, Rhind et al., 1999). However, it is possible that airway inflammation as a result of breathing large volumes of cold dry air may be mistakenly interpreted as URI by athletes (Walsh et al., 2011a).

1.6 SLEEP

Studies suggest that heavy training loads and OT are associated with reduced sleep quality (Hooper et al., 1995, Hausswirth et al., 2014, Killer et al. 2015). Furthermore, athletes may experience impaired sleep following cross meridian travel (Fullagar et al., 2015, Waterhouse et al., 2007) or while sleeping at altitude (Roach et al., 2013, Sargent et al., 2013). Hence, it is perhaps not surprising that the prevalence of poor sleep quality amongst athletes appears to be substantial (Samuels, 2009). Many key metabolic, immunologic and restorative physiological processes are adversely affected by sleep disturbance (Samuels, 2009), such that reductions in sleep quality can result in impaired daytime functioning (Van Dongen et al., 2003) and inadequate recovery between training sessions, as well as potentially exacerbating any immunological disturbances associated with training and competition (Irwin, 2002). Irwin and colleagues (1996) found that one night of partial sleep deprivation in healthy volunteers resulted in reduced NK cell activity and diminished IL-2 production in response to mitogen-stimulation, suggesting that even modest sleep disruption may negatively impact on host defence. Studies have also found that sleep deprivation results in minor reductions in endurance exercise capacity (Oliver et al., 2009, Mougin et al., 1991, Plyley et al., 1987). It is possible that this, in turn, will exacerbate immune disturbances associated with exercise, with sleep-deprived athletes having to work at a higher relative intensity to sustain the same absolute workload. However, Ricardo et al. (2009) found no effect of a 30 h period of sleep deprivation, such as might be experienced by athletes during

28

long-haul air travel, on leukocyte trafficking, neutrophil function or S-IgA either at rest or in response to intense exercise.

Overall, despite a small number of studies reporting modest changes in immune variables, it seems unlikely that short-term sleep disruption meaningfully increases infection susceptibility. However, little is known about the longer-term effects of sleep quantity and quality on immunity. Cohen et al. (2009) found that lower self-reported sleep quality and quantity during the two weeks prior to rhinovirus exposure was associated with greater risk of developing cold symptoms in healthy men and women. Individuals who reported less than seven hours of sleep per night during the two weeks prior to pathogen exposure were approximately three times more likely to develop a cold compared with those who slept more than eight hours per night. Similarly, a sleep efficiency (the percentage of time in bed spent asleep) of <92% was associated with more than five times greater risk of developing a cold compared with a sleep efficiency of >97%. Leeder et al. (2012) found that a cohort of Olympic athletes exhibited similar sleep duration, but significantly lower sleep efficiency compared to matched non-athletic controls (81% compared to 89%). It is therefore possible that lower sleep quality may be a contributing factor towards increased infection susceptibility in athletes.

1.7 SCOPE OF THESIS

There is a general consensus that both acute, prolonged exercise and long-term heavy endurance training can negatively impact on immune function. Although athletes are not clinically immune deficient, it is possible that small changes to multiple aspects of immune function as a result of acute and chronic endurance training may make athletes more susceptible to opportunistic infections, particularly of the upper-respiratory tract. These exercise-induced immune disturbances may be exacerbated when training is undertaken in hypoxic conditions, with suboptimal nutritional status or inadequate sleep and recovery.

Clearly, substantial reductions in training load are not feasible for athletes aiming to optimise performance and compete on the world stage. However, by exploring which modifiable factors influence immunity and infection risk in athletes, it is possible to provide advice to athletes and coaches on effective and feasible strategies to ameliorate exercise- and training-induced immune disturbances and maintain immunocompetence. This thesis presents a series of studies exploring how a number of factors impact on immunity and infection incidence in athletes. Chapter 3 investigates illness incidence in a cohort of world-class endurance athletes, and examines which training-related factors predict onset of illness. Chapter 4 investigates the effect of an intense period of repeated competition on illness incidence and subsequent race performance in the same cohort of athletes. Chapter 5 explores changes in immunological variables during short-term intensified training in cyclists, and whether CHO supplementation can ameliorate associated immune disturbances. Chapter 6 explores the effect of hydration status on immunological responses to prolonged exercise. Finally, chapters 7 and 8 investigate the effect of acute and longer-term hypoxic training on systemic and mucosal immunity.

The following major hypotheses were tested:

1. Heavy training loads impair immunity and increase infection risk in endurance athletes (Chapters 3 and 5)

2. Taking part in competitions increases an athlete's risk of infection (Chapters 3 and 4)

3. International air travel increases an athlete's risk of infection (Chapter 3)

4. Exposure to natural or simulated altitude exacerbates immune disturbances associated with prolonged exercise, and increases an athlete's infection risk (Chapters 3, 7 and 8)

5. Increasing CHO intake before, during and after training sessions helps prevent overreaching and alleviates immunological disturbances associated with intensified training (Chapter 5)

6. Moderate hypohydration, due to inadequate fluid intake before and during exercise, exacerbates exercise-induced immune disturbances and increases an athlete's risk of infection (Chapter 6)

Hence, this thesis endeavours to provide an overview of which factors meaningfully influence immunity and infection risk in endurance athletes, as well as exploring some of the possible underlying mechanisms and potential countermeasures. The overriding aim is to provide a better foundation on which to prescribe effective and practicable competition- and training-related strategies to minimise an athlete's risk of infection.

CHAPTER 2: GENERAL METHODS

2.1 BLOOD SAMPLING & ANALYSES

2.1.1 Blood sampling

On each occasion, a venous blood sample (11 mL) was obtained by venepuncture from an antecubital vein. Blood was collected into two vacutainer tubes (Becton Dickinson, Oxford, UK) containing either lithium heparin or K_3EDTA as anticoagulant.

2.1.2 Haematological analyses

Blood samples in the K_3EDTA vacutainer (4 mL) were used for determination of red blood cell concentration, haematocrit, haemoglobin and total and differential leukocyte counts using an automated cell-counter (Ac.TTM^{5}diff haematology analyser, Beckman Coulter, High Wycombe, UK). The coefficient of variation for all measured variables in our laboratory was <2.0%. Samples were analysed in duplicate, and the average of both values entered into analyses.

2.1.3 Plasma volume changes

Haematocrit and Haemoglobin values from haematological analyses were used to calculate and correct for changes in plasma volume using the equations of Dill & Costill (1974).

2.1.4 Plasma cortisol and ACTH

The remaining K_3EDTA blood sample was centrifuged for 10 min at 1,500 g and 4 °C. Plasma was aliquotted and stored at -20 °C until analysis. Plasma concentrations of cortisol were determined using a commercially available solid phase competitive ELISA (IBL International, Hamburg, Germany), with analytical sensitivity of 2.46 ng/mL and intra-assay coefficient of variation of <3.0%. Plasma concentrations of ACTH were determined using a commercially available two-site ELISA (Biomerica, California), with analytical sensitivity of

0.22 pg/mL and intra-assay coefficient of variation of <6.0%. Plates were read on a microtitre plate reader at 450 nm. All samples were assayed in duplicate.

2.1.2 Plasma cytokine concentrations

Plasma concentrations of IFN-γ, TNF-α, IL-1α, IL-6, IL-8, IL-10, EGF, VEGF and MCP-1 were determined with an Evidence Investigator System using the cytokine biochip array EV 3623 (Randox, County Antrim, UK). The intra-assay coefficient of variation was <5% for all measured cytokines.

2.1.5 Antigen-stimulated cytokine production by whole blood culture

Stimulated whole blood culture production of IFN-γ, TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8 and IL-10 was determined as follows: for each sample, 0.25 mL heparinized whole blood was added to 0.75 mL RPMI medium (Sigma Chemicals, Poole, UK) with no added stimulant (*US*), or stimulant at a dilution of 1:2000 (*S*). The stimulant used was a commercially available multi-antigen vaccine containing diphtheria, tetanus, acellular pertussis, poliomyelitis and Haemophilus influenza type b antigens (Pediacel vaccine, Sanofi Pasteur, Maidenhead, UK). The whole blood culture was then incubated for 24 h at 37 ºC and 5% CO_2. Following centrifugation for 4 min at 13,000 *g* in a microcentrifuge, supernatants were collected and stored frozen at -20 ºC until analysis. Cytokine concentrations were determined with an Evidence Investigator System using the cytokine biochip array EV 3623 (Randox, County Antrim, UK). The intra-assay coefficient of variation was <10% for all measured cytokines. The *US* values were then subtracted from *S* values to isolate cytokine production specifically in response to the antigen-stimulation and account for plasma concentrations and spontaneous production during the culture period.

2.1.6 Lymphocyte subsets

Fluorescent-conjugated monoclonal antibodies were used to identify specific cell surface markers (CD3, CD8, CD4, CD25, CD127) via four colour flow-cytometry with True Volumetric Absolute Counting (Partec CyFlow ML, Germany) and FloMax analysis software (Quantum Analysis GmbH). Briefly, either 10 μL of human regulatory T cell cocktail (Becton Dickinson Biosciences, Oxford, UK) or 10 μL of FITC anti-human CD3 + 10 μL PeCy7 CD4 + 10 μL PE CD8 (Becton Dickinson Biosciences) were added to 120 μL heparinized whole blood and incubated in the dark for 20 min on ice. Erythrocytes were then lysed by adding 1.5 mL of lyse solution (FACS lysis buffer, Becton Dickinson Biosciences). The sample was subsequently incubated for a further 10 min, before centrifugation at 1500 *g* for 6 min. The supernatant was aspirated and the cells re-suspended in phosphate buffered saline solution containing 0.1% bovine serum albumin and 2 mM EDTA. The mixture was centrifuged for a further 6 min at 1500 g, the supernatant aspirated and the cells re-suspended in 1000 μL PBS. Forward-scatter versus side-scatter plots were used to gate lymphocytes based on size and density, with 50,000 lymphocyte events acquired per analysis. CD4+ lymphocytes were further gated to identify CD25+ cells, and those that were also CD127low/- . This was based on the finding that CD127 expression inversely correlates with FoxP3 (Liu et al., 2006) and can therefore be used to identify T regulatory (Treg) cells. CD3+ cells were further gated to identify cells that were CD4+ (T helper) and CD8+ (T suppressor).

2.2 SALIVA SAMPLING & ANALYSES

2.2.1 Saliva collection

Samples were collected in the morning immediately upon waking, following an overnight fast of at least 8 h. After sitting quietly for a few minutes, participants were instructed to swallow to empty the mouth before collecting unstimulated whole saliva into a pre-weighed 7 mL screw-top vial. During the 5 min collection time, participants were instructed to remain

seated, leaning forwards with their head tilted down, allowing the saliva to dribble passively into the collection tube with minimal orofacial movement. Saliva volume was determined by weighing to the nearest milligram, with saliva density estimated to be 1.0 g/ml (Cole and Eastoe, 2014). Saliva flow rate was then calculated by dividing the volume by the collection time. Samples were centrifuged for 3 min at 14,000 g before being stored at -80ºC until analysis.

2.2.2 Salivary AMPs

Commercially available ELISA kits were used to determine salivary concentrations of S-IgA (Salimetrics, Philadelphia, USA), lysozyme (Biomedical Technologies, USA), lactoferrin (Calbiochem, Merck KGaA, Darmstadt, Germany) and LL-37 (Hycult biotech, Uden, Netherlands). For lysozyme and lactoferrin determination, saliva was diluted 500x prior to analysis. For LL-37, saliva was diluted 5x prior to analysis. Secretion rates for each salivary AMP was calculated by multiplying saliva flow rate by AMP concentration. All assays were carried out in duplicate. The intra-assay coefficients of variation for S-IgA, lysozyme, lactoferrin and LL-37 were 1.8%, 5.3%, 3.0% and 3.1%, respectively.

2.2.3 Salivary cortisol and testosterone

Saliva samples were analysed using a solid-phase competitive ELISA to determine concentrations of cortisol and testosterone (Salimetrics, Philadelphia, USA). All samples were assayed in duplicate with analytical sensitivity of 0.2 nmol/L and 3.8 pmol/L, and intra-assay coefficient of variation of 3.5 % and 3.0 % for cortisol and testosterone, respectively.

2.3 DETERMINATION OF MAXIMAL OXYGEN UPTAKE

For determination of maximal oxygen uptake ($\dot{V}O_{2max}$) subjects completed a continuous, incremental exercise test to volitional exhaustion on a magnetically-braked cycle ergometer (IndoorTrainer, SRM International, Jülich, Germany). Subjects arrived at the laboratory at 7:00 am following an overnight fast of at least 10 h, and having abstained from caffeine for a minimum of 24 h. All tests were completed at the same time of day to reduce inter- and intra-trial effects of diurnal variations in cytokine production and plasma cortisol. The test began at 60 W with increments of 35 W every 3 min. Rating of perceived exertion (RPE) was noted and heart rate measured continuously using short-range telemetry (Polar, Kempele, Finland). Expired gas was collected and analysed continuously using a computerized metabolic system with mixing chamber (Moxus, AEI Technologies, Pennsylvania, USA) for determination of $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$. Using this data, fat and CHO oxidation rates were calculated using Stoichiometric equations, assuming that protein oxidation throughout the test was negligible (Brouwer, 1957). The gas analysers were calibrated with certified calibration gases of known concentrations before every test. The flow turbine was calibrated before every test with a Micro Medical SM2125 series 3 L calibration syringe (CareFusion, Hoechberg, Germany). The same metabolic system with identical calibration routines was used for all performance tests. Subjects were asked to record and replicate as closely as possible their dietary intake during the 24 h leading up to each incremental test, and were required to abstain from alcohol or caffeine consumption for 24 h before.

2.4 TRAINING & ILLNESS DATA

2.4.1 Heart rate zones for prescription and categorisation of endurance training

In Chapters 3, 4, 5 and 8, all endurance training was prescribed and analysed using the heart rate zones presented in **Table 2.1.**

Table 2.1. **The 5-zone intensity scale used by athletes to classify endurance training**

Intensity Zone	Heart rate (% max)	Binary model
5	> 94	
4	89-93	HIT
3	84-88	
2	74-83	LIT
1	54-73	

Reference values presented are derived from the average self-reported zone cut-offs of 29 elite cross-country skiers (Tonnessen et al., 2014). LIT: low-intensity training; HIT: high-intensity training.

2.4.2 Self-reported training and illness

Athletes in Chapters 3, 4 and 8 recorded their training daily in spreadsheet training diaries (Microsoft Excel) designed by the Norwegian Ski Federation or, since 2012, in the web-based version developed by the Norwegian Olympic Federation (www.olt-dagbok.net). The training recorded for each session included total training volume and training time distributed across different training forms (endurance, strength, power, sprint) as well as time spent in each intensity zone for all endurance training. In addition, each session included "daily parametrics" and a free text field where athletes recorded additional information, including further details about the training session, illness symptoms and whether training was discontinued or modified due to illness or injury, medication use, days of travel and days spent at altitude.

Based on the definitions proposed by Matthews et al. (2010) self-reported illness was categorized as either "moderate", where an athlete reported symptoms indicative of infectious illness AND training was discontinued, or "mild" where an athlete reported symptoms but continued to do some form of training. An illness episode was defined as a subject reporting "mild" symptoms on two or more consecutive days, or "moderate" symptoms on at least one day. Where athletes reported allergies, asthma, or gastrointestinal distress due to training/nutrition practices, these were not included in the analysis. Only symptoms likely to be due to opportunistic infection were retained for analysis. These included symptoms of upper respiratory infections (blocked or runny nose, sore throat, sneezing), lower respiratory infections (coughing, sputum, chest congestion, wheezing, fever) and gastrointestinal infections (nausea, vomiting, diarrhoea, abdominal pain).

CHAPTER 3: TRAINING- AND COMPETITION-RELATED RISK FACTORS FOR INFECTION IN ELITE CROSS-COUNTRY SKIERS

Ida S. Svendsen[1], Ian M. Taylor[1], Espen Tønnessen[2], Roald Bahr[2,3], Michael Gleeson[1]

1) School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK.
2) Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway
3) Oslo Sports Trauma Research Center, Department of Sports Medicine, Norwegian School of Sport Sciences, Oslo, Norway

3.1 ABSTRACT

PURPOSE: To determine risk factors for illness in elite winter endurance athletes.

METHODS: Self-reported training and illness data for 37 elite male and female cross-country skiers from 2007 to 2015 were analysed. Multilevel logistic regression equations were constructed with illness incidence and symptom duration as outcome variables, and sex, performance level, season, training phase, competition, air travel, altitude exposure, and training characteristics as independent variables. **RESULTS**: Data for 7,016 person-weeks were included, comprising 464 illness events, 110,959 hours of training, 3,812 competitions, 1,769 international flights and 4,879 days at altitude ≥1,500 masl. On average, athletes reported 3-4 events of respiratory and/or gastrointestinal illness per year, with symptoms lasting 5 ± 4 days. During the winter, illness occurred more frequently (odds ratio 2.09, $P<0.001$) and lasted longer ($b=0.043$, $P<0.001$) compared to summer. Recent competition and international air travel increased the risk of illness, with adjusted odds ratios of 2.93 (95% CI: 2.24-3.83) and 4.94 (95% CI: 3.74-6.53), respectively ($P<0.001$). Athletes who scored higher for Training Monotony had significantly lower risk of illness (odds ratio 0.87 (95% CI: 0.73-0.99), $P<0.05$). Other training variables were not associated with illness when accounting for the other significant predictor variables. When illness did occur, athletes who had won an individual Olympic/World Championship medal reported shorter duration of symptoms compared to their less successful counterparts ($b=-0.019$, $P<0.05$).

CONCLUSION: Future research exploring effective strategies to minimise illness risk associated with competition and air travel is warranted. Athletes whose weekly training programmes incorporate large fluctuations in daily training load may be more susceptible to illness. A robust immune system capable of recovering quickly from infection may be associated with success in elite endurance sport.

3.2 INTRODUCTION

Reaching an international level in any sport requires a huge amount of training. Depending on the sport, elite endurance athletes typically train between 500 h (long distance running) (Billat et al., 2001, Billat et al., 2003) to in excess of 1,000 h per year (rowing, cycling, triathlon) (Fiskerstrand and Seiler, 2004, Neal et al., 2011, Zapico et al., 2007), with elite cross country skiers training ~800 h per year (Tonnessen et al., 2014). Cross-sectional studies indicate that individuals with high training loads experience a higher number of upper-respiratory infections (Gleeson et al., 2013a). Furthermore, longitudinal studies with athletes report an increased infection incidence during periods of intense training or competition (Nieman, 2000, Hellard et al., 2015). Malm (2006) suggested that, although high training loads typically suppress immunity, in order to be a successful elite athlete an individual must have an immune system capable of withstanding infections even during severe physiological and psychological stress. However, differences in infection susceptibility between the most successful elite athletes and their less successful counterparts, all of whom have similarly high training loads, has not yet been fully explored. It is possible that the ability to withstand and/or recover quickly from an infection, particularly during key training and competition periods, could be an important predictor of success within an otherwise homogenous athlete group.

A recent study by Hellard et al. (2015) investigated illness incidence amongst international swimmers over a four-year period, and found that illness rates were highest during the winter months and during intensive training periods, with the odds ratio for upper-respiratory and pulmonary infections increasing 1.10 times with each 10% increase in high-load training. However, it is not clear whether the same patterns for illness incidence are present for winter sports athletes, who compete during the winter months but typically undertake the highest training loads in the period June to October (Tonnessen et al., 2014). Furthermore, other than

high training loads, elite athletes are exposed to a number of additional stressors, such as frequent international travel, hypoxic (altitude) exposure and the psychological pressure of competition, which may also impact on immunity and infection susceptibility (Mazzeo, 2005, Wilder-Smith et al., 2012, Clow and Hucklebridge, 2001).

The purpose of the current study was therefore: 1) to describe self-reported illness incidence for elite cross-country skiers across a number of competitive seasons, 2) to examine risk factors for illness in this cohort of world-class winter endurance athletes, and 3) to determine whether there are differences in the number and/or duration of illness events between athletes that have won an individual Olympic or World Championship medal, and those athletes that have not. It was hypothesised that heavy training loads and competition would both significantly increase the risk of illness, and that athletes who had medalled at world or Olympic level would report a lower number of illness events.

3.3 METHODS

3.3.1 Participants

Self-reported training and illness data for the period 01.05.2007 to 30.03.2015 were collected from 39 elite male (n=22) and female (n=17) cross-country skiers. Inclusion criteria were 1) selected to represent the Norwegian national senior or recruit team for cross-country skiing during the analysed time period, 2) over 18 years of age, and 3) had systematically and in detail recorded their day-to-day training from junior through to senior level. As of 30.03.2015, 16 of these athletes (9 males and 7 females) had won at least one individual senior Olympic or World Championship medal (totalling 73 individual Olympic and World Championship medals between them). These athletes were categorised as world-leading (WL), while those athletes that had not won an individual Olympic or World Championship medal were categorised as international level (IL). All athletes provided their written,

informed consent for their data to be included in the study, which was approved by the Loughborough University Ethical Review Committee and registered with the Norwegian Data Protection Authority.

3.3.2 Training and illness data

Athletes recorded their training and illness data daily, as previously described. In total, from 39 athletes, 143 person-years of data were collected, typically consisting of 3-4 years per athlete. Of these, two athletes (eight person-years) were excluded due to inadequate detail in the recording of either training or illness. Self-reported illness episodes were identified as previously described, based on the definitions proposed by Matthews et al. (2010). Each event was broadly categorised as either respiratory symptoms or gastrointestinal symptoms. The number of days of symptoms of each illness event was recorded. Rolling one, three and seven day averages for total training volume, the volume of high-intensity training and the volume of strength/power/sprint training were calculated. Training impulse (Banister and Calvert, 1980) (TRIMP = training duration (min) x intensity (HR zone 1-5), Training Monotony (mean TRIMP / SD TRIMP) and Training Stress (TRIMP x training monotony) scores (Foster, 1998) were also calculated. In addition, the number of competitions, number of international flights and number of days at altitude ($\geq$1,500 masl) during the previous one, three, and seven days were calculated.

2.3.3 Statistical analysis

To explore which factors were associated with onset of illness, multilevel modelling was employed using MLwiN software (version 2.32; University of Bristol). In repeated measures data, measurement time points are nested within participants, which violates an assumption in many single-level analyses that the data are independent. Multilevel modelling overcomes this by creating distinct, but associated, equations at both the within- and between-person

levels, yielding better estimation of the parameters and statistical significance (Hox, 2010). Another advantage of this type of modelling is that it does not require an equal number of time points for every participant; therefore, missing data are not a statistical issue.

Multilevel logistic regression equations were constructed to predict illness incidence, which was treated as a binary (0=did not become ill, 1=did become ill) outcome variable. Firstly, we explored whether illness events occurred more frequently for males compared with females (0=males, 1=female), for athletes of different performance levels (0=IL, 1=WL), during different times of year (spring (March-May), summer (June-August), autumn (September-November) and winter (December-February), with summer used as the reference category) and during different training phases (transition phase, general preparation phase, specific preparation phase, competition phase, regeneration phase, with the general preparation phase used as the reference category) by including these as categorical independent variables in separate multilevel models. Training variables, air travel, altitude exposure and competition were entered into separate models as independent variables to determine unadjusted odds ratios. In order to disaggregate within-person and between-person associations, each participant's aggregate for each variable was centred on the grand mean and entered into the level 2 equation in order to determine whether an individual with higher average values for that independent variable had a higher incidence of illness, compared to an individual with lower average values (between-person relationships). Each variable was also centred on each participant's unique mean and entered into the level 1 equation in order to determine whether fluctuations around an individual's average levels were associated with changes in illness incidence (within-person relationships). Odds ratios for training variables represent the increase in risk of an illness event occurring as a result of a 1 h/week increase in that training variable, or a 10% increase in Training Monotony, Training Stress and TRIMP. As a final step, based on the unadjusted models described above, we then constructed an adjusted

44

logistic multilevel model with all significant independent variables from the unadjusted models included. Multilevel regression equations were also constructed to determine whether sex, performance level, type of illness, season, training phase or training load during illness were significantly associated with the duration of symptoms, which was treated as a continuous variable. Significance was accepted at the $P<0.05$ level.

3.4 RESULTS

In total, data for 7,016 person-weeks were analysed. These included 464 events of illness, 110,959 h of training, 3,812 competitions, 1,769 international flights and 4,879 days at altitude >1,500 m above sea level. Of all the training recorded, 91% was endurance training and 9% was strength/power/sprint training (**Table 3.1**). Of endurance training, 90% was at low intensity (I-zone 1 and 2), and 10% was at high intensity (I-zone 3-5).

3.4.1 Illness incidence

Athletes reported symptoms on 2,347 (4.8%) of the 49,147 days monitored, equivalent to an average of 3.4 events and 17.5 days of illness per year. For the 37 athletes included in the current study, average individual illness rates ranged from 1-7 events per year, despite modest between-athlete differences in training load, travel and competition schedule or training environment. Of all the illness events reported, 19% were gastrointestinal symptoms and 81% were respiratory symptoms. The average duration of symptoms was 5 ± 4 days, with this being significantly longer for respiratory symptoms compared to gastrointestinal symptoms (5 ± 4 vs. 3 ± 2 days, b=-1.294, $P<0.001$).

Table 3.1: Training variables and illness events by sex, performance level, training phase and season during 7,016 person-weeks for 37 elite cross-country skiers

| FACTOR | ENDURANCE | | | | | Str/Pw | Sprint | Daily training volume (h:min) | Daily TRIMP | Illness events (% of weeks) | Weeks (% of factor) |
	I-zone 1	I-zone 2	I-zone 3	I-zone 4	I-zone 5						
TOTAL	79%	3%	4%	3%	2%	8%	2%	02:20 ± 01:19	192 ± 114	6.6	7016 (100%)
SEX											
Male	78%	4%	4%	3%	2%	7%	1%	02:11 ± 01:20	181 ± 116	7.4	3402 (48%)
Female	79%	3%	3%	3%	2%	8%	2%	02:28 ± 01:18	203 ± 111	5.9	3614 (52%)
PERF. LEVEL											
IL	78%	4%	4%	3%	2%	7%	2%	02:14 ± 01:20	187 ± 115	7.4	3298 (47%)
WL	80%	3%	3%	3%	2%	7%	2%	02:24 ± 01:19	197 ± 112	5.9	3718 (53%)
PHASE											
Transition	76%	5%	4%	2%	1%	11%	1%	02:15 ± 01:16	183 ± 108	7.3	629 (9%)
General prep.	78%	4%	4%	3%	1%	8%	2%	02:42 ± 01:24	221 ± 116	5.3	3150 (45%)
Specific prep.	81%	2%	3%	3%	3%	7%	1%	02:16 ± 01:08	186 ± 96	6.7	1252 (18%)
Competition	81%	2%	3%	4%	4%	5%	1%	01:53 ± 01:03	162 ± 108	8.6	1673 (24%)
Regeneration	76%	5%	3%	4%	3%	9%	0%	01:10 ± 01:07	102 ± 116	7.7	312 (4%)
SEASON											
Spring	77%	4%	4%	3%	3%	8%	1%	01:50 ± 01:14	155 ± 118	8.0	1404 (20%)
Summer	79%	4%	4%	2%	1%	8%	2%	02:44 ± 01:26	222 ± 117	4.1	1887 (27%)
Autumn	79%	3%	4%	3%	1%	8%	2%	02:34 ± 01:18	210 ± 109	6.5	1901 (27%)
Winter	81%	2%	3%	3%	4%	6%	2%	01:06 ± 01:07	171 ± 99	8.3	1824 (26%)

For endurance training in each of the five intensity zones, strength/power (Str/Pw) and sprint training values are percentages of total training volume. Daily training volume and daily Training Impulse (TRIMP) are presented as mean ± SD.

IL: International level athletes; WL: World-leading athletes (minimum one individual senior Olympic/World Championship medal)

3.4.2 Risk factors for illness

Unadjusted and adjusted odds ratios for illness for all the candidate risk factors are presented in Tables 3.2 and 3.2. Analysing respiratory symptoms separately from gastrointestinal symptoms did not affect which variables were associated with illness.

3.4.2.1 Sex, performance level and family situation

Sex was not a significant risk factor for either illness incidence or symptom duration, and nor was living in a household with young children (**Table 3.2**). However, performance level was significantly associated with the duration of symptoms, with this being lower for WL athletes (b=-0.019, P=0.034). Consequently, WL athletes reported a lower total number of days of illness per year compared with IL athletes (15 ± 8 days/year vs. 23 ± 11 days/year), despite no significant between-group differences in training load, number of flights, number of competitions or number of days spent at altitude.

3.4.2.2 Time of year

Athletes experienced significantly more illness events during autumn, winter and spring compared with summer, with the highest probability of illness during the winter months (**Table 3.3**). However, when adjusted for other independent variables, the highest risk of illness was in the spring. Regarding training phase, the highest probability of illness was during the Competition Phase which was from January to March (**Table 3.2**), but training phase was no longer a significant risk factor when accounting for other variables. Season was also significantly associated with symptom duration, with illnesses contracted during autumn (b=0.024, P<0.01), winter (b=0.043, P<0.001) and spring (b=0.037, P<0.001) typically persisting for longer than those reported during the summer.

Table 3.2: Unadjusted odds ratios for illness for predictor variables

Predictor variable	Estimated odds ratio (95% CI)	
	Within subject	Between subject
Sex (M=0)		0.78 (0.60-1.02)
Age	1.01 (0.95-1.07)	1.02 (0.99-1.05)
Team (Recruit=0)		0.87 (0.68-1.11)
Performance level (IL=0)		0.83 (0.63-1.10)
Family situation (No children=0)		0.98 (0.67-1.44)
Summer	1.00	
Autumn	**1.63 (1.23-2.16)***	
Winter	**2.09 (1.59-2.73)***	
Spring	**2.00 (1.50-2.65)***	
General Preparation Phase	1.00	
Specific Preparation Phase	1.28 (0.99-1.65)	
Competition Phase	**1.68 (1.35-2.09)***	
Regeneration Phase	**1.56 (1.02-2.37)#**	
Transition Phase	**1.40 (1.01-1.92)#**	
International air travel	**4.53 (3.49-5.88)***	1.23 (0.19-7.91)
Altitude exposure	**1.05 (1.01-1.10)#**	0.81 (0.52-1.27)
Competition	**2.74 (2.18-3.45)***	0.53 (0.19-1.45)
Total training volume	0.98 (0.97-1.00)	**0.91 (0.85-0.98)#**
High-intensity endurance exercise	**1.16 (1.03-1.31)#**	0.57 (0.30-1.09)
Strength/Power/Sprint exercise	**0.83 (0.75-0.91)***	0.70 (0.48-1.02)
Training monotony	1.01 (0.99-1.02)	**0.86 (0.79-0.93)***
Training impulse	1.00 (0.98-1.01)	1.06 (1.00-1.12)
Training stress	1.00 (1.00-1.00)	**0.97 (0.96-0.99)#**

Data for 7016 person-weeks from 37 elite cross-country skiers. * $P<0.001$; # $P<0.05$

Table 3.3: Adjusted odds ratios for illness for predictor variables retained in the final multilevel model

Predictor variable	Estimated odds ratio (95% CI)	
	Within subject	Between subject
FIXED EFFECTS		
Intercept	-5.116*	
PREDICTOR VARIABLES		
Summer	1.00	
Autumn	**1.65 (1.20-2.25)***	
Winter	**1.91 (1.11-3.27)***	
Spring	**2.09 (1.09-3.99)***	
General Preparation Phase	1.00	
Specific Preparation Phase	0.70 (0.46-1.04)	
Competition Phase	0.78 (0.46-1.34)	
Regeneration Phase	0.97 (0.46-2.04)	
Transition Phase	0.88 (0.44-1.75)	
International air travel	**4.94 (3.74-6.53)***	2.65 (0.16-44.7)
Altitude exposure	1.05 (1.00-1.10)	0.76 (0.38-1.53)
Competition	**2.93 (2.24-3.83)***	0.55 (0.16-1.92)
Total training volume	1.01 (0.98-1.04)	1.06 (0.90-1.15)
High-intensity endurance exercise	1.08 (0.95-1.24)	0.80 (0.37-1.76)
Strength/Power/Sprint exercise	0.93 (0.83-1.05)	0.82 (0.51-1.33)
Training monotony	1.01 (1.00-1.01)	**0.87 (0.73-1.00)#**
RANDOM EFFECTS		
Intercept	0.066	
R^2	0.347	

Data for 7016 person-weeks from 37 elite cross-country skiers.

* *P*<0.001; # *P*<0.05

3.4.2.3 Competition, air travel and altitude exposure

International air travel was associated with unadjusted odds ratios of 4.53 (3.49-5.88) of becoming ill within the next day, 2.73 (2.25-3.31) of becoming ill within the next three days and 1.78 (1.52-2.06) of becoming ill within the next seven days (all $P<0.001$). When accounting for other independent variables, air travel was associated with adjusted odds ratios of 4.94 (3.74-6.53), 2.66 (2.17-3.26) and 1.59 (1.36-1.85) of becoming ill within one, three and seven days, respectively (all $P<0.001$). Indeed, 35% of all recorded events were associated with an international flight during the preceding 72 h. Of the 1769 flights recorded, 53% were outbound and 47% were homebound, while 95% were continental and 5% intercontinental. Homebound flights were more likely to be associated with the athlete subsequently reporting symptoms compared with outbound flights (14% vs. 5%) as were intercontinental flights compared with continental flights (17% vs. 9%).

Taking part in a competition was associated with unadjusted odds ratios of 2.74 (2.18-3.45) of becoming ill within the next day, 1.75 (1.57-1.95) of becoming ill within the next three days and 1.45 (1.35-1.55) of becoming ill within the next seven days (all $P<0.001$). When accounting for other independent variables, competition was associated with adjusted odds ratios of 2.93 (2.24-3.83), 1.78 (1.56-2.03) and 1.51 (1.38-1.66) of becoming ill within one, three and seven days, respectively (all $P<0.001$).

Exposure to altitude of ≥1,500 masl was associated with an unadjusted odds ratio of 1.05 (1.01-1.10) ($P<0.05$) of becoming ill within the next seven days, but was no longer significantly associated with illness when accounting for other independent variables (**Table 3.4**).

3.4.2.4 Training

The only training variable that was a significant risk factor illness in both unadjusted and adjusted models was Training Monotony. A 10% increase in between-person Training Monotony was associated with an odds ratio of 0.87, remaining significant when accounting for other variables (**Table 3.4**). In unadjusted models, within-person increases in the volume of strength/power/sprint exercise was negatively associated with illness incidence, while within-person high-intensity endurance exercise was positively associated with illness incidence (**Table 3.3**). However, both became non-significant when accounting for other independent variables (**Table 3.4**). Between-person total training volume and Training Stress were both negatively associated with illness incidence in unadjusted models, but became non-significant when adjusted for other independent variables. TRIMP was not a significant risk factor for subsequent illness, nor was TRIMP during illness significantly associated with symptom duration.

The final adjusted multilevel model included season, training phase, competition, aircraft travel, altitude exposure, total training volume, volume of high-intensity training, volume of strength/power/sprint training and training monotony (**Table 3.4**).

3.5 DISCUSSION

Our data indicate that elite winter endurance athletes experience three to four episodes of respiratory and/or gastrointestinal illness per year, with symptoms typically lasting five days. Few other studies have examined illness rates in athletes for periods of more than a few weeks or months. However, Hellard et al. (2015) reported a similar frequency of infectious illness for elite swimmers who experienced, on average, four episodes of respiratory, gastrointestinal and/or urogenital infection per person-year. This suggests that elite endurance athletes experience broadly similar illness rates, regardless of whether their competition

season falls during the winter or summer months. Although we unfortunately do not have equivalent data for a sedentary or recreationally active control group in Norway during the same time period, these illness rates do not appear to differ markedly from those of adults in the general population, who typically experience approximately two to four respiratory episodes per year (Heikkinen and Jarvinen, 2003, Monto, 2002). However, it is worth noting that there appears to be high inter-individual variability in infection susceptibility, even within a homogenous group of elite athletes.

Cross-country skiers experience the highest frequency of illness during the winter months, similar to elite swimmers (Hellard et al., 2015) and mirroring seasonal patterns for respiratory infections observed in the general population (Monto, 2002). However, when adjusting for other variables, such as the number of competitions, spring was associated with the highest risk of illness. It should be noted, however, that since these athletes typically train and compete on snow until mid-April, the months categorised as "spring" bear much resemblance to winter. Furthermore, it is during these months that vitamin D levels would be expected to be at their lowest (Klingberg et al., 2015), with recent research indicating that vitamin D deficiency is an important predictor of infection in endurance athletes (He et al., 2013). Since training phase was not a significant risk factor when adjusted for season, and since patterns of illness appear to be similar for both summer and winter sport athletes, this suggests that time of year is a more important in determining illness risk than which training phase the athlete is in.

Of the candidate factors, the single biggest risk factor for illness was international air travel, making the athlete approximately five times more likely to experience illness the subsequent day. Athletes remained at an enhanced risk of illness for seven days after an international flight. This is in agreement with previous studies in non-athletes which have reported that approximately 20% of individuals experience symptoms of respiratory illness within one

week of commercial aircraft travel (Zitter et al., 2002) and that cabin staff are more predisposed to respiratory infections than the general population (Whelan et al., 2003). Schwellnus et al. (2012) found that athletes traveling to a foreign destination more than five time zones difference from their home country had a 2-3 fold increase in risk of illness. However, in contrast to the current study, homeward travel was not associated with an increased risk of symptoms. The majority of homebound flights included in the present analyses were following competition weekends, with athletes typically flying home the same day and often within a few hours of finishing the final competition. As such, it is possible to speculate that infection risk was further exacerbated because athletes were traveling home in an already somewhat immunocompromised state, since it is well-documented that acute bouts of strenuous and prolonged physical activity result in immune disturbances that persist for a number of hours (Walsh et al., 2011). Air travel is associated with a number of stressors which may increase infection risk. These include: time-zone changes, sleep disruption, increased pathogen exposure due to crowded conditions in airports and on aircraft, mild hypoxia and drying of oral and nasal mucosa, as well as psychological stress. It is therefore not surprising that simulated long-haul flights have been found to result in a transient impairment of some aspects of immune function (Wilder-Smith et al., 2012). Hence, although often unavoidable, athletes are advised to minimise air travel as much as possible, particularly immediately prior to important competitions and major championships. When competing in central Europe, driving between competition venues may be a preferable alternative to flying, provided the driving distance is feasible. Where this is not possible, flying out as early as possible prior to a major competition will reduce the likelihood of the athlete experiencing illness during the event itself, although this of course does not ameliorate the risk of illness disrupting the training/tapering process. Following competitions abroad, delaying homeward travel until the subsequent day may be preferable to flying home on the

53

same day. Further research is necessary in order to identify the mechanisms underlying the increased infection risk associated with air travel. Including measures of psychological stress in such a study would be of particular interest.

The training distribution of the athletes followed a 90:10 distribution of low intensity: high intensity endurance training, similar to that previously reported for endurance athletes training more than 500 h per year (Seiler and Kjerland, 2006). In contrast to the findings of Foster (1998) neither TRIMP nor Training Stress were associated with increased illness incidence at the between- or within-person level, when accounting for other risk factors. Although within- person changes in Training Monotony was not significantly associated with illness, those athletes with higher average Training Monotony were somewhat less susceptible to illness than athletes with lower Training Monotony. This finding suggests that a microcycle that avoids large fluctuations in total daily training load may be preferable to one that alternates between very hard and very easy days, at least in terms of reducing illness risk. This is an interesting finding and contrasts with current recommendations that athletes should add variety to limit training monotony (Walsh et al., 2011a). However, typical practice in cross-country skiing does traditionally incorporate "hard" and "easy" training days, so these findings may be indicative that it is more extreme fluctuations above this norm which increase infection risk. Rather than measuring only daily variation in TRIMP, a calculation that also accounts for differences in activity type (e.g. skiing vs. running vs. cycling) would give a more accurate indication of the true training monotony. It is also possible to question the validity of TRIMP as an accurate measure of training load, since it does not account for activity type and assumes that the relationship between heart rate and intensity is linear, rather than exponential, and may therefore somewhat underestimate the load associated with high-intensity training.

None of the other measured training variables were significantly associated with illness incidence when accounting for other independent variables. This is in contrast to Hellard et al. (2015) who found that increases in high-load and resistance training augmented the risk of upper-respiratory and pulmonary infections. In the current study, high-intensity endurance exercise was significantly associated with illness only in the unadjusted models. This can likely be explained by the fact that high-intensity endurance exercise was a sum of all recorded time spent in Intensity Zones 3-5, including interval training, intensive continuous training and competition. However, since competitions are associated with additional physiological and psychological stress above that typically encountered even during the most intense training sessions, competition was also treated as a separate binary variable (did the athlete compete? Yes/No). A high-pressure competition during which the athlete remains continuously in Intensity Zone 4 for 40 min likely represents a somewhat different physiological and psychological stimulus to an interval session composed of ten 4 min repetitions in the same intensity zone. Indeed, since only competition remained significant in the adjusted model, it appears that some other factor than merely time spent at high-intensity contributes to the increase in illness susceptibility following competition. Whether this is related to greater psychological stress, greater physiological stress, environmental factors such as crowd exposure, or a combination of these, is not clear. Whatever the underlying mechanisms, the finding that taking part in competitions increases the risk of illness is in agreement with previous studies (Nieman, 2007), although Hellard et al. (2015) found that the risk of upper respiratory tract and pulmonary infections in swimmers was higher in the periods of intensive training than in both competition and post-competition periods. However, unlike swimmers, cross-country skiers perform their most intensive training during the summer and primarily compete during the winter months (Tonnessen et al., 2014) which may account for this discrepancy.

In an attempt to examine whether athletes who rested completely recovered more quickly than those who continued to train through illness, we explored whether average daily training load during illness was associated with symptom duration. However, this was confounded by symptom severity, since athletes typically continued to do some form of training when they experienced light symptoms, while taking complete rest during more severe symptoms, with severe symptoms also being associated with more prolonged illness. Finding a valid means of exploring what effect training following onset of symptoms has on illness duration remains a challenging but important area for future research.

It is well known that ascent to high altitude alters both physiological and metabolic function, and can influence immune function (Mazzeo, 2005). However, it is not clear whether the moderate altitudes to which elite endurance athletes are typically exposed results in clinical changes in immunity and infection susceptibility. Tiollier et al. (2005) found a cumulative negative effect of physical exercise and hypoxia on S-IgA levels in elite cross-country skiers during 18-days of live high, train low altitude training. Since reduced S-IgA levels have been consistently linked to increased risk of upper-respiratory symptoms (Gleeson et al., 1999, Neville et al., 2008) it is likely that such a reduction would make athletes more susceptible to illness. However, the current results indicate that altitude exposure does not significantly increase the risk of an athlete becoming ill when other factors, such as air travel to and from the altitude camp, are accounted for. However, it is worth noting that, in contrast to the majority of previous research on altitude and immune function, athletes in the current study regularly trained and competed at altitude, with the average athlete reporting 35-40 days per year at ≥1,500 masl. It is possible that this may have limited any adverse effects of hypoxia, either because athletes were already partially acclimatised, or as a result of athletes and coaches developing effective strategies to minimise infection risk at altitude through many years of practical experience.

In the current study, the athletes of the highest performance level experienced a lower total number of annual illness days compared to athletes of a somewhat lower performance level. This was principally due to significantly shorter duration of symptoms, although there was also a non-significant tendency for WL to report a lower number of events. Although it is not possible to determine cause and effect, this is in accordance with the findings of Hellard et al. (2015) that swimmers of a higher performance level had a lower risk of upper-respiratory tract infections, suggesting that a robust immune system capable of withstanding and recovering quickly from infection, even during periods of high physiological, psychological and environmental stress, may be a predictor of success in an otherwise homogenous group of elite endurance athletes. Although there were no significant differences between the two groups in terms of training load, number of flights, number of competitions or number of days spent at altitude, athletes in the WL group were slightly older than those in the IL group (25 ± 4 years vs. 23 ± 5 years). It is unlikely that such a small difference in age would have any meaningful effect on immune function, but it is possible to speculate that two additional years of experience may be associated with behavioural differences (e.g. better routines in terms of hygiene, nutrition and hydration) which could influence infection incidence.

Although in the general population, men appear to be somewhat more susceptible to viral and bacterial infections than women (Klein, 2000) recent studies of athletes suggest a higher incidence of respiratory illnesses amongst females compared to males (Engebretsen et al., 2010, Engebretsen et al., 2013, He et al., 2014, Soligard et al., 2015). However, in the current study, sex was not significantly associated with either illness incidence or symptom duration.

The current dataset includes a large amount of data for some of the world's best winter endurance athletes. However, a clear limitation is that both training and illness data were self-reported. Although elite cross-country skiers have previously been found to self-report their training accurately (Sylta et al., 2014), only 79% of 159 respiratory illnesses reported by

athletes during the Sochi Olympic Games were due to infection (Soligard et al., 2015). Since not all symptoms in the current study were verified by a physician, or better still confirmed via pathological examination of nasal and throat swabs, it is likely that some of the illness events included in analyses were due to non-infectious causes, such as airway inflammation or allergen exposure. It is also possible that elite athletes may be more/less apt to self-report illness than the general population. However, since the current analyses are based only on comparisons within or between individuals from the same athlete group, a reporting bias is unlikely to be a major issue.

3.6 CONCLUSION

World-class cross-country skiers experience, on average, 3-4 respiratory and/or gastrointestinal illness events per year, with symptoms typically lasting 5 days. The highest illness rates for these athletes occur during the winter months (Dec-Feb). However, world-leading athletes experience fewer illness days per year compared to athletes of a lower performance level. International air travel and taking part in competitions both significantly increase the risk of illness. Since both are inherent components of international-level sport, future research exploring effective strategies to minimise illness risk associated with competition and air travel is warranted. In terms of reducing infection susceptibility, a microcycle that avoids large fluctuations in total daily training load may be preferable.

.

CHAPTER 4: EFFECT OF AN INTENSE PERIOD OF COMPETITION ON SUBSEQUENT RACE PERFORMANCE AND SELF-REPORTED ILLNESS IN ELITE CROSS-COUNTRY SKIERS

Ida S. Svendsen[1], Michael Gleeson[1], Thomas A. Haugen[2], Espen Tønnessen[2]

1) School of Sport, Exercise and Health Sciences, Loughborough University, UK.
2) Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway

4.1 ABSTRACT

PURPOSE: To determine whether participating in a cross-country skiing stage race (Tour de Ski) affects subsequent illness incidence, training and race performance. **METHODS:** Self-reported training and illness data from 44 male and female elite cross-country skiers were included. In total, 125 person-years of data were collected (2-3 seasons per athlete). Illness incidence, training load and performance in international competitions were calculated for athletes who did and did not participate in Tour de Ski. **RESULTS:** 48% of athletes reported becoming ill during or in the days immediately after taking part in Tour de Ski, vs. 16% of athletes who did not participate. A larger proportion of the male athletes, both in the TDS (53% vs. 44%) and CON (21% vs. 8%) groups, became ill in this time period compared to the female athletes. There was no significant change in race performance in the 6 weeks following Tour de Ski for female athletes. However, for the male athletes, average placing in international races was significantly worse following participation in Tour de Ski (9th (95% CI: 6th-15th) vs. 18th (95% CI: 14th-23rd), p=0.003). Similarly, best placing achieved in international competition was significantly worse for the 6 weeks after taking part in Tour de Ski compared to the 6 weeks before (3rd (95% CI: 2nd-6th) vs 10th (95% CI: 14th-23rd), p=0.03). Furthermore, there was a significant interaction effect when examining performance in World Championship and Olympic Games for men and women who took part in Tour de Ski compared to CON (F(1,49)=4.187, p=0.046). While female athletes who took part in TDS typically performed relatively better in these major championships compared to CON, male athletes who took part in Tour de Ski typically performed worse in Olympic/World Championships than CON. **CONCLUSION:** Participating in Tour de Ski appears to result in ~3-fold increase in risk of illness in this period. Male athletes appear more prone to illness and also see a drop in race performance following Tour de Ski.

4.2 INTRODUCTION

Elite cross-country skiers typically perform approximately 800 hours of training per year, more than 90% of which is endurance training (Tonnessen et al., 2014). With such large volumes of training, these athletes frequently tread a fine line between the optimal training load to stimulate performance adaptation, and excessive overload leading to maladaptive outcomes (Meeusen et al., 2013). Previous studies suggest that intense periods of training and competition may result in adverse changes to a number of immune variables (Gleeson et al., 1995, Mackinnon and Hooper, 1994). This in turn appears to lead to an increased susceptibility to opportunistic infections, in particular upper-respiratory tract infections such as colds and influenza (Nieman, 2000). However, limited data exist on the effects of intensified periods of competition on illness incidence in world-class endurance athletes already training in excess of 700 hours per year.

The Tour de Ski is an event that has been part of the cross-country skiing calendar every year since its introduction by the International Ski Federation (FIS) in 2006. The race consists of six to nine stages during late December and early January. These stages are held in the Czech Republic, Germany, Italy, and Switzerland, with the competition typically running over an eight to eleven day period, including one or two rest days. For those athletes that take part, it undoubtedly represents the most intense period of competition of the season. Not only are competitors required to push their bodies to the physical limit on multiple consecutive days, but they are also exposed to the additional stressors of travel, cold temperatures (typically ranging from +4°C to -10°C) and moderate altitude (1000-1300 m above sea level), as well as the repeated psychological pressure of competition. Stage races such as this provide a unique opportunity to examine the impact of short-term OR in elite athletes, something that is often not feasible or achievable in a laboratory setting. It seems conceivable that taking part in a

stage race such as Tour de Ski could have an immunodepressive effect, resulting in increased illness incidence during both the competition period itself, and in the days that follow. Indeed, anecdotal reports from athletes and coaches suggest that this may well be the case, although no empirical evidence is currently available.

Furthermore, a large number of elite cross-country skiers choose to take part in Tour de Ski in the same year that they also participate in Olympic Games or World Championships. Recent studies have examined the training and peaking practices of elite athletes leading up to major championships, and underlined the importance of an appropriate training volume and intensity distribution (Tonnessen et al., 2014, Tonnessen et al., 2015). These studies also found that competitions are a key component in an athlete's preparation for a major championship, making up a substantial proportion of all high-intensity training in this period. However, it is not known whether a stage race such as Tour de Ski, characterized by a marked shift in the normal intensity distribution towards more high-intensity activity (of which all is performed as competitions rather than interval training) and relatively little low-intensity training, could affect subsequent training and race performance. It is possible that taking part in such an event could impair an athlete's ability to achieve peak performance in major championships 5-7 weeks later, or even lead to a state of non-functional OR or OT (Meeusen et al., 2013). On the other hand, it is equally conceivable that taking part in Tour de Ski might in fact play a beneficial role in the preparation process of these athletes.

Hence, the aims of the current study were to determine, 1) whether athletes experience increased illness during and in the days immediately after taking part in Tour de Ski, compared to those athletes who do not take part in Tour de Ski, 2) whether taking part in Tour de Ski affects subsequent training and race performance, particularly in major championships (Olympic Games and FIS Nordic Ski World Championships), and 3) whether any of these effects are influenced by sex.

4.3 METHODS

4.3.1 Subjects

Self-reported training and illness data from 27 male and 17 female elite cross-country skiers were included in the current study. In total, 127 person-years of data were collected, typically consisting of 2-3 seasons per athlete from 2006-2014 (**Table 4.1**). Of the data collected, 10 seasons were excluded due to inadequate detail in the recording of either training or illness, or due to periods of missing data. A further two seasons were excluded as outliers due to the athletes experiencing repeated and unusually prolonged episodes of illness, resulting in the annual number of illness days being far above (>3 SDs) the group mean. Of the remaining 115 seasons, 42 included participation in Tour de Ski. In total, data for 38,525 person-days were collected, including 380 episodes of illness and 86,739 hours of training.

Table 4.1: Characteristics of athletes included in the study at the time of the analysed season.

	Male n=27 (63 seasons)	**Female** n=17 (52 seasons)
Age (yrs)	24 ± 3	24 ± 4
Body mass (kg)	76.0 ± 6.1	60.1 ± 4.8
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	78.2 ± 5.1	70.6 ± 5.2
$\dot{V}O_{2max}$ (L·min^{-1})	5.9 ± 0.5	4.2 ± 0.4
Annual training volume (h)	716 ± 91	816 ± 103
Took part in Tour de Ski	n=17 (27%)	n=25 (48%)

Data are presented as mean ± SD.

Inclusion criteria were 1) currently, or during the analysed time period, selected to represent the Norwegian national senior or recruit team for cross country skiing, 2) over 18 years of

age, and 3) systematic and detailed recording of their day-to-day training from junior through to senior level. In total, these athletes had won 106 World Championship and Olympic medals (individual and relay events). The regional ethics committee of Southern Norway reviewed the study and concluded that, due to the nature of the investigation, it did not require their approval. The study was therefore submitted to and approved by the Norwegian Data Protection Authority (NSD), and all athletes gave their oral and written informed consent for their data to be included in the study.

4.3.2 Training and illness monitoring

Athletes included in the study recorded their day-to-day training and illness data as previously described. To determine whether taking part in this event might increases the risk of illness, the number of athletes that experienced an episode of illness either during, or within 10 days after completion of the Tour de Ski, was determined. The percentage of athletes that became ill during Tour de Ski or in the ten days immediately after taking part in Tour de Ski was then compared to the percentage of athletes who did not compete in Tour de Ski and who became ill in the same time period (control).

4.3.4 Training load

To determine whether taking part in Tour de Ski resulted in a significant increase in exercise load above "normal" and whether taking part would impact on subsequent training, average daily training impulse (TRIMP) (Banister and Calvert, 1980) was determined for 6 weeks immediately before Tour de Ski, during Tour de Ski (including races, warm-ups, cool downs and training on rest days), and for 6 weeks after Tour de Ski. This was calculated by multiplying duration in minutes by the intensity zone (1-5; **Table 2.1**) reported in the training diaries. TRIMP values, total race time and distance covered during Tour de Ski were also compared between the sexes.

4.3.5 Race performance

In addition, to determine whether participation in Tour de Ski might have an impact on subsequent race performance, average FIS points and placing per race, and best placing were determined for the 6 weeks immediately before Tour de Ski and 6 weeks immediately after Tour de Ski for both males and females for the Tour de Ski group (TDS) and a matched control group (CON). This 6-week time-frame was chosen as it typically represents the race period between Tour de Ski and that season's major championships (World Championships/Winter Olympic Games), while also being long enough to include a minimum of one World Cup race for every athlete. Where possible, a different season for the same athlete was used as the control. In two cases this was not possible as the athletes in question had competed in Tour de Ski in all available seasons, and therefore control athletes were matched as closely as possible based on sex, age, discipline and performance level. Only international competitions were included in these analyses (World Cup, World Championship and Olympic races), and only performance in each athlete's own discipline (i.e. sprint races for sprint skiers, distance races for distance skiers). Results were taken from the FIS website (www.fis-ski.com/cross-country/). FIS points were calculated using the following formula:

$$(F \cdot Tx) / To - F$$

Where: F = FIS Factor (800 for interval starts, 1200 for sprints / pursuits with a break, 1400 for mass starts and pursuits without a break); Tx = competitor's race time in seconds; To = winner's race time in seconds.

In addition, to determine whether taking part in Tour de Ski might adversely affect an athlete's subsequent performance in Olympic Games or World Championships 1-2 months later, placing in these events was compared for both males and females for TDS and CON. In

order to account for differences in performance level and indicate whether or not the athletes were able to reach peak performance at this time, placing in World Championships/Olympic Games were normalised as a percentage of each athlete's mean placing in international competition for that whole season. For example, where an athlete's mean result for the season was 9th, and they finished 6th in the Olympic Games, this was expressed as 66.7%.

4.3.6 Statistical analyses

Normally distributed data are presented as means ± SD. Data that were not normally distributed data were treated using a Box-Cox transformation (Box and Cox, 1964) to meet the assumption of normality prior to analysis, and are presented as back transformed geometric mean ± 95% CI. Data were analysed using an independent or mixed-model ANOVA with a Bonferroni post-hoc correction. Differences in total race time and total race distance between men and women were analysed using an independent samples T-test. Significance was accepted at the $p < 0.05$ level.

4.4 RESULTS

4.4.1 Training and competition load

Taking part in Tour de Ski resulted in a significant increase in average daily TRIMP compared to 6 weeks before Tour de Ski for both men and women (**Figure 4.1**). The female athletes had significantly higher training loads than the male athletes both 6 weeks before and 6 weeks after Tour de Ski, but there was no sex-difference in TRIMP during the competition period itself (**Figure 4.1**). However, total Tour de Ski competition time was significantly higher for men than women, as was total Tour de Ski race distance (**Figure 4.2**).

4.4.2 Illness incidence

In 20 / 42 (48%) seasons which included participation in Tour de Ski, athletes reported becoming ill during or in the first 10 days immediately after the competition. Only 12 / 73 (16 %) of the athletes who did not take part in Tour de Ski reported becoming ill in the same time period (**Figure 4.3**). Out of the 20 episodes of illness reported, 85 % were symptoms indicative of upper respiratory infection (sore throat, blocked/runny nose, coughing, sneezing, headache, fever). When comparing men and women separately, a larger proportion of the male athletes, both in the TDS and CON groups, became ill in this time period compared to the female athletes (**Figure 4.3**).
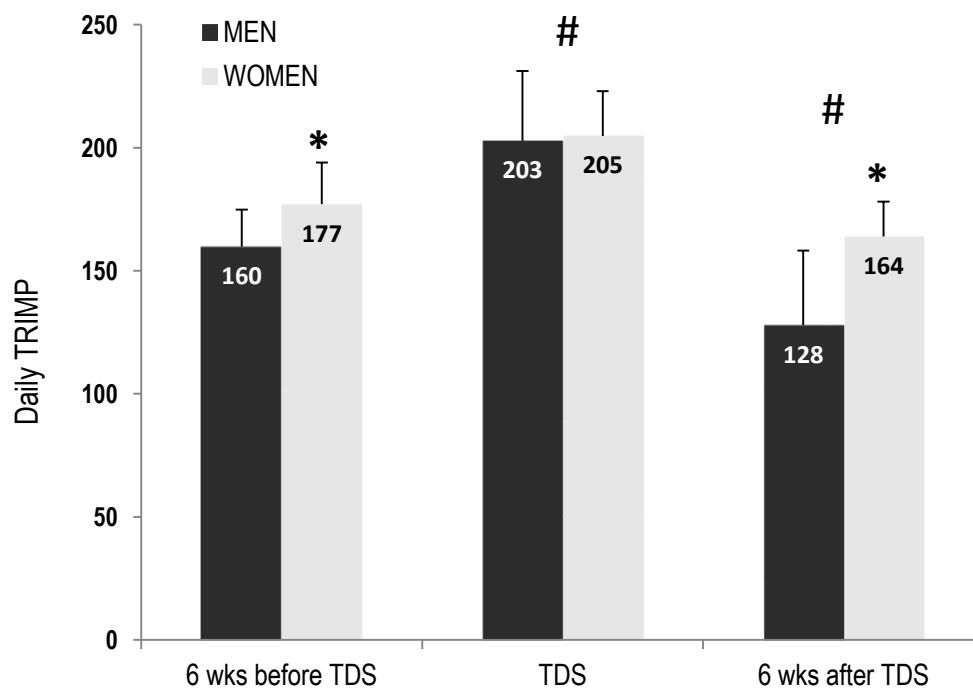
Figure 4.1: Average daily TRIMP score for 6 weeks before Tour de Ski, during Tour de Ski, and 6 weeks after Tour de Ski.

#*significantly different from 6 weeks before TDS for both men and women (p<0.01);* *******significantly different from men (p<0.01). Data are Mean+SD.*
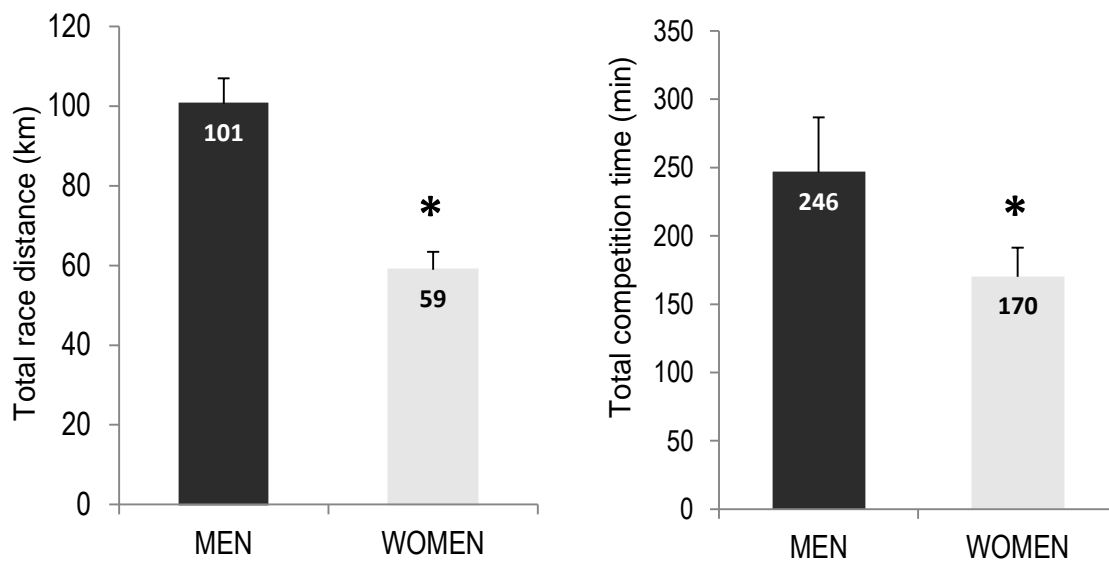
Figure 4.2: Total race distance (A) and total competition time (B) in men's and women's Tour de Ski. * *Significantly different from men (p<0.001). Data are Mean+SD.*

4.4.3 Race Performance

There was no significant change in race performance in the 6 weeks following Tour de Ski for female athletes. However, for the male athletes, average placing in international races was significantly worse following participation in Tour de Ski (**Figure 4.4**), increasing from 9th to 18th with no significant change in CON (Interaction effect: $F(1,34)=9.884$, $p=0.003$). Similarly, best placing achieved in international competition was significantly worse for the 6 weeks after taking part in Tour de Ski compared to the 6 weeks before (**Figure 4.5**), going from 3rd to 10th, despite no significant change in CON (Interaction effect: $F(1,34)=5.130$, $p=0.03$). There was also a borderline significant effect for average FIS points per race (Interaction effect: $F(1,26)=4.152$, $p=0.05$). Despite no change in CON, pairwise comparisons showed that average FIS points per race went up significantly in the TDS group ($p=0.007$) from 13 (95% CI: 9.1-17.5) to 22 (95% CI: 16.1-28.9), a difference equivalent to skiing ~26 seconds slower in a 15 km classic style race.

Finally, there was a significant interaction effect when examining performance in World Championship and Olympic Games for men and women who took part in Tour de Ski compared to CON ($F_{(1,49)}=4.187$, $p=0.046$). While female athletes who took part in Tour de Ski typically performed relatively better in these major championships compared to CON, male athletes who took part in Tour de Ski typically performed worse in Olympic/World Championships than CON (**Figure 4.6**).
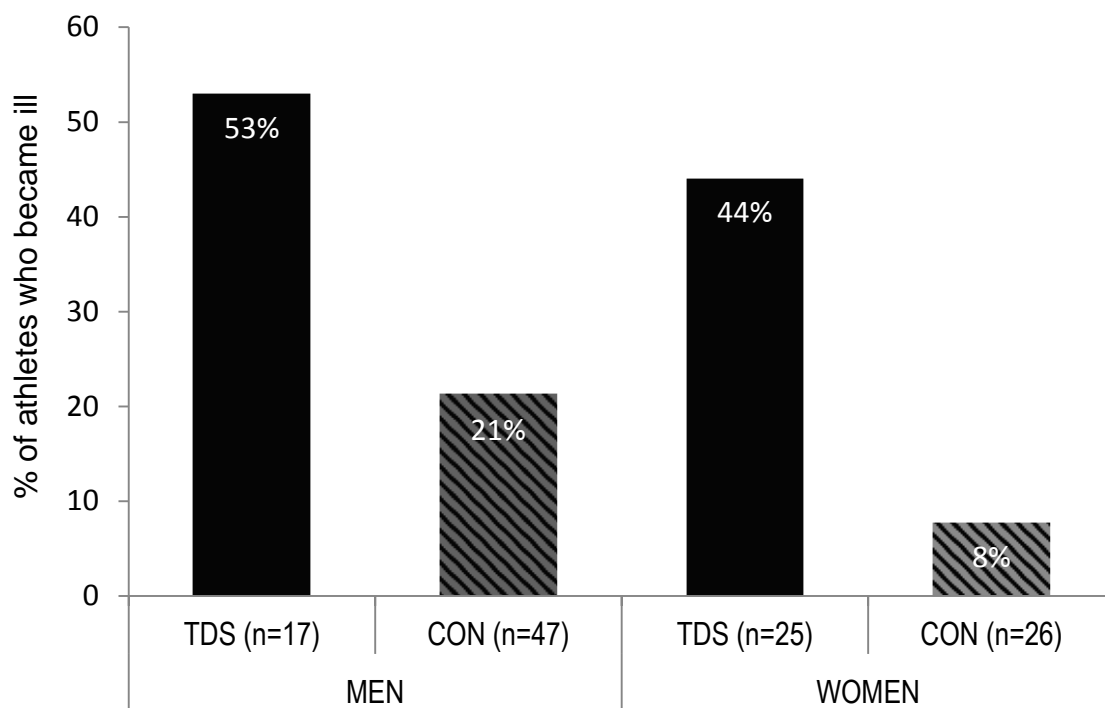
Figure 4.3: Percentage of male and female athletes who became ill during or within 10 days after Tour de Ski, or during the same time period for the control group.

TDS = *took part in Tour de Ski*
CON = *did not take part in Tour de Ski*
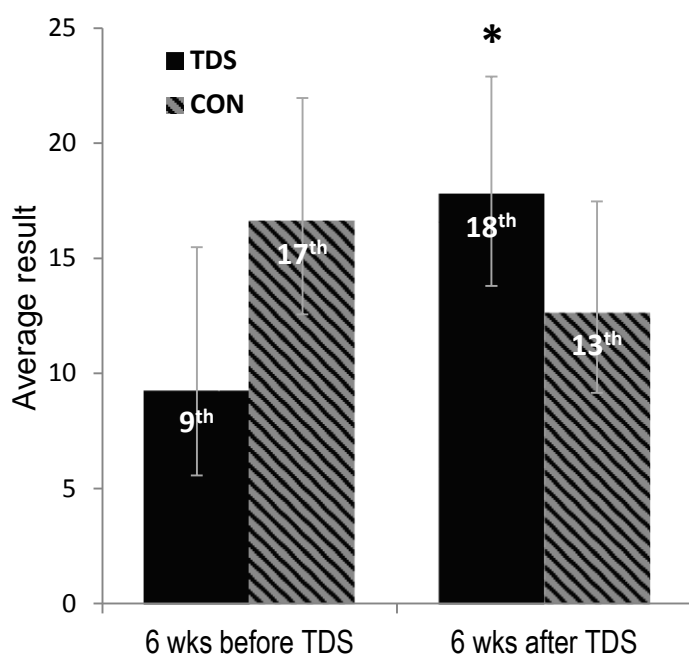
Figure 4.4: Average placing achieved by male athletes in international competition (World Cup/Olympic Games/World Championships) 6 weeks before and 6 weeks after Tour de Ski.

Data are geometric mean ± 95% CI.

TDS = *took part in Tour de Ski*, **CON** = *did not take part in Tour de Ski*

* *Significantly different vs. 6 weeks before TDS (p=0.003). No change in CON.*
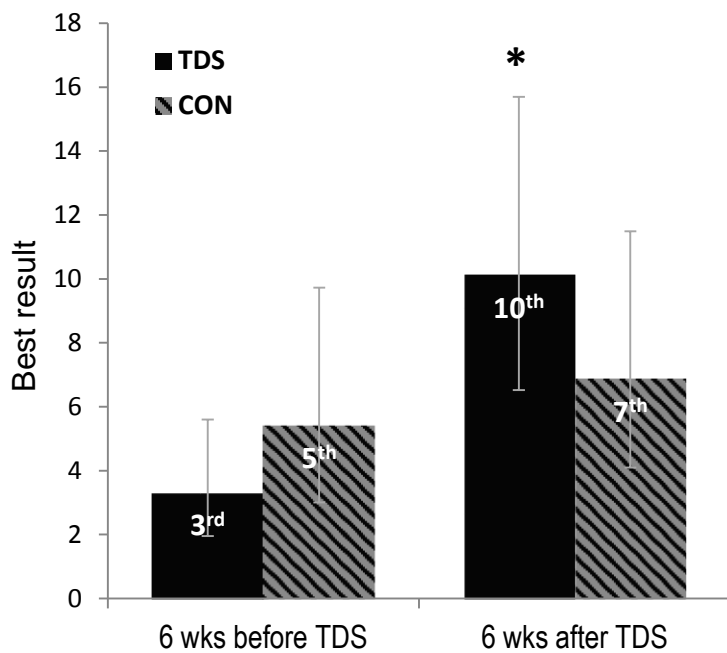
Figure 4.5: Best placing achieved by male athletes in international competition (World Cup / Olympic Games / World Championships) 6 weeks before and 6 weeks after Tour de Ski.

Data are geometric mean ± 95% CI.

TDS = *took part in Tour de Ski*, **CON** = *did not take part in Tour de Ski*

* Significantly different vs. 6weeks before TDS (p<0.001). No change in CON.
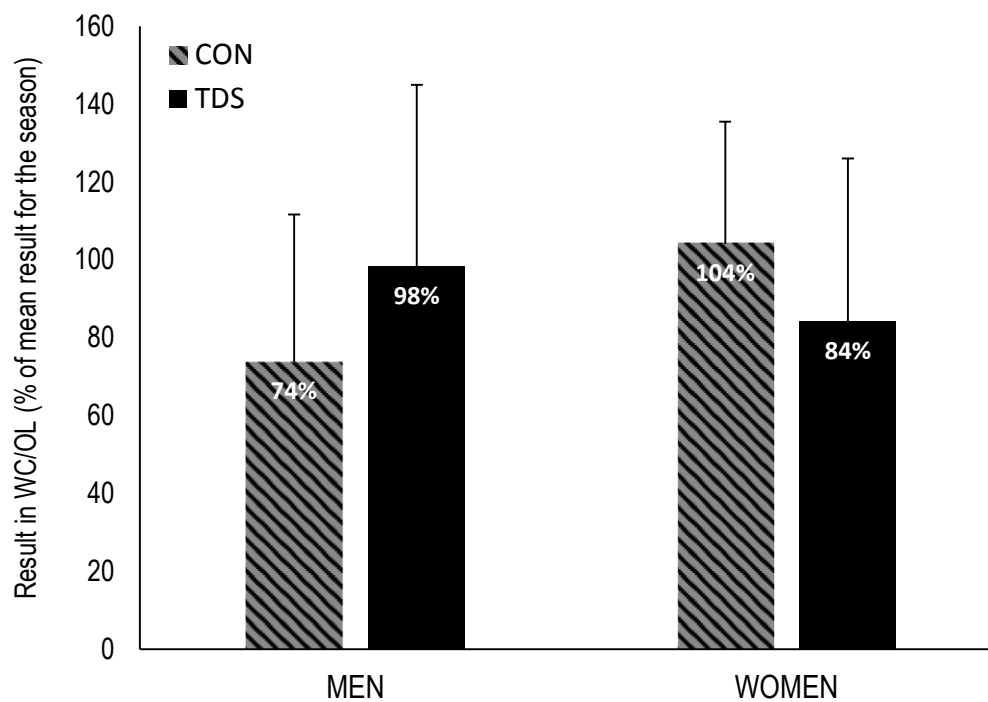
Figure 4.6: Placing in World Championships (WC) & Olympic Games (OL) normalised as a percentage of each athlete's mean placing for the season (i.e. less than 100% = better than average performance).

Data are mean+SD.
TDS = *took part in Tour de Ski,* **CON** = *did not take part in Tour de Ski.*
Significant interaction effect *(Gender*TDS, p=0.046)*

4.5 DISCUSSION

To the authors' knowledge, this is the first study to investigate the effects of an intense period of repeated competition on subsequent illness incidence, training and race performance in a large cohort of world-class winter sports athletes. A main finding of this study is that athletes taking part in a cross-country skiing stage race resulting in a significant increase above normal training load, had a ~3-fold higher risk of contracting an illness during and in the days immediately after the competition, compared to control athletes, with male athletes in both

groups appearing somewhat more susceptible to illness than female athletes. Another major finding is that male athletes typically see a drop in race performance for at least 6 weeks following participation in Tour de Ski, alongside a concurrent reduction in training load.

The finding of increased illness following participation in Tour de Ski is in accordance with previous studies reporting a relationship between upper respiratory illness (URI) risk and a high level of exercise stress (Nieman, 1994, Moreira et al., 2009, Gleeson et al., 2011a). The results also support earlier findings that URIs are the most common medical complaint affecting athletes (Reeser et al., 2003, Fricker et al., 2005), with 17 / 20 cases reported following Tour de Ski being symptomatic of URI. However, it is worth bearing in mind that the observed increase in illness could also be due to factors in addition to the physiological stress of the competition itself. For example, it is possible that athletes may have had a higher-than-normal exposure to pathogens due to increased contact with crowds and media during the competition period.

Compared to female athletes, a greater proportion of male athletes reported becoming ill, in both the TDS and CON groups (30% of all male athletes vs. 25% of all female athletes). This difference does not appear to be explained by differences in absolute training load, since there was no significant difference in TRIMP between males and females during the race period. However, because the male athletes had a lower baseline training load going into the race, the increase in TRIMP as a result of taking part in Tour de Ski was greater (27% increase in TRIMP compared to 16% for the female athletes). Furthermore, evidence suggests that men typically have a somewhat lower immune response than women, and therefore may be more susceptible to opportunistic infections such as colds and influenza (Engler et al., 2008), possibly due to an immunosuppressive effect of testosterone (Furman et al., 2014). On the other hand, in contrast to the general population, data from medical reports following major sporting events such as the Olympic Games have generally found a higher incidence of

illness among female compared to male athletes (Engebretsen et al., 2010, Engebretsen et al., 2013, Soligard et al., 2015, He et al., 2014), which does not correspond with the findings of the current study.

The male athletes experienced a significant drop in performance in international races in the 6 weeks following participation in Tour de Ski, while there was no change for female athletes. Furthermore, male athletes typically performed relatively worse in World Championships and Olympic Games after taking part in Tour de Ski in the same season (~5-7 weeks earlier), while female athletes typically performed somewhat better following Tour de Ski participation. This is likely, at least in part, due to a marked (20%) reduction in training load for the men after compared to before Tour de Ski, while the female athletes appear better able to recover and maintain a similar training load in the weeks following the race. The reduction in TRIMP observed for the men from before to after Tour de Ski is equivalent to 3.7 hours less per week of low-intensity training (zone 1), or 56 minutes less per week of high-intensity training (zone 4). It is possible that such a reduction in training stimulus could impact negatively on the resulting physiological adaptation and thereby explain the drop in race performance. It is interesting that the male athletes had a significantly lower training load going into Tour de Ski, so that the increase in training load above baseline during Tour de Ski was greater for the male athletes than it was for the female athletes. This may have increased the risk of Tour de Ski participation leading to non-functional OR or even OT in the male athletes (Meeusen et al., 2013), and may help to explain why they were subsequently unable to maintain their normal training load after the competition.

As well as differences in training load before and after the event, the observed sex differences in subsequent race performance may be partly explained by greater competition load in men's compared to women's Tour de Ski. The total race distance for the men is ~71% further than in women's Tour de Ski, with each stage typically being substantially longer. Similarly, total

competition time is ~46% longer for the men. However, it is interesting that this did not result in a significant difference in TRIMP, due to the female athletes typically engaging in markedly more low-intensity physical activity around and between races (e.g. longer warm-ups and cool-downs), and more easy training on the rest days during Tour de Ski. Another possible explanation for the drop in performance observed for the male, but not the female athletes, is that there are a greater number of male athletes competing at the very top end of cross-country skiing. Hence, the time differences between places is typically smaller, and a reduction in performance resulting in a small time decrement can mean a loss of a greater number of places compared to the women's elite races where the time differences between competitors are typically larger. For example, for the last three Olympic Games, the average time difference between 1st and 20th position in the men's 50 km event was 47 seconds, compared to an average time difference of 3:32min between 1st and 20th position in the equivalent women's 30 km event (www.fis-ski.com).

As a greater proportion of the male athletes reported illness during and following Tour de Ski this may also have affected their subsequent performance, due to loss of training days during this time. A final explanation could be that a greater proportion of top level female cross-country skiers choose to take part in Tour de Ski, and that any absolute performance decrements are therefore not reflected in race positions or FIS points, because the majority of the other competitors have been similarly affected. For example, of the 19 athletes who came top 10 in the three women's individual cross distance events in the 2014 Olympic Games (10 km classical, 15 km skiathlon, 30 km freestyle), 68% had participated in Tour de Ski earlier in the same season. Of the men, only 38 % of the athletes who claimed the top ten places in the individual cross-country distance events (15 km classical, 30 km skiathlon, 50 km freestyle) had also competed in Tour de Ski in the same season.

A strength of this current study is that it includes a considerable amount of data for a large cohort of world-class endurance athletes. However, because the data are based on athlete self-report, and illness episodes were therefore not verified by a medical professional, it is not possible to ascertain whether the symptoms reported were truly due to an infection. Indeed, Spence et al. (2007) found that pathogens were isolated in fewer than 30% of symptomatic URI cases in elite triathletes, suggesting that a large proportion may be due to non-infectious causes. However, it is worth noting that these findings are likely partly explained by low incidence of common cold during the summer months when this study was conducted. Similar concerns may be raised regarding the accuracy of the training data. However, Sylta et al. (2014) concluded that elite endurance athletes self-report their training with high accuracy. The same level of accuracy and validity likely holds true for this data set, as both athlete groups used similar reporting routines and a number of the athletes are, in fact, included in both papers. In addition, athletes recorded both their training and illness symptoms on a daily basis, which likely reduced reporting error. It should be recognised that the analysis method used in the current study, based on seasons rather than individuals, is susceptible to the influence of a small number of outliers. However, all of the individuals included in the current study were part of the Norwegian national team and therefore represent a highly homogenous group of elite athletes. During the analysed time period, cross-country skiing training culture in Norway has changed very little. Hence, both inter- and intra-individual differences between seasons in terms of training variables are small, and outliers with abnormal annual illness rates were excluded from the analyses.

Taking part in an 8-11 day cross-country skiing stage race appears to result in a ~3-fold increase in the risk of contracting an illness, primarily URI, in this period. Whether taking part in the competition or not, male athletes appear somewhat more prone to illness than female athletes. Furthermore, unlike their female counterparts, male athletes see a drop in

race performance for at least 6 weeks following participation in Tour de Ski, possibly due to suboptimal training loads going into and coming out of the event. Male athletes also typically perform worse in subsequent World Championships / Olympic Games when taking part in Tour de Ski earlier in the season. Female athletes, on the other hand, appear to perform somewhat better after participating in Tour de Ski.

4.6 CONCLUSION

In accordance with previous studies linking training load and infection susceptibility, this study provides evidence that an intense period of competition leads to increased illness incidence, even in elite athlete populations who already have an extremely high training load. The study also provides new insight into how participation in such an event might influence subsequent training and race performance. This information can aid in making an informed decision about whether or not an athlete should take part in a competition such as Tour de Ski in the same season that they are also aiming to reach peak performance for Olympic Games or World Championships. When making this decision, coaches and athletes should reflect carefully on the potential impact the race may have on the rest of the competition season. Male athletes, in particular, may wish to consider foregoing Tour de Ski in an Olympic year. However, if the decision is made to participate, careful consideration should be given regarding how best to train in the weeks and days before and after the race, in order to prevent maladaptive outcomes and instead turn the event into a beneficial component of the athlete's championship preparation.

CHAPTER 5: IMPACT OF INTENSIFIED TRAINING AND CARBOHYDRATE SUPPLEMENTATION ON IMMUNITY AND MARKERS OF OVERREACHING IN HIGHLY TRAINED CYCLISTS

Ida S. Svendsen[1], Sophie C. Killer[1], James Carter[2], Rebecca K. Randell[2], & Michael Gleeson[1]

1) School of Sport, Exercise and Health Sciences, Loughborough University, UK.

2) Gatorade Sports Science Institute, Beaumont Park, Leicester, UK

5.1 ABSTRACT

Upper respiratory infections are particularly prevalent during intensified training (IT) or competition and in athletes suffering from overreaching (OR). High CHO intake may play a role in the prevention of OR and ameliorate associated immune disturbances. **PURPOSE**: To determine the effects of IT and CHO supplementation on markers of OR and immunity. **METHODS:** In a randomised, double-blind, crossover design, 13 male cyclists (age 25 ± 6 years, $\dot{V}O_{2max}$ 72 ± 5 ml/kg/min) completed two 8-day periods of IT while ingesting either high (6%) (H-CHO) or low (2%) (L-CHO) carbohydrate beverages before, during and after every training session. Blood samples were collected before and immediately after an incremental test to fatigue on days 1 and 9. **RESULTS:** IT resulted in decreased peak power in the incremental test (375 ± 37 vs. 391 ± 37 W, $P<0.001$), reduced maximal heart rate (179 ± 8 vs. 190 ± 10 bpm, $P<0.001$), reduced resting haematocrit (39 ± 2 vs. 42 ± 2 %, $P<0.001$) and increased plasma volume (PV) ($P<0.001$), with no difference between trials. Resting plasma cortisol was increased while plasma ACTH was reduced following IT ($P<0.05$), with no differences between trials. Following IT, resting whole blood culture production of IL-1α in response to antigen-challenge was higher in L-CHO than H-CHO (0.70 (95% CI 0.52-0.95) pg/ml vs. 0.33 (0.24-0.45) pg/ml, $P<0.01$), as was production of IL-1β (9.3 (95% CI 7-10.4) pg/ml vs. 6.0 (5.0-7.8) pg/ml, $P<0.05$). Circulating total leukocytes ($P<0.05$) and neutrophils ($P<0.01$) at rest were increased with IT, as was neutrophil:lymphocyte ratio and percentage CD4+ lymphocytes ($P<0.05$), with no difference between trials. There were no significant changes in numbers of Treg cells between trials, or following IT. **CONCLUSION:** IT resulted in changes consistent with symptoms of OR, although immunological and performance changes were modest. Increasing CHO intake was not able to better maintain peak power, or alleviate physiological/immunological disturbances.

.

5.2 INTRODUCTION

Overload is a key training principle used by athletes and coaches to improve physical performance. Periods of intensified training (IT) are therefore commonly incorporated over the course of a training season. However, when high training load is combined with insufficient recovery and/or other stressors such as lack of sleep, psychological stress, environmental stress or inadequate energy intake, it can lead to a state of OR and place athletes at risk of developing Overtraining Syndrome (OTS) (Meeusen et al., 2013). Although Chapter 3 did not find a significant relationship between overall training volume or intensity and infection risk, high day-today fluctuations in training load were associated with an increased risk. Furthermore, repeated competition over a number of consecutive days was associated with increased infection incidence, and a reduction in race performance in Chapter 4, suggesting that this competition period induced a state of overreaching in many of the athletes.

Much anecdotal evidence indicates increased URS incidence in athletes with OTS. This is not altogether surprising, since previous studies have also reported reduced S-IgA (Gleeson et al., 1995, Mackinnon and Hooper, 1994), reduced numbers of natural killer (NK) cells (Gleeson et al., 1995, Meyer et al., 2004) and CD3+ and CD4+ cells (Baj et al., 1994b), impaired neutrophil function (Robson-Ansley et al., 2007, Baj et al., 1994b), reduced glutamine/glutamate ratio (Halson et al., 2003, Rowbottom et al., 1995, Coutts et al., 2007, Parrybillings et al., 1992) and elevated plasma concentrations of IL-6 and creatine kinase at rest (Robson-Ansley et al., 2007) following IT. In addition, studies suggest that the capacity of immune cells to produce cytokines in response to an immune challenge may be impaired (Baj et al., 1994b, Lancaster et al., 2004, Morgado et al., 2012). Cytokines play a key, pleiotropic role in orchestrating the responses of lymphocytes, macrophages and other

immune cells during an infection (Trinchieri, 1997, Biron et al., 1999, Samuel, 2001). Disturbances to the profile of cytokine production in response to IT may therefore increase infection susceptibility. Gleeson et al. (2011a) found that athletes with a high training load (≥11 h moderate-high intensity training per week) had more than twice as many URI episodes, alongside approximately three-fold higher resting IL-2, IL-4 and IL-10 production in response to antigen challenge compared to athletes with a low training volume (<6 h per week). An elevated anti-inflammatory response, and specifically increased IL-10 production, appears to be a risk factor for URS in athletes (Gleeson et al., 2012). IL-10 is primarily produced by lymphocytes and, in particular, regulatory T (Treg) cells (Gleeson et al., 2011b); circulating numbers of Treg cells appear to be higher in physically active compared with sedentary individuals (Handzlik et al., 2013) but no studies to date have investigated changes in this cell population during IT.

CHO intake may play a role in the prevention of OR and alleviate associated immune disturbances (Achten et al., 2004, Halson et al., 2004, Costa et al., 2005), possibly via attenuation of the stress hormone response to exercise (Costa et al., 2005). Most competitive endurance athletes typically ingest some CHO during prolonged bouts of training or competition. However, it is not clear whether increasing CHO ingestion before, during and after exercise in line with American College of Sports Medicine (ACSM) guidelines (Rodriguez et al., 2009) can provide additional protection against OR and immune disturbances compared to more modest CHO intakes in and around the exercise occasion.

The aim of the current study was therefore to determine the effects of eight days of intensified cycling training with a self-selected diet supplemented with beverages with either a low or high CHO concentration before, during and after the training, on circulating leukocytes and lymphocyte subsets, antigen-stimulated cytokine production, and plasma hormones in competitive cyclists.

5.3 METHODS

5.3.1 Subjects

Thirteen highly trained, competitive male cyclists provided their written, informed consent to participate in the study, which was approved by the Loughborough University ethical advisory committee. Prior to the start of the study, participants completed a health screen questionnaire. Subjects could be included if they were between 18 and 35 years of age, had a $\dot{V}O_{2max}$ of $\geq$ 64 mL/kg/min, had been actively competing in cycling for at least two years and were currently engaging in a minimum of 6 h of cycling training per week. Participants were also required to be currently healthy and without upper-respiratory symptoms or use of any medication during the past four weeks. Exclusion criteria were: smoking, suffering from, or with a history of, cardiac, hepatic, pulmonary, renal, neurological, haematological, psychiatric or gastrointestinal illness, or baseline haematological values (leukocyte and erythrocyte counts) outside the normal range. Baseline characteristics were as follows (mean $\pm$ SD): age 25 $\pm$ 6 years, body mass 69.7 $\pm$ 6.3 kg, height 1.78 $\pm$ 0.03 m, body fat percentage 13.4 $\pm$ 4.1%, and $\dot{V}O_{2max}$ 72.2 $\pm$ 4.9 mL/kg/min, 5.0 $\pm$ 0.5 L/min.

5.3.2 Study design

The study was conducted using a double-blind, placebo-controlled, crossover design. Each participant completed two 8-day periods of intensified training in a randomized and counterbalanced order, separated by a 15-day washout period (Figure 1). During one trial, participants were provided with high concentration CHO solutions to drink before, during and after every training session (H-CHO). In the other trial they were given a taste-matched low CHO solution (L-CHO).

5.3.3 Baseline training

Prior to the start of the study, participants were asked to record all of their training for a minimum of two weeks. Session structure and duration, type of exercise, heart rate and power output data (where available) were uploaded to an online training diary (TrainingPeaks, Peaksware LLC, Colorado). These data were analysed to give a baseline total weekly number of hours of cycling, and training volume at different intensities, and used to calculate and prescribe individual training plans for each IT period.
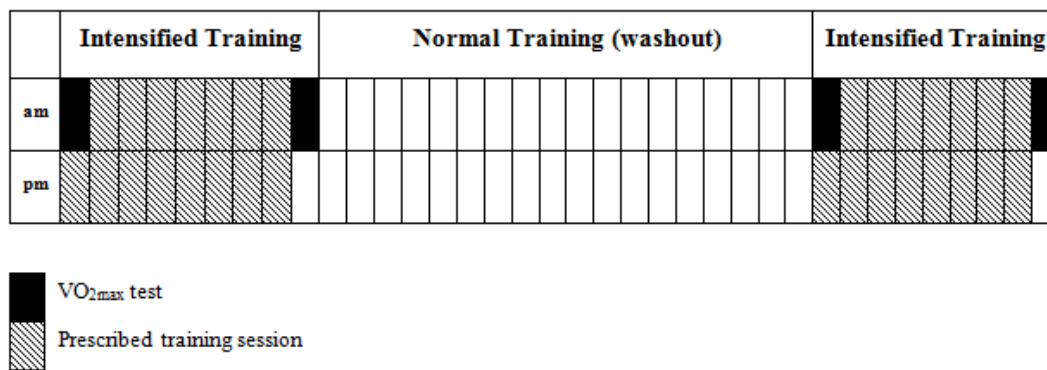
Figure 5.1: Diagrammatical representation of the intensified training protocol.

5.3.4 Intensified training

During each 8-day IT period participants were prescribed an individualised training plan with targets for training time, heart rate and power output set for every session. HR and power targets were based on results from the initial $\dot{V}O_{2max}$ test. If performance in the baseline $\dot{V}O_{2max}$ test of the second trial differed significantly from that of the first trial (i.e. if the athlete's fitness has improved or deteriorated between the two trials), power targets were adjusted accordingly so that relative training intensity was identical for both trials . During each IT period, total training volume was doubled compared to baseline training, and time spent at high-intensity more than trebled, with high intensity training comprising ~35% of

total training time. Training was categorized into five heart rate zones as previously described.

All participants were prescribed two sessions per day, with every session including a high-intensity interval training component (Zones 3-5). In addition to maximal incremental tests, participants attended the laboratory for supervised training sessions on days 1, 2, 5, 6, and 8 of both trials.

All training was performed either out on the road or in the laboratory on the participant's own bike equipped with a power meter (SRM PowerMeter Shimano DA7950 compact, SRM International, Jülich, Germany) and mounted on a stationary turbo trainer. Heart rate and power output were recorded continuously for every session with a sampling frequency of 4 Hz and stored on the SRM PowerControl 7. All training data were subsequently uploaded to and analysed in an online training diary (TrainingPeaks, Peaksware LLC, Colorado). Participants were required to complete every session prescribed in their training plan in order to be included in the study. The two IT periods were separated by a 15-day washout, during which participants were encouraged to take time to recover before returning to normal training. Participants were asked to continue recording and uploading training data during the washout period.

5.3.5 Nutritional intervention

For both trials, participants were provided with a specified volume and composition of drink before, during and after every training session (**Table 5.1**). Drinks ingested before and during exercise were taste-matched, and participants were informed that the only difference in the nutritional intervention between the two trials was that in one they would be provided with two recovery drinks, whereas during the other, one of these drinks would be replaced with a

'recovery pill'. They were informed that the purpose of the study was to investigate the efficacy of a new amino acid pill versus a drink, on recovery from exercise.

For a typical 2 hour training session, this provided a CHO intake of 187.5 g in H-CHO compared to 52.4 g in L-CHO (an additional 17 g protein was provided after exercise in the H-CHO group, with no protein received in the L-CHO group). The composition of the drinks provided in the H-CHO trial were chosen to conform with the current ACSM guidelines on recommended CHO intake for endurance exercise (Rodriguez et al., 2009). Participants were asked to refrain from ingesting any other sports nutrition products or nutritional supplements for the duration of the study and were provided with a diet diary in which to record weighed food and fluid intake for 3 days prior to and throughout the course of each trial.

Table 5.1: Volumes and compositions of drinks provided before, during and after training sessions in H-CHO and L-CHO trials.

	H-CHO	L-CHO
Before exercise	118 mL 20% CHO solution *(24g)*	118 mL 2% CHO solution *(2.4g)*
During exercise	1 L per hour 6% CHO solution *(60g/h)*	1 L per hour 2% CHO solution *(20g/h)*
After exercise	1x 500 mL 6% CHO solution *(30g)* 1x 500 mL 2.7% CHO / 3.3% PRO solution *(13.5g)*	1x 500 mL 2% CHO solution *(10g)* 1 x cellulose capsule *(0g)*

Values in parentheses indicate CHO content in grams.

CHO: carbohydrate; PRO: protein

5.3.5 Maximal incremental test

On days 1 and 9 of each trial, subjects completed a continuous, incremental exercise test to volitional exhaustion on a magnetically-braked cycle ergometer (IndoorTrainer, SRM International, Jülich, Germany) for determination of $\dot{V}O_{2max}$ as previously described.

5.3.6 Blood sampling and analyses

On days 1 and 9 of each trial, blood samples were collected at rest immediately before the start of the maximal exercise test (PRE), and immediately after cessation of exercise (POST). On each occasion, a venous blood sample (11 mL) was obtained by venepuncture from an antecubital vein. Blood was collected into two vacutainer tubes (Becton Dickinson, Oxford, UK) containing either lithium heparin or K_3EDTA as anticoagulant. Haematological analysis was immediately performed on the K_3EDTA sample, with the remaining sample used for determination of plasma cortisol and ACTH, as outlined previously. The heparinized sample was used for determination of antigen-stimulated cytokine production by whole blood culture and lymphocyte subsets via four colour flow-cytometry, as previously described.

5.3.7 Statistical analysis

Data are presented as means $\pm$ SD unless otherwise stated. All statistical analyses were performed using IBM SPSS Statistics 22.0. The Shapiro–Wilk test was used to determine whether data were normally distributed. A two-way repeated measures ANOVA was used to determine whether there were significant differences between L-CHO and H-CHO trials before and after intensified training. A post hoc test with Bonferroni correction for multiple comparisons was used to determine the location of variance. Variables found to be significantly non-normal were transformed to normality using a log transformation prior to analysis, and are presented as back-transformed geometric mean with 95% CI. Based on the

results of Mauchly's sphericity test, Greenhouse-Geisser corrections were applied for epsilon <0.75 and Huynd-Feldt corrections applied for epsilon >0.75 where violations of sphericity were identified. Statistical significance was accepted at the P<0.05 level. Pearson product-moment correlation analyses were used to determine whether changes in any of the measured immune variables correlated significantly with changes in performance, in order to determine whether any could be used as potential markers of overreaching.

5.4 RESULTS

During the 8-day IT period, total training volume was increased from 9.3 ± 2.4 h/wk at baseline, to 23.5 ± 3.4 h/wk, with high-intensity training increased from 2.6 ± 2.5 to 6.5 ± 4.0 h/wk, with no differences between trials. Over the 8-day period, participants completed 24.3 ± 3.3 MJ of cycling, with no significant difference between the trials. Total daily energy intake was significantly lower in L-CHO compared to H-CHO (14.65 ± 2.68 MJ/d vs. 17.36 ± 3.21 MJ/d, $P<0.001$). This difference was primarily due to lower CHO intake in L-CHO (505 ± 107 g/d vs. 679 ± 105 g/d, $P<0.001$; 7.2 ± 1.6 g/kg/d vs. 9.7 ± 1.5 g/kg/d, p<0.05). However, in both trials participants remained weight stable with a mean body mass change of -0.19 ± 1.08 kg.

There was no change in $\dot{V}O_{2max}$ following IT. However, in both L-CHO and H-CHO trials, IT resulted in decreased peak power in the maximal incremental exercise test compared to baseline (375 ± 37 W vs. 391 ± 37 W, $p=0.001$) (**Figure 5.2**), reduced maximal HR (179 ± 8 bpm vs. 190 ± 10 bpm, $P<0.001$), reduced haematocrit (39 ± 2 % vs. 42 ± 2 %, P<0.001) and a 14 ± 10 % increase in plasma volume (PV) at rest ($P<0.001$) (**Table 5.2**), with no significant differences between trials.

Table 5.2: Haematological variables and plasma volume changes before and after acute maximal exercise, and before and after the 8 day intensified training intervention.

		PRE IT			POST IT	
		Pre-exercise	Post-exercise		Pre-exercise	Post-exercise
RBC $(x10^9/L)$	H-CHO	**4.60** ± 0.22	**4.87** ± 0.28 *		**4.25** ± 0.19 †	**4.47** ± 0.23 †*
	L-CHO	**4.60** ± 0.16	**4.87** ± 0.16 *		**4.29** ± 0.23 †	**4.52** ± 0.23 †*
Hb (g/dL)	H-CHO	**14.3** ± 0.8	**15.1** ± 0.9 *	*8-days intensified training*	**13.2** ± 0.8 †	**13.9** ± 0.9 †*
	L-CHO	**14.1** ± 0.8	**14.9** ± 0.9 *		**13.0** ± 0.8 †	**13.6** ± 0.9 †*
HCT (%)	H-CHO	**41.9** ± 2.4	**45.0** ± 2.7 *		**39.0** ± 1.7 †	**41.3** ± 1.8 †*
	L-CHO	**42.0** ± 1.3	**45.1** ± 1.7 *		**39.2** ± 2.0 †	**41.7** ± 2.0 †*
PV (% of baseline)	H-CHO	**100** ± 0	**89** ± 4 *		**114** ± 11 †	**104** ± 12 †*
	L-CHO	**100** ± 0	**90** ± 5 *		**114** ± 10 †	**104** ± 9 †*

Data are mean ± SD. **RBC**: red blood cell count; **Hb**: Haemoglobin; **HCT**: Haematocrit; **IT**: intensified training; **PV**: Plasma volume. * Significant effect of acute maximal exercise (P<0.001), † significantly different from same time point pre-intensified training (P<0.001).
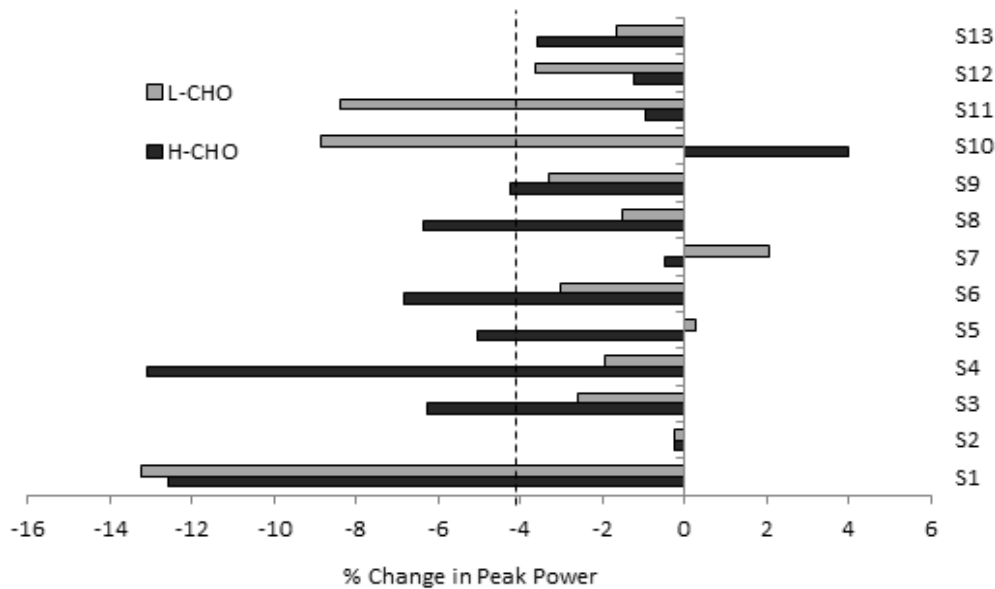
Figure 5.2: Percentage change in peak power achieved during the incremental, maximal exercise test for each participant following the intensified training period with L-CHO and H-CHO. Dashed line indicates significant mean change (P<0.01)

Circulating total leukocytes and neutrophils at rest were increased after IT (**Table 5.3**), as was the percentage of lymphocytes that were CD4+ (29 ± 6 % vs. 26 ± 7 %, *P*<0.05), with no difference between trials. Seven of the 13 participants had somewhat lower than normal numbers of CD4+ cells (<0.5 x10^9 cells and <30% of total lymphocytes) at baseline. The exercise-induced increase in CD4+ cells was significantly reduced following IT (0.10 ± 0.14 x10^9 vs. 0.19 ± 0.11 x10^9, *P*<0.01). There were no significant changes in numbers of CD24+CD25+CD127$^{low/-}$ Treg cells following intensified training in either trial (**Table 5.3**). Resting neutrophil:lymphocyte ratio was significantly increased following IT in both trials (**Figure 5.3**).
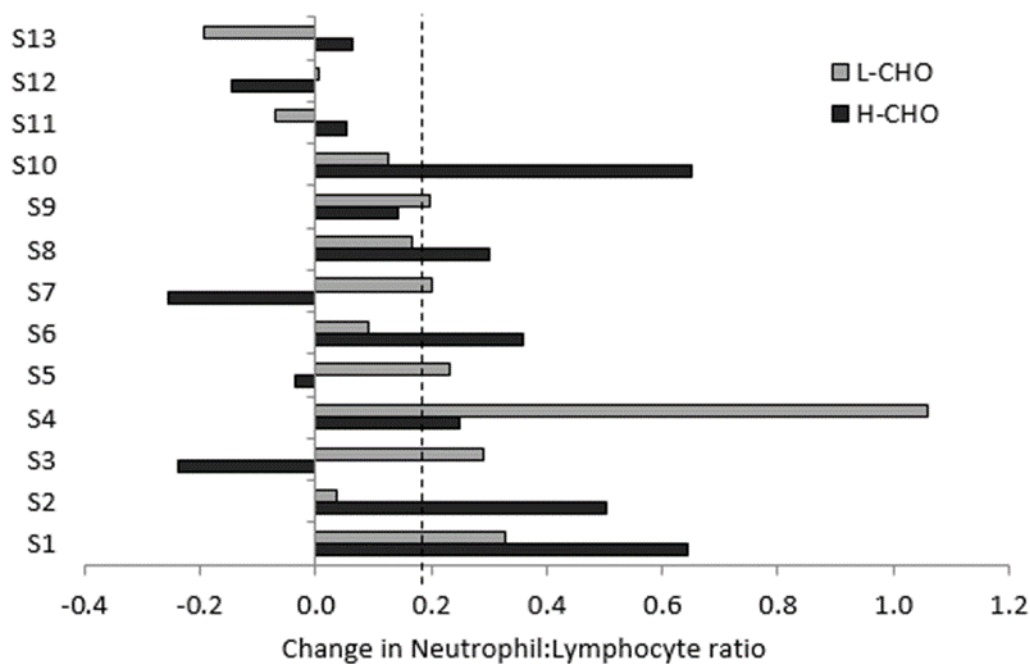
Figure 5.3: Change in neutrophil: lymphocyte ratio for each participant following intensified training with L-CHO and H-CHO. Dashed line indicates significant mean change (P<0.05)

Table 5.3: Leukocyte counts before and after acute maximal exercise, and before and after 8 days intensified training in L-CHO and H-CHO trials.

		PRE IT		POST IT	
		Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
WBC	HCHO	5.30 ± 0.85	8.34 ± 1.49*	5.99 ± 1.00 †	9.14 ± 1.70 *†
	LCHO	5.43 ± 0.93	7.93 ± 1.27*	5.92 ± 0.94 †	8.31 ± 1.17 *†
NEUT	HCHO	2.45 ± 0.49	3.17 ± 0.75*	2.93 ± 0.78 †	3.99 ± 1.08 *†
	LCHO	2.58 ± 0.69	3.26 ± 0.77*	3.00 ± 0.78 †	3.74 ± 0.81 *†
MONO	HCHO	0.51 ± 0.12	0.75 ± 0.22*	0.59 ± 0.15	0.89 ± 0.33*
	LCHO	0.53 ± 0.13	0.72 ± 0.18*	0.55 ± 0.14	0.75 ± 0.21*
LYMPH	HCHO	2.17 ± 0.61	4.08 ± 1.07*#	2.26 ± 0.65	4.03 ± 0.98*#
	LCHO	2.12 ± 0.47	3.65 ± 0.66*	2.17 ± 0.47	3.53 ± 0.95*
CD4+	HCHO	0.55 ± 0.21	0.77 ± 0.23*#	0.58 ± 0.20	0.71 ± 0.20*†#
	LCHO	0.54 ± 0.20	0.69 ± 0.23*	0.58 ± 0.20	0.65 ± 0.18*†
CD4+CD25+	HCHO	0.14 ± 0.04	0.22 ± 0.07*	0.14 ± 0.05	0.19 ± 0.05*
	LCHO	0.14 ± 0.05	0.18 ± 0.06*	0.13 ± 0.04	0.16 ± 0.05*
CD4+CD25+CD127$^{low/-}$	HCHO	0.05 ± 0.02	0.06 ± 0.03*	0.05 ± 0.02	0.06 ± 0.02*
	LCHO	0.05 ± 0.02	0.06 ± 0.02*	0.05 ± 0.02	0.05 ± 0.02*

Data are mean ± SD. Cell counts are corrected for changes in plasma volume.

IT: intensified training; **WBC**: total white blood cells; **NEUT:** neutrophils; **MONO**: monocytes; **LYMPH**: lymphocytes**; CD4+:** lymphocytes expressing CD4 (T helper cells); **CD4+CD25**+: lymphocytes expressing CD4 and CD25; **CD4+CD25+CD127low/-**: Lymphocytes expressing CD4 and CD25, but not CD127 (Treg cells).

* Significant effect of acute maximal exercise (P<0.01), **†** significant difference from same time point pre-intensified training (P<0.01), # significant difference from L-CHO at same time point (P<0.05).

Resting plasma ACTH was significantly lower following IT, while resting plasma cortisol concentration was increased (**Figure 5.4**). There was no significant effect of IT on the exercise-induced change in ACTH or cortisol. Nor were there any significant differences between L-CHO and H-CHO in either hormone. Following IT, production of IL-1α at rest in response to antigen challenge was higher in L-CHO compared to H-CHO, as was production of IL-1β (**Figure 5.5**). Production of TNFα in response to antigen challenge also tended to be higher in L-CHO post IT (14.7 (11.0-19.6) vs. 8.6 (5.0-14.9) pg/ml, *P*=0.063). Antigen-stimulated production of IL-2 (Pre IT: LCHO 21.6 (10.9-41.8) pg/ml, HCHO 23.0 (12.1-42.7) pg/ml; Post IT: LCHO 16.0 (6.7-36.5) pg/ml, HCHO 28.4 (13.2-59.6) pg/ml), IFN-γ (Pre IT: LCHO 1.9 (0.8-3.8) pg/ml, HCHO 1.9 (0.8-3.6) pg/ml; Post IT: LCHO 2.5 (1.0-5.1) pg/ml, HCHO 1.6 (0.6-3.3) pg/ml), IL-6 (Pre IT: LCHO 67 (34-133) pg/ml, HCHO 58 (28-117) pg/ml; Post IT: LCHO 83 (48-144) pg/ml, HCHO 29 (14-61) pg/ml), and IL-4 (Pre IT: LCHO 1.9 (1.3-2.6) pg/ml, HCHO 1.9 (1.3-2.6) pg/ml; Post IT: LCHO 2.7 (2.1-3.6) pg/ml, HCHO 3.0 (2.2-3.8) pg/ml) were not significantly altered by the intensified training and did not differ between trials.

Submaximal fat oxidation during the first five stages of the incremental test was significantly increased following IT in both trials, with this increase being greater in L-CHO (**Figure 5.6**). The workload at which maximal fat oxidation occurred was also increased following IT (226 ± 45 W vs. 181 ± 41 W, *P*<0.01) with no difference between trials.

None of the measured variables correlated significantly with changes in performance following intensified training.
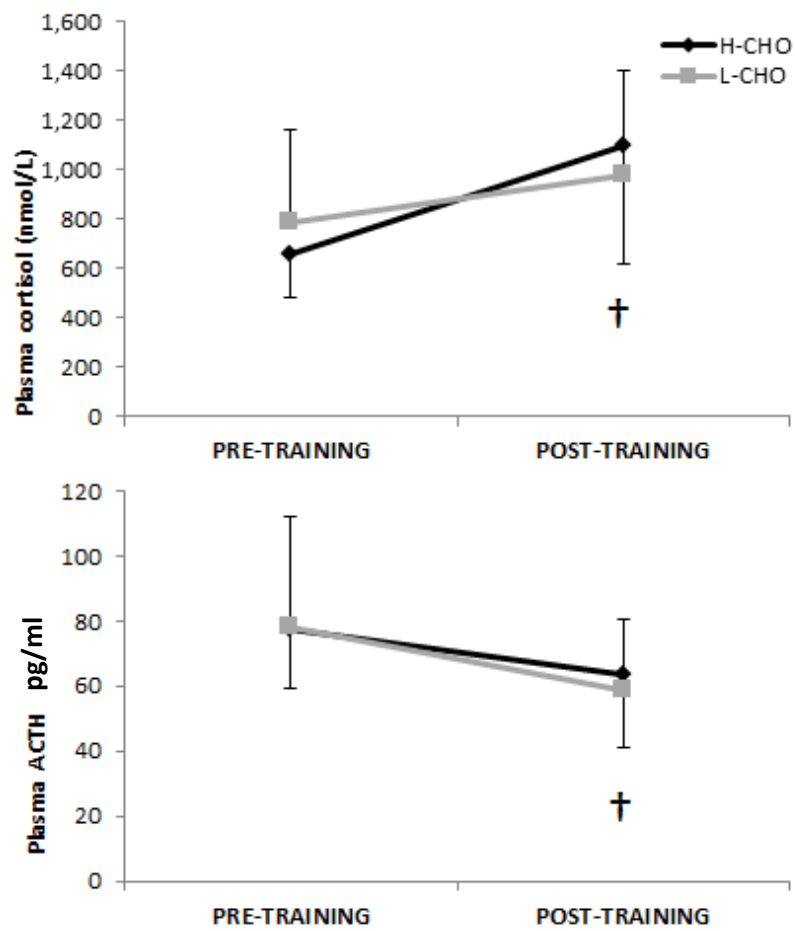
Figure 5.4: Resting plasma cortisol and ACTH following intensified training.

Values are mean ± 95% CI. † Indicates significant difference from pre-intensified training, P<0.05. Corrected for change in plasma volume.
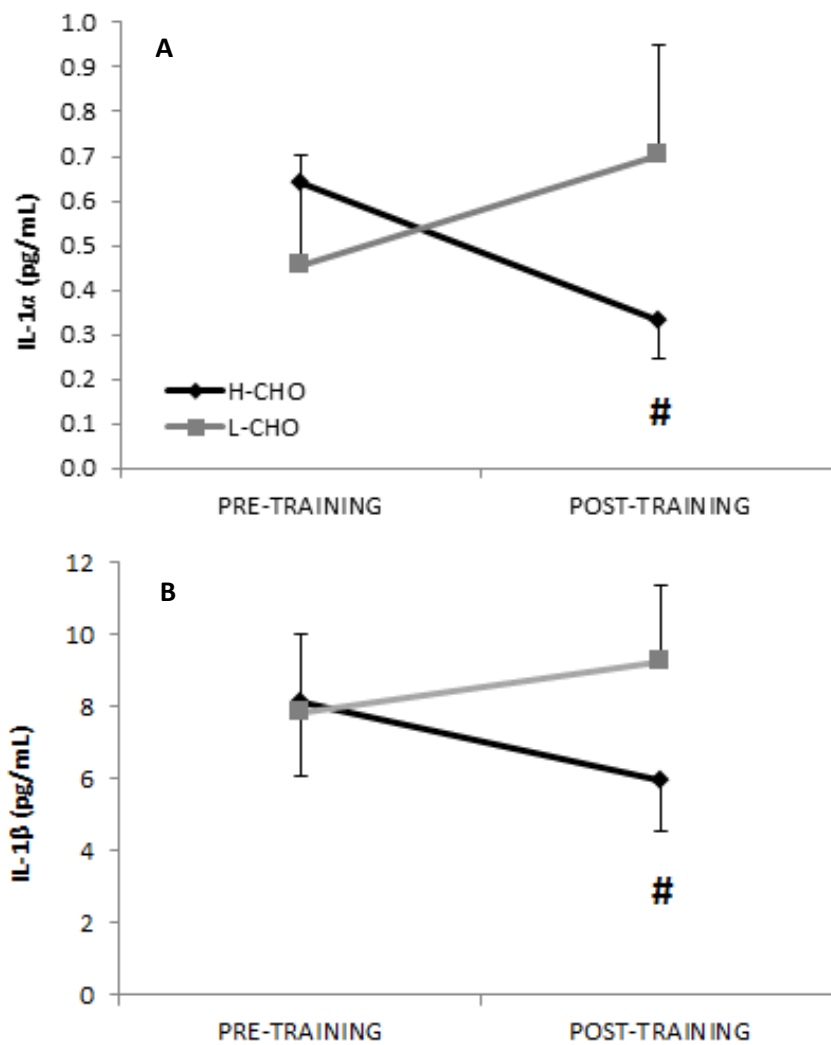
Figure 5.5: Resting antigen-stimulated production of IL-1α and IL-1β by whole blood culture before and after intensified training with L-CHO and H-CHO.

Values are mean ± 95% CI. #significant difference between L-CHO and H-CHO (P<0.05). Corrected for change in plasma volume.
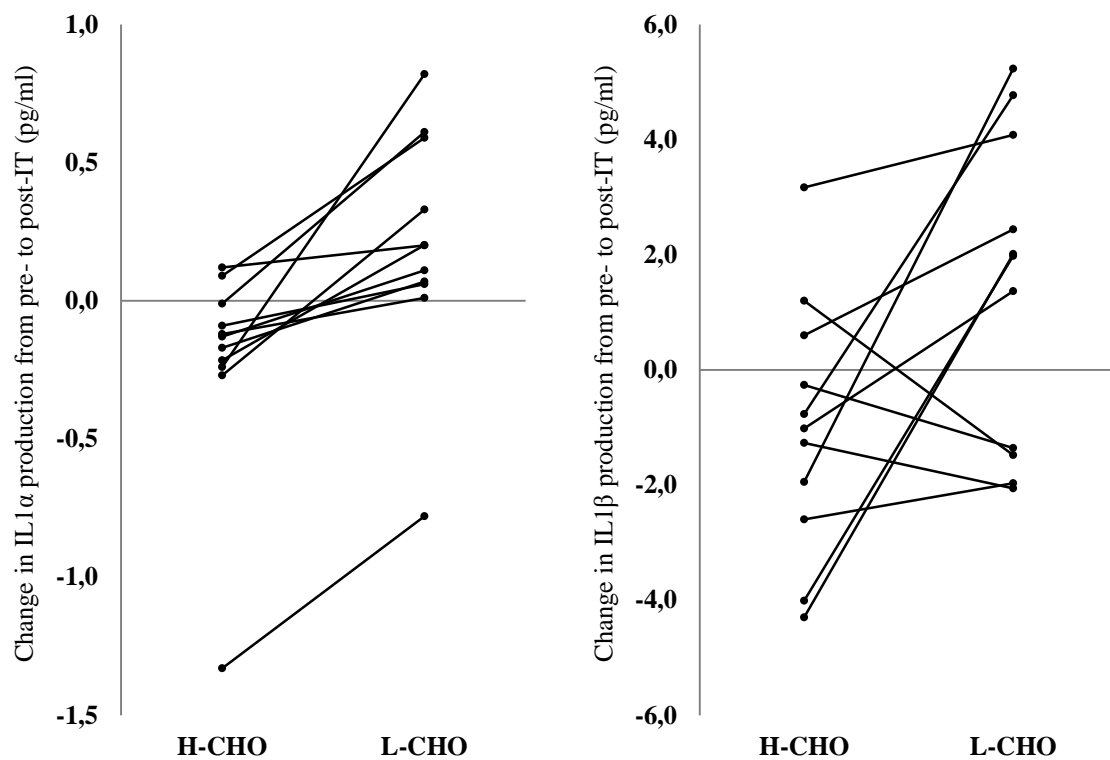
Figure 5.6: Individual changes in antigen-stimulated production of IL-1α and IL-1β by whole blood culture from pre- to post-intensified training in L-CHO and H-CHO trials.
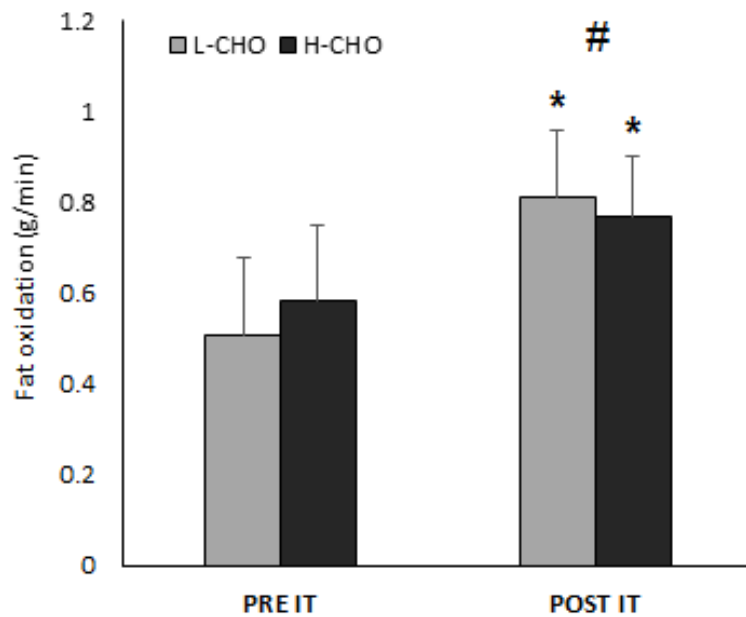
Figure 5.7: Average fat oxidation during the first five stages (60-235 W) of the incremental test before and after intensified training in L-CHO and H-CHO trials.

Values are mean + SD. *significant difference from pre-intensified training in both trials, $P<0.01$. #significant interaction effect, $P<0.05$.

5.5 DISCUSSION

The main finding of the current study is that an 8-day period of IT in highly trained cyclists induces some immunological, haematological, physiological and exercise-related changes consistent with the symptoms of OR. However, high-dose CHO supplementation in and around the exercise training occasion, in addition to a self-selected diet, did not reduce the associated maximal power output decrement or ameliorate hormonal and immunological disturbances compared to a more moderate CHO intake. This is in agreement with Snyder et al. (1995) who found that even when muscle glycogen was maintained via CHO supplementation, competitive cyclists still displayed symptoms of OT following 15 days of IT. In contrast, Achten and colleagues (2004) found that an increase in dietary CHO from 5.4

to 8.5 g CHO/kg bm/day better maintained physical performance in runners undergoing a period of IT, reducing the symptoms of OR. Similarly, Costa et al. (2005) found that consumption of a very high CHO diet (12 g CHO/kg bm/day) during IT attenuated cortisol release and increased salivary secretory immunoglobulin-A concentration compared to a self-selected, and more moderate, CHO intake of 5.9 g/kg bm/day. Halson et al. (2004) found that ingestion of a high CHO beverage before, during and after training sessions over the course of an 8-day period of intensified cycling training better maintained performance and reduced symptoms of OR compared to a low CHO placebo (9.4 vs. 6.4 g/kg bm/day). However, in the current study, total daily CHO intake in the L-CHO trial was higher than in these studies at 7.2 g/kg bm/day; a daily amount classed as 'high' in the International Olympic Committee's latest consensus on sports nutrition (Burke et al., 2011). It is possible that this intake was already sufficient to provide some protection against immunological disturbances and other symptoms of OR. Another possible explanation is differences in training during the IT period. Compared with the study of Halson et al. (2004) participants in the current study completed a greater total volume of cycling (23.5 h/wk vs. 15.3 h/wk) but less high-intensity training (6.5 h/wk vs. 10.5 h/wk). Since muscle glycogen utilization is related to exercise intensity (Saltin and Karlsson, 1971), it is possible to speculate that the lower number of hours of high intensity training in the current study may have resulted in lower glycogen depletion, and that CHO supplementation was therefore less critical in maintaining performance. In addition, participants in the current study were highly trained, with greater aerobic capacity than participants included in the above mentioned studies, and may therefore have been more resilient to detrimental effects of the IT period. Indeed, despite showing some symptoms of OR, the performance decrement in the current study was relatively modest (~4%), and it is possible that the training load during IT was not, in fact, sufficient to induce a true state of OR in these well-trained athletes.

The IT did, however, result in a marked reduction in resting haematocrit, haemoglobin and red blood cell concentration, likely primarily indicative of a pronounced plasma volume expansion. It is well reported that physical training stimulates an increase in plasma volume (Convertino, 1991) and it is likely that the changes observed in the current study are primarily indicative of adaptation rather than OR. However, it is also possible that these changes were due, at least in part, to changes in red cell destruction or production as a result of IT. Indeed, strenuous endurance exercise has been associated with increased intravascular haemolysis, due to oxidative damage to red blood cells (Smith, 1995).

Whether or not the changes in immune variables observed in the current study following IT might make athletes more susceptible to URI is not clear. The ~315 nmol/L increase in resting plasma cortisol may have negative implications for host defence, particularly since mean concentration at baseline was already towards the upper end of the normal range. Several studies have investigated the effect of IT on plasma cortisol concentrations, with the majority reporting no significant change in resting plasma cortisol following IT (Urhausen et al., 1998, Mackinnon et al., 1997, Hooper et al., 1993). It is possible that the increase observed in the current study, along with elevations in circulating leukocytes, neutrophils, CD4+ lymphocytes and neutrophil:lymphocyte ratio, may be only a transient effect caused by incomplete recovery from the previous exercise bout ~13 h earlier.

Handzlik et al. (2013) recently reported that endurance trained athletes with a high training load (~15 h/wk) had a significantly higher proportion of regulatory T cells as a percentage of total lymphocytes and higher antigen-stimulated *in vitro* IL-10 production compared with sedentary individuals. However, in the current study, there was no significant effect of IT on absolute or relative numbers of regulatory T cells or antigen-stimulated IL-10 production, suggesting that these variables are not altered by short-term increases in training load in individuals already training ~9 h/wk. However, antigen-stimulated production of IL-1α and

97

IL-1β were both reduced following IT in H-CHO and increased following IT in L-CHO. Type 1 cytokines, such as IL-1, promote cellular immunity by stimulating the functional activity of T cytotoxic cells, NK cells and activated macrophages (Gleeson et al., 2013b). Reduced production of these cytokines in response to an immune challenge may therefore have negative implications for host defence. Similar to the results observed for the H-CHO trial, (Morgado et al., 2012) reported impaired production of IL-1β from dendritic cells and monocytes in swimmers following IT. However, that IL-1α and IL-1 β production should be increased following IT in L-CHO appears counterintuitive, and further research is necessary to corroborate these findings. Nevertheless, it is clear that increasing CHO intake during exercise, from 20 to 60 g/h, does not provide any additional benefit in preserving the measured immune parameters during IT in this cohort of highly trained cyclists. In fact, since neither maximal power output nor immunity were better maintained with a high CHO intake, and increases in fat oxidation were lower, it could be argued that ingesting a moderate (~20 g/hr) CHO dose during short-term IT may be more advantageous than a higher CHO dose, at least when athletes already have a high daily intake of dietary CHO (>7 g CHO/kg bm/day). This intake during exercise appears sufficient to help prevent large maximal power output decrements, and preserve immunity, while facilitating greater adaptation in terms of fat oxidation. Whether this improved capacity to oxidise fat has subsequent performance benefits once recovery from IT is complete is unknown.

The mean $\dot{V}O_{2max}$ of participants in the current study was 72.2 mL/kg/min, and to our knowledge, no previous study has attempted to intentionally overreach such highly endurance trained athletes. The current data indicate that these athletes are relatively robust to increases in training load and can tolerate multiple consecutive days of IT without large reductions in maximal power output or severe immune disturbances. However, since many of the participants had lower than expected numbers of CD4+ cells and higher than expected plasma

cortisol already at baseline, it is possible that their habitually high training loads may already be associated with some mild immunodepression.

5.6 CONCLUSION

Eight days of IT results in a number of changes consistent with the symptoms of short-term OR in highly trained cyclists, although both immunological and exercise-related changes are relatively modest. When ingesting an otherwise self-selected diet, increasing CHO intake immediately before, during and after training sessions did not better maintain maximal power output, or alleviate physiological/immunological disturbances compared to a more moderate CHO intake. The results also highlight the importance of considering plasma volume changes when interpreting results from training studies, since even highly trained individuals appear to demonstrate a pronounced plasma volume expansion following just eight days of IT.

CHAPTER 6: INFLUENCE OF HYDRATION STATUS AND PROLONGED EXERCISE ON MARKERS OF SYSTEMIC IMMUNITY

Ida S. Svendsen, Sophie C. Killer, and Michael Gleeson

School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire LE11 3TU, UK

6.1 ABSTRACT

PURPOSE: There is some evidence that performing acute exercise in a hypohydrated state can exacerbate immune disturbances compared to when euhydration is maintained before and during exercise. The purpose of this study was therefore to determine the effects of prolonged exercise and hydration status on plasma cortisol and antigen-stimulated cytokine production. **METHODS:** Twelve healthy males cycled for 120 min at 60% $\dot{V}O_{2\,max}$ on two occasions, either euhydrated or moderately hypohydrated (induced by fluid restriction for 24h). Blood samples were collected before and after exercise and following 2 h recovery for determination of cell counts, plasma cortisol and *in vitro* antigen-stimulated cytokine production by whole blood culture. **RESULTS:** Fluid restriction resulted in mean body mass loss of 1.3% and 3.9% before and after exercise, respectively. Exercise elicited a significant leukocytosis and elevated plasma cortisol (623 ± 101 nmol/L pre-exercise vs. 729 ± 127 nmol/L post-exercise, p<0.05), with no differences between trials. IL-6 production was significantly reduced 2 h post-exercise (4.1 (1.5-10.0) pg/ml 2h post-sexercise vs. 9.0 (3.7-22.4) pg/ml pre-exercise, *p*<0.05), while IL-10 production was elevated post-exercise (0.68 (0.43-0.94) pg/ml post-exercise vs. 0.42 (0.10-0.82) pre-exercise, *p*<0.05). IFN-γ and IL-2 production tended to decrease post-exercise, although this did not reach statistical significance. No significant effect of hydration status was observed for any of the measured variables. **CONCLUSION:** Prolonged exercise appears to result in augmented anti-inflammatory cytokine release in response to antigen challenge, possibly coupled with acute suppression of pro-inflammatory cytokine production, corresponding with studies using mitogen or endotoxin as stimulant. Moderate hypohydration does not appear to influence these changes.

6.2 INTRODUCTION

Studies suggest that athletes frequently commence training or competition in a mildly or moderately hypohydrated state (Da Silva et al., 2012, Fernando Aragon-Vargas et al., 2009, Maughan et al., 2005, Volpe et al., 2009), and that they typically fail to ingest sufficient fluid to offset losses through sweating during exercise (Da Silva et al., 2012, Maughan et al., 2004, Maughan et al., 2005, Beis et al., 2012). Compared with exercise in a euhydrated state, moderate dehydration would be expected to result in an augmented stress response and thus likely greater disturbances to immune function. For example, Maresh et al. (2006) found that athletes hypohydrated to ~5% body mass loss had significantly higher plasma cortisol concentrations prior to and 20 min following acute submaximal exercise. The aims of the current study were therefore to determine, 1) the acute effects of a single bout of prolonged exercise on plasma cortisol and antigen-stimulated cytokine production by whole blood culture, and 2) how these effects differ depending on whether exercise is undertaken in a euhydrated or moderately hypohydrated state. It was hypothesised that immediately following 2 h of cycling exercise at 60 %$\dot{V}O_{2max}$, there would be no significant change in antigen-stimulated production of type 2/anti-inflammatory cytokines, but that there would be a transient suppression of type 1/pro-inflammatory cytokine production relative to baseline, with values returning towards resting levels within 2 h of recovery. In addition, it was hypothesised that exercise in a moderately hypohydrated state would lead to a somewhat augmented cortisol response, and greater disturbances of cytokine production compared to exercise in a euhydrated state.

6.3 METHODS

6.3.1 Subjects

Twelve healthy, male university students provided their written, informed consent to participate in the study, which was approved by the Loughborough University ethical advisory committee. Prior to the start of the study, participants completed a health screening questionnaire. Subjects could be included if they were between 18 and 35 years of age, currently healthy and without URS or use of any medication during the past four weeks. Exclusion criteria were: smoking, suffering from, or with a history of, cardiac, hepatic, pulmonary, renal, neurological, haematological, psychiatric or gastrointestinal illness, or haematological values (leukocyte and erythrocyte counts) outside the normal range. Baseline characteristics were as follows (mean ± SD): age 21 ± 1 years, body mass 73.6 ± 7.7 kg, height 1.77 ± 0.07 m, $\dot{V}O_{2max}$ 57.6 ± 6.1 ml/kg/min.

6.3.2 Laboratory visits

On the first laboratory visit, 1-2 weeks prior to the first experimental trial, subjects completed a continuous, incremental exercise test to volitional exhaustion for determination of $\dot{V}O_{2max}$, as previously described. The work rate corresponding to 60% $\dot{V}O_{2max}$ was then calculated from the $\dot{V}O_2$– work rate relationship using a linear equation. After a 15-minute recovery, participants cycled for 20 min at a steady-state work rate equivalent to 60% $\dot{V}O_{2max}$ with expired gas samples collected after 5, 15 and 20 min in order to familiarize the subjects with the exercise protocol for subsequent trials, and to ensure that the calculated work rate elicited the desired relative exercise intensity. The final three days prior to commencing the first experimental trial, participants attended the laboratory in the morning following an overnight

fast. On each occasion nude body mass was measured, and the average of these taken as baseline euhydrated body mass.

For each experimental trial, subjects arrived at the laboratory at 08:00 following an overnight fast of at least 10 h. All trials were completed at the same time of day to reduce inter-trial effects of diurnal variations in cytokine production and plasma cortisol (Kanaley et al., 2001, Petrovsky et al., 1998). Subjects were requested to refrain from strenuous exercise and, in an effort to standardize their nutritional status, asked to record and replicate as closely as possible their dietary intake during the 24 h leading up to each trial, and instructed to refrain from foods with a high water-content. The two trials were conducted in a randomized and counterbalanced order, and separated by six or seven days. For the dehydrated trial (DH), fluid intake was restricted to 500 mL of water during the 24-h period prior to the start of the trial, and no fluid was ingested in the morning before or during the exercise test. In the euhydrated trial (EU), subjects were asked to drink normally in the 24 h leading up to the trial and to consume 500 mL of water in the morning 1-2 h before the start of exercise test to ensure adequate hydration. In the EU trial subjects were also provided with 250 mL of water every 20 min during exercise to offset fluid losses through sweating. Before each trial, subjects provided a first pass morning urine sample. The exercise protocol was identical for both trials.

Prior to the start of exercise, subjects voided and nude body mass was recorded. After sitting quietly for 10 min, an initial resting blood sample was obtained. Subjects then cycled for 120 min at 60 % $\dot{V}O_{2max}$ on a stationary cycle ergometer in a laboratory maintained at 21 ± 1ºC. Expired gas was collected after 15, 45, 75 and 105 min of exercise to determine $\dot{V}O_2$. Heart rate was measured using short-range telemetry (Polar, Finland) at 15-minute intervals. Immediately after completion of exercise, blood and urine samples were collected, and body mass measured before participants were provided with 500 mL of water. Subjects then sat

quietly for 120 min, before providing a final blood sample. Urine osmolality before and after exercise was determined via freezing-point depression using a single-sample osmometer (Osmomat 030, Gonotec, Berlin, Germany).

6.3.3 Blood sampling and analyses

Blood samples were collected at rest immediately before the start of exercise (t0), immediately after cessation of exercise (t120), and after a 2-hour recovery period (t240). Haematological variables, plasma concentrations of cortisol and antigen-stimulated cytokine production were determined as previously outlined. Haematocrit and Haemoglobin values were used to calculate changes in plasma volume using the equations of Dill & Costill (1974).

6.3.4 Statistical Analysis

Data are presented as Mean ± SD. Statistical analyses were performed using IBM SPSS Statistics 19. Changes in total and differential leukocyte counts, plasma cortisol and antigen-stimulated cytokine production were analysed using a 2-way repeated measures ANOVA with trial (DH and EU) and time (pre-exercise, post-exercise and 2 h post-exercise) as factors. Although ANOVA are generally considered robust to minor violations of normality (Maxwell and Delaney, 2004), cytokine data that were found to be significantly non-normal were transformed to normality using a log transformation prior to analysis. The statistical analysis and calculation of summary statistics was subsequently carried out on the transformed data with summary statistics transformed back to the original scale for presentation as geometric means with 95% CIs. A Bonferroni adjustment was used to correct for multiple comparisons. Between-trial differences in mean heart rate, RPE and $\%\dot{V}O_{2max}$ during exercise were determined using a paired samples t-test. Significance was accepted at the $p<0.05$ level.

6.4 RESULTS

Compared to baseline euhydrated body mass, fluid restriction in the DH trial resulted in body mass loss of $1.3 \pm 0.7\%$ and $3.9 \pm 1.0\%$ before and after exercise, respectively, compared to $+0.2\% \pm 0.6\%$ and $-1.1 \pm 0.5\%$ before and after exercise in EU. Urine osmolality decreased following exercise and was significantly ($P<0.01$) higher in DH compared to EU at both time points (972 ± 134 vs. 707 ± 229 mOsm/kg, and 933 ± 138 vs. 555 ± 254 mOsm/kg at pre- and post-exercise, respectively). Subjects cycled at an average power output of 175 ± 22 W for the 2-h exercise period. This elicited an exercise $\dot{V}O_2$ of 60 ± 4 % $\dot{V}O_{2max}$, which did not differ significantly between trials. Mean heart rate was significantly ($P<0.001$) lower during exercise in EU than DH (154 ± 10 bpm vs. 161 ± 10 bpm). Similarly, RPE was significantly lower for EU than DH (13 ± 2 vs. 14 ± 3, $P<0.01$).

Red blood cell concentration, haematocrit and haemoglobin concentration were all significantly higher post-exercise and at 2 h post-exercise (**Table 6.1**). The post-exercise increase in haemoglobin was significantly higher in DH than EU. Plasma volume was significantly reduced both immediately post and 2 h post-exercise, but did not significantly differ between trials. Nor did adjusting for plasma volume change the outcomes of statistical tests, and hence the data presented are uncorrected values. Analyses of leukocyte counts demonstrated a significant main effect of time for total and differential leukocytes, with no significant difference between trials. Under both conditions, exercise resulted in a significant increase in total leukocytes, neutrophils and monocytes above baseline, which persisted during the 2-h period of recovery (**Figure 6.1**). Lymphocytes also increased significantly immediately following exercise, before dropping below baseline after 2 h. Plasma cortisol was significantly elevated by approximately 22 % post-exercise in both trials, with no difference between trials. **Figure 6.2** shows plasma cortisol concentration pre-, post- and 2 h post-exercise.

Table 6.1: **Haematological variables pre-, post- and 2 h post-exercise in euhydrated and dehydrated trials**

		Pre-exercise	Post-exercise	2h post-exercise
RBC (x 10^{12}/L)	*EU*	**4.76** (0.35)	**4.95** (0.36) *	**4.96** (0.44) *
	DH	**4.83** (0.40)	**5.10** (0.40) *	**4.95** (0.48) *
Hct (%)	*EU*	**45.9** (3.2)	**47.8** (3.6) *	**47.4** (4.4) *
	DH	**46.8** (3.0)	**49.6** (4.1) *	**47.9** (3.5) *
Hb (g/dL)	*EU*	**15.7** (0.7)	**16.4** (0.7) *	**16.3** (1.1) *
	DH	**15.9** (1.0)	**16.9** (1.2) *†	**16.3** (1.1) *

Data are Mean (SD) * Indicates significant difference from pre-exercise ($P<0.001$); † Indicates significant interaction effect (Trial*Time) ($P<0.05$).

RBC: Red blood cells; Hct: Haematocrit; Hb: Haemoglobin.

Stimulation with antigens resulted in significantly higher levels of all measured cytokines compared to unstimulated samples. Cytokine concentrations following stimulation are presented in **Table 6.2**. IL-6 production was significantly (P=0.010) reduced 2 h post-exercise (**Figure 6.3**). IL-10 production also showed a main effect of time (P=0.023) with higher production immediately post-exercise. Although not quite reaching statistical significance, antigen-stimulated IFN-γ and IL-2 release tended (0.05<P<0.10) to decrease following exercise (**Table 6.2**). IL-1β and IL-1α production were not significantly altered by exercise. No significant effect of hydration status was observed for any of the measured cytokines.
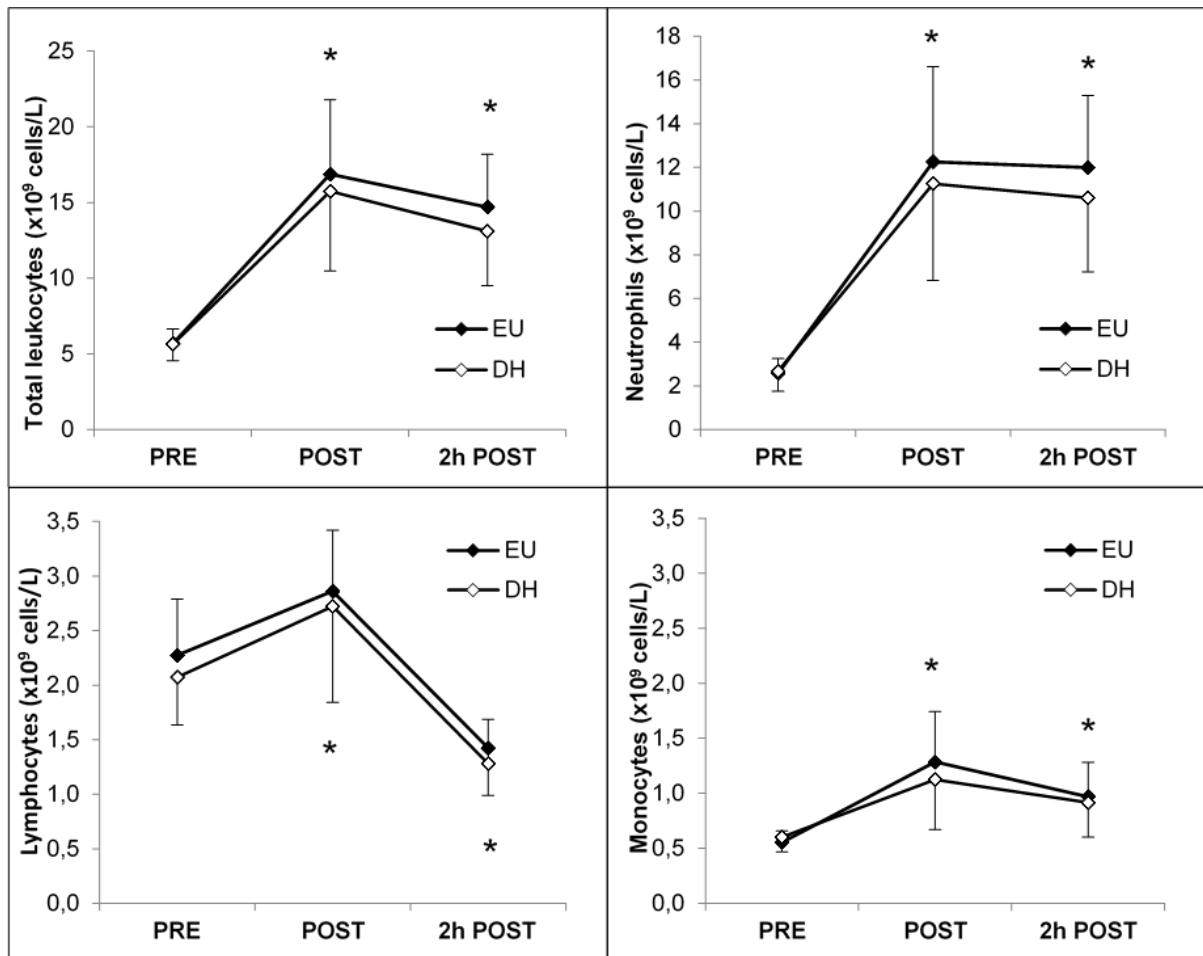
Figure 6.1: Total leukocyte, neutrophil, lymphocyte and monocyte counts before, immediately after and after 2 h recovery following 2 h cycling exercise in euhydrated (EU; filled circles) and dehydrated (DH; open circles) trials.

Data are Mean ± SD. * Indicates significant difference for both trials from pre-exercise ($P<0.05$).
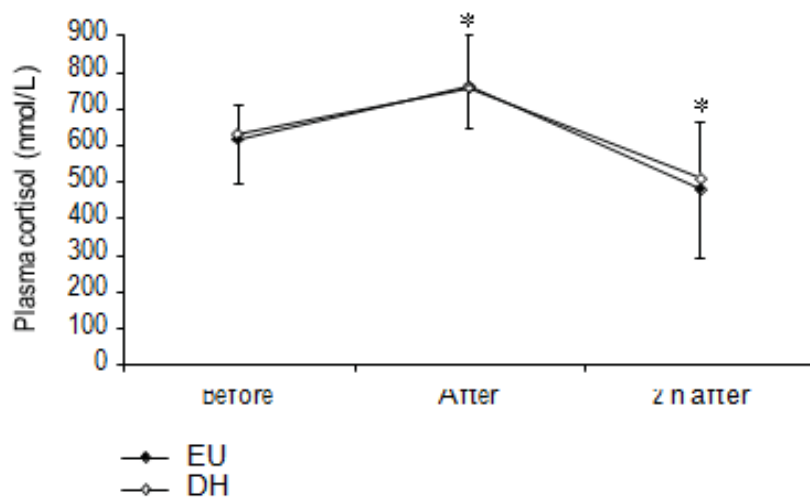
Figure 6.2: Plasma cortisol concentration before, after and after 2 h recovery following 2 h cycling exercise in euhydrated (EU; filled circles) and dehydrated (DH; open circles) trials. Data are presented as Mean ± SD. * Indicates significant difference for both trials from pre-exercise ($P<0.001$).
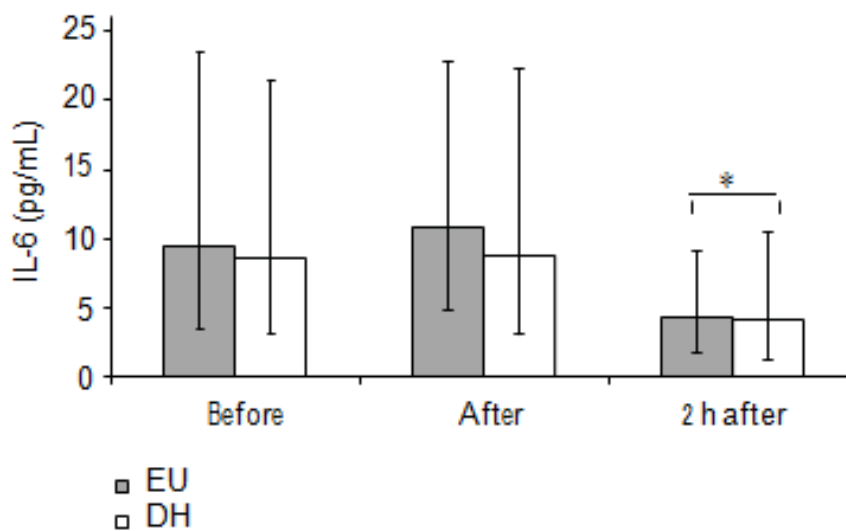
Figure 6.3: IL-6 production by antigen-stimulated whole blood culture pre-, post- and 2 h post-exercise in euhydrated (EU; filled bars) and dehydrated (DH; open bars). Data are geometric mean with 95% *CI*. * Indicates significant difference for both trials from pre -exercise ($P=0.01$).

Table 6.2: **24-h cytokine production in response to *in vitro* antigen stimulation of whole blood collected pre-, post- and 2 h post-exercise in euhydrated and dehydrated trials.**

Data are presented as geometric means (95% *CI*).

	Euhydrated Trial			Dehydrated Trial			Statistics
	Pre-exercise	Post-exercise	2h Post-exercise	Pre-exercise	Post-exercise	2h Post-exercise	
IL-1α (pg/ml)	**0.40** (0.11-0.76)	**0.22** (0.07-0.38)	**0.25** (0.01-0.56)	**0.27** (0.02-0.56)	**0.22** (0.08-0.38)	**0.19** (0.07-0.32)	
IL-1β (pg/ml)	**1.80** (0.78-3.42)	**2.50** (1.35-4.21)	**1.81** (1.12-2.71)	**1.95** (0.74-4.02)	**1.73** (0.63-3.59)	**1.38** (0.53-2.69)	
IL-2 (pg/ml)	**8.01** (2.45-22.5)	**6.93** (2.44-17.3)	**4.80** (1.82-10.9)	**5.56** (1.37-17.2)	**4.31** (1.19-11.9)	**3.23** (1.16-7.30)	
IL-4 (pg/ml)	**0.65** (0.16-1.14)	**0.73** (0.34-1.11)	**0.86** (0.27-1.44)	**0.58** (0.25-0.92)	**0.68** (0.32-1.04)	**0.55** (0.31-0.79)	
IL6 (pg/ml)	**9.45** (3.47-23.5)	**10.8** (4.80-22.8)	**4.24** (1.70-9.16)	**8.54** (3.04-21.5)	**8.81** (3.13-22.3)	**4.05** (1.22-10.47)	**Main effect of time** $F_{(1.93)}$=5.840, *p*=0.010 Observed power= 0.801
IL-10 (pg/ml)	**0.43** (0.09-0.88)	**0.77** (0.57-1.01)	**0.42** (0.16-0.73)	**0.40** (0.11-0.78)	**0.59** (0.34-0.90)	**0.38** (0.15-0.66)	**Main effect of time** $F_{(2)}$=4.495, *p*=0.023 Observed power= 0.707
IFN-γ (pg/ml)	**0.76** (0.18-1.63)	**0.58** (0.17-1.14)	**0.48** (0.02-1.13)	**0.87** (0.26-1.79)	**0.45** (0.05-1.00)	**0.52** (0.16-1.00)	
TNF-α (pg/ml)	**2.68** (1.04-5.62)	**2.24** (1.38-3.43)	**2.40** (1.17-4.32)	**2.36** (0.77-5.36)	**2.12** (0.78-4.49)	**1.95** (0.80-3.83)	

6.5 DISCUSSION

The main findings of the current study are that moderate hypohydration equivalent to ≤4% body mass loss does not appear to influence resting or exercise-induced changes in plasma cortisol, total and differential leukocyte counts or antigen-stimulated cytokine production. However, they do provide evidence that prolonged exercise may up-regulate the production of IL-10 in response to antigen challenge, while inhibiting the production of IL-6 during recovery from a single bout of endurance exercise. Although there was also a consistent trend for suppressed type 1 cytokine production following exercise, due to large individual variations these changes did not quite reach statistical significance.

An enhanced anti-inflammatory response to antigen challenge, as evidenced by potentiated IL-10 production post- exercise, indicates a shift in the type 1/type 2 cytokine balance towards type 2. This corresponds with previous studies that have reported exercise-induced changes in T cell number and function resulting in a relatively type 2 dominant cytokine profile (Ibfelt et al., 2002, Lancaster et al., 2005b, Steensberg et al., 2001, Lancaster et al., 2004). However, contrary to the current findings, these studies observed that this shift was primarily due to a reduction in type 1 cytokine production, with no significant change in the production of type 2 cytokines. Although evidence indicates that intracellular expression of IL-4 in both unstimulated and stimulated cultures is increased in certain cell subsets following exercise (LaVoy et al., 2013, Zaldivar et al., 2006) other studies have found that acute endurance exercise has little or no effect on IL-4 release in response to mitogen stimulation (Lancaster et al., 2004, Lancaster et al., 2005b), while inducing a temporal suppression of type 1 cytokine production, specifically IFN-γ (Lancaster et al., 2004, Baum et al., 1997, Starkie et al., 2001, Weinstock et al., 1997), IL-2 (Weinstock et al., 1997, Starkie et al., 2001, Lewicki et al., 1988), and TNF-α (Baum et al., 1997, Weinstock et al., 1997,

111

Drenth et al., 1995, Kvernmo et al., 1992, Smits et al., 1998, Starkie et al., 2000). To the authors' knowledge, only two previous studies have investigated the acute exercise effects on IL-10 release in humans in response to stimulation. In line with our results, a recent study by LaVoy and colleagues (2013) reported significantly increased intracellular expression of IL-10 in CD27-CD8+ T-cells in response to phytohemaglutinin 1 h after a 40-km cycling time-trial. Conversely, Smits et al. (Smits et al., 1998) reported suppressed *in vitro* IL-10 production in response to LPS following 15-20 min of rowing to volitional exhaustion in competitive oarsmen. Although these results appear contrary to those of the current study, it is difficult to draw direct comparisons due to dissimilarities in the training status of participants, exercise mode, intensity and duration. Furthermore, the primary cellular source of IL-10 is the lymphocyte, particularly the Treg cell subset. LPS-stimulation primarily activates monocytes, with any lymphocyte response being highly monocyte dependent. The pattern of IL-10 release elicited by LPS will therefore likely differ somewhat from that induced by antigen-stimulation. Van der Poll et al. (van der Poll et al., 1996) found that prior exposure to adrenaline potentiates LPS-induced IL-10 production in humans, both *in vivo* and *in vitro*, and, alongside the anti-inflammatory effects of elevations in plasma cortisol, may provide a plausible mechanism through which acute exercise augments IL-10 release.

Type 1 cytokines promote cellular immunity by stimulating the functional activity of T cytotoxic cells, NK cells and activated macrophages (Gleeson et al., 2013b). A shift towards a more type 2 dominant cytokine response as seen in the current study will likely compromise cellular immunity while temporarily enhancing humoral immunity, particularly because the two are mutually inhibitory. Dysregulation of the type 1/type 2 cytokine balance has been associated with susceptibility to infectious disease (Lucey et al., 1996). Viral infection generally leads to a predominantly type 1 cytokine response (Mosmann and Coffman, 1989). Pitkaranta et al. (1999) found that lower virus-induced IFN-γ production *in vitro* was

associated with frequently recurring respiratory infections in children, whereas blocking of Type 2 cytokine expression via prior administration of antibodies to IL-10 and IL-4 reduces symptom severity following respiratory syncytial virus infection (Connors et al., 1994, Tang and Graham, 1994). Similarly, URS risk in athletes is associated with increased antigen-stimulated IL-10 production at rest (Gleeson et al., 2012). Hence, a lower type 1/type 2 cytokine ratio in response to immune challenge may compromise host defence against respiratory infections.

Another interesting finding of the current study is that antigen-stimulated IL-6 production was inhibited 2 h post-exercise. It is well recognised that IL-6 is released from contracting skeletal muscle resulting in elevated plasma concentrations following exercise (Ostrowski et al., 1998, Steensberg et al., 2000). However, the results of the current study indicate that the capacity of immune cells to produce IL-6 in response to antigen challenge is inhibited following prolonged exercise. The results of previous studies investigating changes in stimulated IL-6 release following endurance exercise are inconsistent. Smits et al. (1998) reported suppressed post-exercise IL-6 production in response to *in vitro* stimulation with LPS. Weinstock et al. (1997) found that although total LPS-induced IL-6 release appeared unchanged by exercise, when corrected for the number of monocytes in the culture, IL-6 production per cell was significantly lower compared with pre-exercise. However, Baum et al. (1997) found no significant changes in IL-6 production from whole blood cultured with LPS, and Starkie et al. (2000) reported no exercise-induced changes in IL-6 production from monocytes, although it is worth noting that the exercise protocol used in that study was not of sufficient intensity to stimulate an increase in plasma cortisol. Haahr et al. (1991) reported an increase in IL-6 production 2 h post-exercise in response to incubation of PBMCs with LPS. These inconsistencies can likely be explained by variations in exercise protocols and training status of participants, as well as the immunological stimulant, duration of cell culture (Reddy

et al., 2004) and culture technique used (e.g. PBMCs vs. whole blood culture). Well-regulated cytokine responses are critical for host defence (Cross et al., 1995), and IL-6 plays a key multifunctional role in orchestrating the acute phase reaction and various immune responses (Kopf et al., 1994), eliciting both pro- and anti-inflammatory effects (Xing et al., 1998). The observed exercise-induced inhibition of IL-6, coupled with an enhanced anti-inflammatory cytokine response, may therefore be indicative of somewhat compromised host-defence to viral infection.

It is well-documented that prolonged exercise results in changes in the total and differential numbers of circulating leukocytes along with changes in lymphocyte subsets (Ricken et al., 1990, Shek et al., 1995). Depending on the cytokine and its principal cellular source, shifts in cell numbers will have clear implications for total cytokine production. However, a weakness of the current study was that, since the principal cellular source of IL-10 and IL-6 in antigen-stimulated culture is not known, it was not possible to correct absolute production for such changes. As well as major cell types, shifts in circulating cell (e.g. lymphocyte) subsets with exercise (Ricken et al., 1990) may also have contributed towards changes in total cytokine production, as it is well-established that different subsets also vary in their proclivity to secrete different cytokines (Street and Mosmann, 1991).

The observed changes in cytokine production can likely in part be explained by the exercise-induced elevation in plasma cortisol. A glucocorticoid released from the adrenal cortex in response to stress, cortisol has immunosuppressive and anti-inflammatory effects (Auphan et al., 1995), acting to mediate the recovery from immune activation early in the stress response, thus preventing an "overshoot" of the immune reaction (Sorrells and Sapolsky, 2007). Cortisol acts to suppress the production of pro-inflammatory cytokines (Petrovsky et al., 1998), as well as up-regulate the expression of certain anti-inflammatory cytokines (Elenkov and Chrousos, 2002), thus functioning as a negative feedback mechanism protecting against

excessive inflammation. Studies on the effect of elevated plasma cortisol on stimulated cytokine production are somewhat inconsistent. However, elevations in plasma cortisol within the physiological range, such as achieved during strenuous exercise, appear to suppress LPS-induced TNF-α, IL-1β and IFN-γ production, but interestingly not IL-6 production (Petrovsky et al., 1998, DeRijk et al., 1997). In the current study there was no effect of fluid restriction on plasma cortisol. This is somewhat unexpected as heart rate and RPE data suggest that exercise in DH was more physiologically stressful than in the EU condition. Bishop et al. (2004) found that fluid ingestion during prolonged exercise attenuated the cortisol response. Mitchell et al. (2002), on the other hand, found that although hypohydration resulted in elevated plasma cortisol when exercise was performed in the heat, there was no effect of hydration status on cortisol concentration, total or differential leukocyte numbers or lymphocyte function when exercising in temperate ambient conditions. The relatively modest level of hypohydration induced in the current study appeared insufficient to influence the plasma cortisol response to exercise at 21ºC, and subsequently had no significant effect on the measured immune variables. Although it was beyond the scope of the current study to measure multiple hormones, it is worth noting that, in addition to cortisol, several other hormones known to increase with exercise also have immunomodulatory effects, notably adrenaline, noradrenaline, growth hormone and prolactin (Pedersen and Hoffman-Goetz, 2000).

Induction of cytokine production by antigen challenge proceeds with a slower kinetic than that associated with powerful T-cell mitogens used in a number of previous studies, and induces lymphocyte responses more directly than LPS challenge. A strength of the current study is that the cytokine response to the multi-antigen vaccine used as stimulant can be considered closer to the natural response to such opportunistic infections as may be encountered during the recovery from exercise. It is acknowledged that measuring cytokine

production from whole blood culture rather than a known number of PBMCs may be confounded by changes in numbers and proportions of circulating lymphocytes and monocytes with exercise. However, a disadvantage of isolating PBMCs is that this insulates the cells from the influence of other cells, hormones and cytokines, while whole blood culture more closely replicates the natural milieu. Although changes in leukocyte subsets with exercise will alter the number of cells in whole blood culture and thereby influence total cytokine production, changes in absolute cytokine production in response to an immune challenge are likely to have implications for disease susceptibility, regardless of the underlying mechanism. A weakness of the current study is that very large individual variations in cytokine responses, although perhaps an interesting finding in itself, somewhat limit the interpretation of results. Future studies examining cytokine responses to antigen-challenge may benefit from larger sample sizes in order to improve statistical power.

6.6 CONCLUSION

Contrary to our hypothesis, moderate hypohydration elicited by a 24-h fluid restriction protocol does not appear to influence plasma cortisol levels, or exacerbate exercise-induced immune disturbances. However, the effects of more severe levels of hypohydration on such immune markers remain unclear. The current study does, however, provide further evidence of a temporal shift towards a more type 2 dominant cytokine response to immune challenge, perhaps indicative of compromised host-defence to viral infection following prolonged exercise. Although the clinical significance of the magnitude of changes observed are not clear, these results may further contribute towards explaining the apparent "open-window" of infection that is evident during short-term recovery from endurance exercise.

CHAPTER 7: EFFECT OF ACUTE EXERCISE AND HYPOXIA ON MARKERS OF SYSTEMIC AND MUCOSAL IMMUNITY

Ida S. Svendsen[1], Erlend Hem[2], & Michael Gleeson[1]

1) School of Sport, Exercise and Health Sciences, Loughborough University, UK.

2) Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway

7.1 ABSTRACT

PURPOSE: To determine how immune markers are affected by acute exercise in normoxia and hypobaric hypoxia equivalent to 2000 m above sea-level (masl). **METHODS**: twelve males (28 ± 4 years, $\dot{V}O_{2max}$ 63.7 ± 5.3 mL/kg/min) cycled for 75 min at 70% altitude-specific $\dot{V}O_{2max}$, once in normoxia (N) and once in hypoxia (H). Blood and saliva samples were collected pre-, post- and 2h post-exercise. **RESULTS**: Participants cycled at 10.5% lower power output in H vs. N, with no differences in heart rate, blood lactate or RPE. Post-exercise plasma cortisol was higher in H vs. N (683 (95% CI 576-810) nmol/l vs. 549 (469-643) nmol/l, P<0.05), with no differences 2 hr post-exercise. There were no between-trial differences for ACTH, plasma cytokines or antigen-stimulated cytokine production. The exercise-induced decrease in CD4:CD8 ratio was greater in H vs. N (-0.5 ± 0.2 vs. -0.3 ± 0.2, P<0.05). There were no between-trial differences for salivary IgA or lactoferrin. However, there was a main trial effect for concentration (F(11)=5.99, P<0.05) and secretion (F(11)=5.01, P<0.05) of salivary lysozyme, with this being higher in N at every time-point. **CONCLUSION**: The observed differences are unlikely of sufficient magnitude to meaningfully impair host defence, particularly as they are transient in nature and since the other measured immune markers are unaffected by hypoxia when exercising at the same relative intensity.

7.2 INTRODUCTION

Hypoxic training is routinely used by competitive endurance athletes, both to improve exercise performance at sea level (Levine and StrayGundersen, 1997, Stray-Gundersen et al., 2001) and to acclimatise prior to competition at altitude (Fulco et al., 2000). However, both anecdotal evidence from athletes and coaches, and scientific literature point towards an increased incidence of opportunistic infectious illness during and immediately following a period of altitude training (Bailey et al., 1998). Exercise in hypoxic conditions typically causes greater physiological stress than exercise at sea level (Bailey and Davies, 1997). Taking into account this augmented stress response to exercise, coupled with reports of increased resting levels of adrenaline, cortisol and interleukin-6 at high altitude (Mazzeo et al., 2001) it is not altogether surprising that both acute and chronic hypoxic training have been found to impact negatively on mucosal (Tiollier et al., 2005) and cell-mediated immunity (Facco et al., 2005, Oliver et al., 2013, Pyne et al., 2000). However, it is still unclear how many aspects of immune function respond to hypoxic exercise, and particularly to the types of exercise and levels of hypoxia that athletes are typically exposed to during training or competition at altitude. Furthermore, it is well known that $\dot{V}O_{2max}$ is reduced at altitude, with the magnitude of this reduction being proportional to the level of hypoxia (Ferretti et al., 1997). Some previous studies that have investigated differences in immune responses to an acute bout of exercise performed in normoxic and hypoxic conditions have matched trials based on absolute workload, such as a specific running speed or power output (Mazzeo et al., 2001, Klokker et al., 1995). However, because of the reduction in $\dot{V}O_{2max}$, the same absolute workload performed in hypoxic conditions will result in higher relative exercise intensity (%$\dot{V}O_{2max}$). To determine whether the differences in responses observed are due to hypoxia per se, rather than this increase in relative exercise intensity, it is useful to instead match trials based on a fraction of altitude-specific $\dot{V}O_{2max}$. This is also of greater

relevance to endurance athlete populations, since they will typically train and compete at a similar relative exercise intensity at altitude as when they are at sea level. The aim of this study was therefore to determine how markers of systemic and mucosal immunity are affected by an acute bout of endurance exercise in hypoxic conditions equivalent to 2000 masl, compared to exercising at the same relative intensity in normoxia.

7.3 METHODS

7.3.1 Participants

Twelve healthy, physically active males volunteered their written, informed consent to take part in the study (mean ($\pm$SD) age: 28 ± 4 years, body mass 79.1 ± 5.9 kg, $\dot{V}O_{2max}$ 63.7 ± 5.3 mL/kg/min, 5.06 ± 0.45 L/min), which was approved by the regional ethics committee of southern Norway. All participants were engaged in regular endurance training at least three times per week. Prior to the start of the study, participants completed a health-screening questionnaire. Participants could be included if they were between 18 and 40 years of age, participating in regular endurance exercise and currently healthy and without URS or use of any medication during the past four weeks. Exclusion criteria were smoking, suffering from, or with a history of, cardiac, hepatic, pulmonary, renal, neurological, haematological, psychiatric, or gastrointestinal illness, or exposure to natural or simulated altitude (equivalent to $\geq$1,000 m above sea level) during the past eight weeks.

7.3.2 Laboratory visits

On the first laboratory visit, 1-2 weeks prior to the first experimental trial, participants completed a continuous, incremental exercise test to volitional exhaustion at sea level for determination of $\dot{V}O_{2max}$ as previously described.. The work rate corresponding to 70% $\dot{V}O_{2max}$ was then calculated from the $\dot{V}O_2$-work rate relationship using a linear equation. The same relative workload for the hypoxic trial was calculated using the same linear equation

and based on previous tests in the recompression chamber showing that $\dot{V}O_{2max}$ decreases by, on average, 10.4 % in hypobaric hypoxia equivalent to 2000 masl (unpublished data). For both main experimental trials, participants arrived at the laboratory at 07:45 am following an overnight fast of at least 10 h, and having abstained from caffeine for a minimum of 12 h. Participants were also required to abstain from alcohol or strenuous exercise for at least 24 h before each trial and, in an effort to standardize nutritional status, asked to record and replicate as closely as possible their dietary intake the day before. For both trials, participants completed a standardised 10 minute warm-up at 55% of altitude specific $\dot{V}O_{2max}$, followed by 75 min at 70% altitude specific $\dot{V}O_{2max}$, on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) inside a recompression chamber (Norsk undervannsteknikk A/S, Haugesund, Norway). Following the exercise bout, participants remained inside the recompression chamber for a 2 h resting recovery. During the normoxic trial (N), the recompression chamber was switched off, and the doors remained open. During the hypoxic trial (H), the pressure was adjusted to 800 mbar (equivalent to 2000 masl at 17ºC). Oxygen and carbon dioxide levels inside the chamber were continuously monitored throughout the trials. Carbon dioxide build up was controlled via three CO_2 scrubbers containing soda lime, and oxygen was supplied continuously at a rate sufficient to maintain normal ambient composition (~20.9% O_2, ~0.04% CO_2). To confirm that the same relative exercise was achieved in both trials, heart rate was measured continuously via short range telemetry (RCX3, Polar, Kempele, Finland), RPE was recorded every 10 min, and blood lactate concentration was measured from a finger prick blood sample using a portable blood lactate analyser (Lactate Pro LT-1710, Arkray, Kyoto, Japan) every 20 min during exercise.

7.3.3 Saliva sampling and analyses

Saliva samples were collected at rest immediately before entry into the recompression chamber, immediately after cessation of exercise, and after a 2 h recovery period and analysed to determine concentrations and secretion rates of S-IgA, lysozyme and lactoferrin, as previously described.

7.3.4 Blood sampling & analyses

Blood samples were collected at the same time points as saliva, and analysed to determine plasma concentrations of cortisol, ACTH and cytokines (IFN-γ, TNF-α, IL-1α, IL-1β, IL-2, IL-4, and IL-10) as well as antigen-stimulated cytokine production by whole-blood culture, as previously described. For a subset of 8 participants, fluorescent-conjugated monoclonal antibodies were used to identify CD4+ CD25+ CD127low/- (Treg), CD3+CD4+ (T helper) and CD3+CD8+ (T suppressor) cells via four colour flow-cytometry as previously described.

7.3.5 Statistical analyses

Data are presented as means ± SD unless otherwise stated. All statistical analyses were performed using IBM SPSS Statistics 22.0. The Shapiro–Wilk test was used to determine whether data were normally distributed. A two-way repeated measures ANOVA was used to determine whether there were significant differences between H and N pre-, post- and 2h post-exercise. A post hoc test with Bonferroni correction for multiple comparisons was used to determine the location of variance. Variables found to be significantly non-normal were log transformed to normality prior to analysis (Box & Cox, 1964), and are presented as back-transformed geometric mean with 95% CI. Based on the results of Mauchly's sphericity test, Greenhouse-Geisser corrections were applied for epsilon <0.75 and Huynd-Feldt corrections applied for epsilon >0.75 where violations of sphericity were identified. Statistical significance was accepted at the P<0.05 level.

7.4 RESULTS

On average, participants cycled at 10.5% lower power output in H compared to N (191 ± 26 W vs. 213 ± 30 W). There were no significant differences in heart rate (N: 157 ± 11 vs. H: 156 ± 12 bpm), blood lactate concentration (N: 3.1 ± 1.5 vs. H: 3.2 ± 1.7 mmol/L) or RPE (N: 15 ± 1 vs. H: 15 ± 1) between trials.

7.4.1 Plasma cytokines, ACTH and cortisol

In both trials, plasma cortisol and ACTH concentrations were significantly lower 2 h post exercise compared to pre-exercise and immediately post-exercise (**Figure 7.1**). Plasma cortisol immediately post-exercise was significantly higher in H compared to N. There were no significant differences between trials at any time point for plasma ACTH. **Table 7.1** shows plasma cytokine concentrations before, immediately after and 2 h after exercise in normoxic and hypoxic conditions. There were no significant differences between trials at any time point for any of the measured cytokines. However, plasma IL-6, IL-8 and MCP1 concentrations were significantly elevated post and 2 h post exercise in both H and N, with only IL-6 remaining elevated at 2 h post-exercise. Plasma VEGF was significantly reduced immediately post exercise in both conditions, before returning to baseline 2 h post exercise.
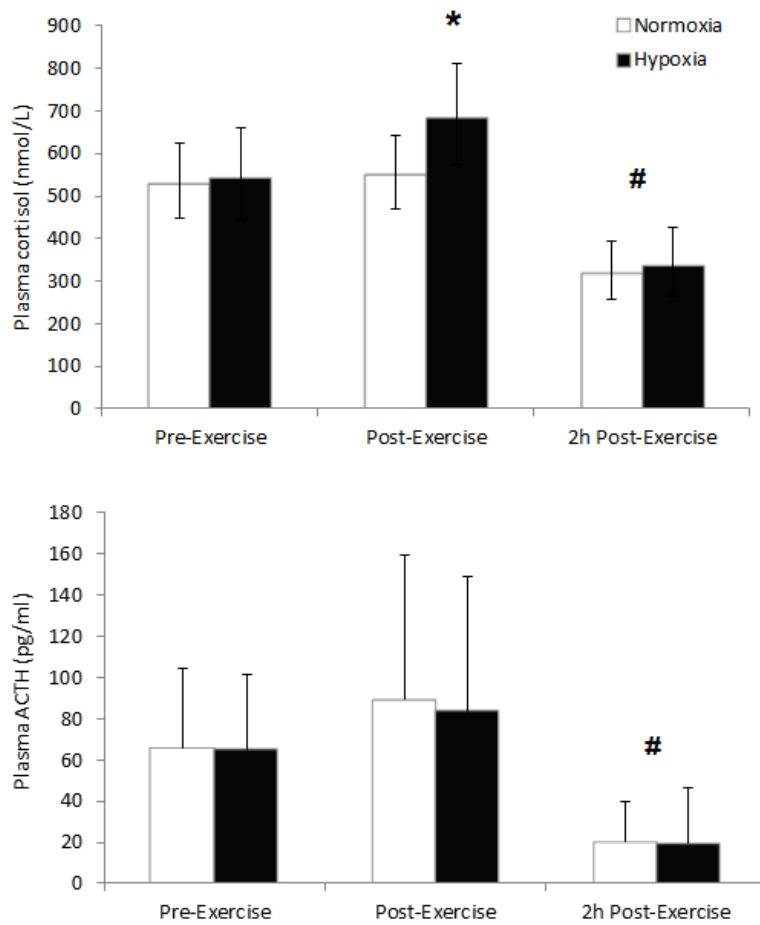
Figure 7.1: Plasma cortisol and ACTH before, immediately after, and 2 h after cycling exercise in normoxia and hypoxia.

Data are geometric mean ± 95% CI. *significantly different from normoxia, ($P<0.05$).

#Both trials significantly different from pre-exercise and post-exercise ($P<0.01$)

Table 7.1: Plasma cytokine concentrations before, immediately after and 2 h after exercise in normoxic and hypoxic conditions.

	PRE	POST	2 h POST
IL-1a (pg/ml)			
Normoxia	0.30 (0.17-0.42)	0.25 (0.15-0.36)	0.26 (0.17-0.36)
Hypoxia	0.29 (0.21-0.37)	0.31 (0.22-0.41)	0.37 (0.17-0.36)
IL-6 (pg/ml)			
Normoxia	0.70 (0.58-0.85)	2.71 (2.11-3.49)#	1.10 (0.85-1.44)#†
Hypoxia	0.70 (0.55-0.88)	2.53 (2.00-3.20)#	1.09 (0.83-1.41)#†
IL-8 (pg/ml)			
Normoxia	2.26 (1.90-2.62)	3.47 (2.90-4.04)#	2.71 (2.10-3.33)†
Hypoxia	2.19 (1.84-2.55)	3.31 (2.53-4.09)#	2.71 (2.01-3.40)†
IL-10 (pg/ml)			
Normoxia	1.10 (0.84-1.44)	1.50 (1.10-2.11)	1.10 (0.89-1.35)†
Hypoxia	0.69 (0.39-1.16)	1.55 (1.12-2.21)	1.10 (0.82-1.49)†
TNFα (pg/ml)			
Normoxia	2.44 (1.99-2.88)	2.44 (2.09-2.80)	2.24 (1.86-2.62)
Hypoxia	2.18 (1.88-2.48)	2.27 (1.99-2.55)	2.48 (1.98-2.48)
EGF (pg/ml)			
Normoxia	11.3 (8.9-14.0)	17.4 (12.1-23.8)	17.9 (13.5-22.9)
Hypoxia	12.7 (8.9-17.2)	16.3 (10.1-23.9)	11.6 (7.2-16.9)
VEGF (pg/ml)			
Normoxia	14.7 (12.2-17.8)	11.5 (8.8-15.0)#	14.9 (11.5-19.3)†
Hypoxia	13.3 (10.3-17.3)	12.0 (9.0-16.0)#	14.1 (11.4-17.4)†
MCP1 (pg/ml)			
Normoxia	107 (93-120)	124 (111-137)#	105 (93-117)†
Hypoxia	103 (92-113)	121 (108-135)#	111 (94-127)†

Values are mean (95% CI). #significantly different from pre-exercise ($P<0.01$). †significantly different from post-exercise ($P<0.01$).
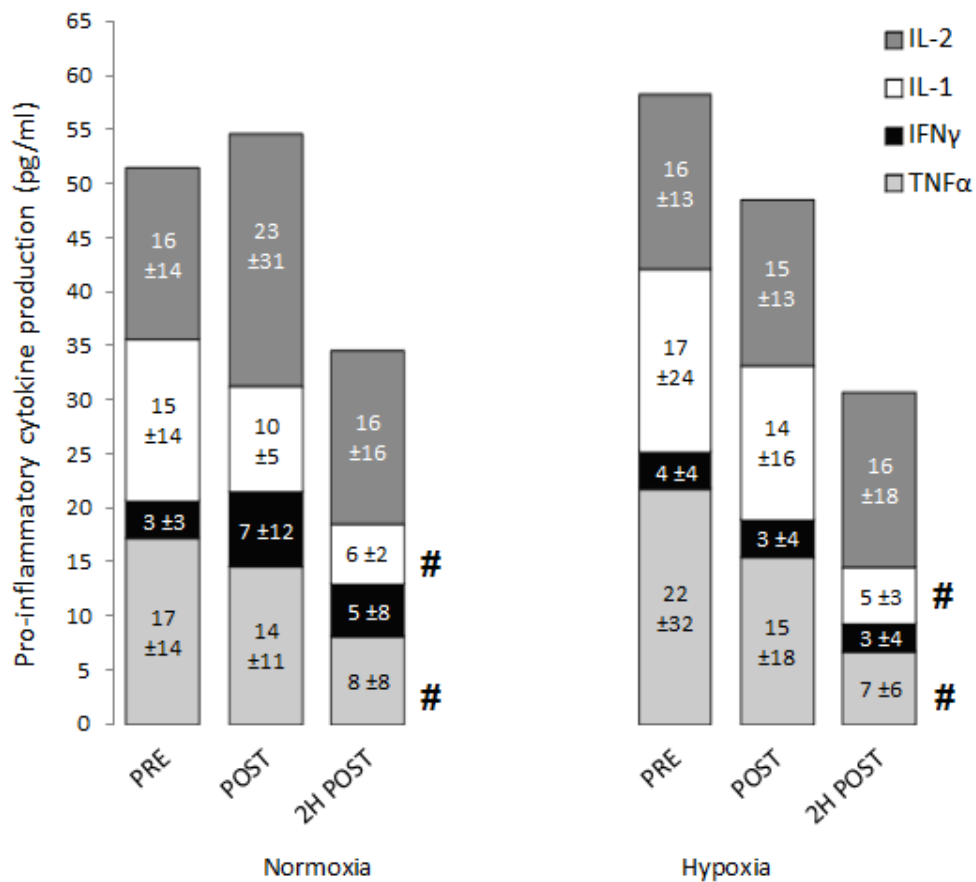
Figure 7.2: Pro-inflammatory cytokine production by whole blood culture in response to a multi-antigen challenge.

IL-2: interleukin 2; IL-1: interleukin 1 ($\alpha+\beta$); IFNγ: Interferon gamma; TNFα: Tumour necrosis factor alpha. Data are mean ±SD. # significant main effect of time for TNFα and IL-1 ($P<0.05$).

NB: ANOVA were run for each cytokine separately, and for total pro-inflammatory cytokine production (IL-2+IL-1+IFNγ+TNFα)
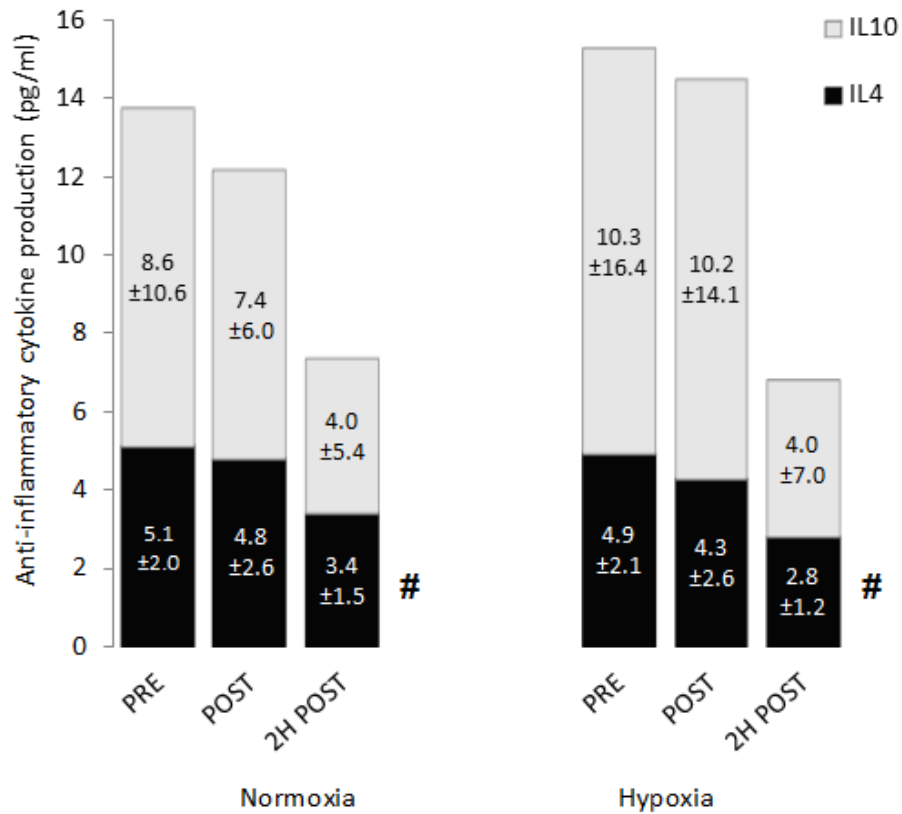
Figure 7.3: Anti-inflammatory cytokine production by whole blood culture in response to a multi-antigen challenge. IL-10: interleukin 10; IL-4: interleukin 4. Data are mean ±SD. # significant main effect of time for IL-4 ($P<0.01$).

NB: ANOVA were run for each cytokine separately, and for total anti-inflammatory cytokine production (IL-10+IL-4)

7.4.2 Antigen-stimulated cytokine production

Figure 7.2 and **Figure 7.3** show in vivo pro-inflammatory and anti-inflammatory cytokine production in whole blood culture in response to a multi-antigen challenge. Production of TNFα, IL-1 and IL-4 were significantly reduced 2 h post-exercise. There were no significant differences between the two trials.

7.4.3 Salivary AMPs

There were no significant differences in S-IgA concentration or secretion rate (**Table 7.2**). Both concentration and secretion rates of salivary lysozyme and lactoferrin were significantly increased post-exercise, with lactoferrin remaining significantly elevated 2 h post-exercise. There was a significant main effect of trial for both concentration ($F(11)=5.99$, *P*=0.032) and secretion rate ($F(11)=5.01$, *P*=0.047) of salivary lysozyme, with this being higher in N at every time-point.

7.4.4 Lymphocyte subsets

Total numbers of circulating lymphocytes, T cells (CD3+), T helper cells (CD3+CD4+) and T suppressor cells (CD3+CD8+) were significantly reduced 2 h Post exercise (**Table 7.3**). The relative number of lymphocytes as a percentage of total leukocytes was also significantly reduced post and 2 h post exercise in both trials. There was no change in the absolute or relative number of Treg cells. The exercise-induced decrease in CD4:CD8 ratio was significantly greater in H compared to N (**Figure 7.4**) but, in both trials, this returned to baseline by 2 h post exercise. There were no significant differences between conditions for any of the other variables.

Table 7.2 Concentrations and secretion rates of salivary AMPs before, immediately after and 2 h after exercise in normoxia and hypobaric hypoxia.

Salivary IgA								
	Concentration (mg/L)				Secretion rate (µg/min)			
	PRE	**POST**	**2h POST**		**PRE**	**POST**	**2h POST**	
Normoxia	328 ± 187	290 ± 99	257 ± 82		108 ± 68	96 ± 42	100 ± 52	
Hypoxia	334 ± 212	335 ± 135	228 ± 58		91 ± 51	105 ± 45	88 ± 42	
Salivary Lysozyme								
	Concentration (mg/L)			Sig. main effect of trial (*P*=0.032)	Secretion rate (µg/min)			Sig. main effect of trial (*P*=0.047)
	PRE	**POST**	**2h POST**		**PRE**	**POST**	**2h POST**	
Normoxia	2.3 ± 1.7	3.2 ± 1.4#	2.3 ± 1.7		0.7 ± 0.5	1.1 ± 0.6#	0.8 ± 0.5	
Hypoxia	1.6 ± 1.4	3.1 ± 1.5#	1.9 ± 1.1		0.4 ± 0.1	1.0 ± 0.6#	0.6 ± 0.3	
Salivary Lactoferrin								
	Concentration (mg/L)				Secretion rate (µg/min)			
	PRE	**POST**	**2h POST**		**PRE**	**POST**	**2h POST**	
Normoxia	3.0 ± 1.2	9.5 ± 3.2#	5.5 ± 1.6#†		1.1 ± 1.0	3.5 ± 2.6#	2.2 ± 1.1#	
Hypoxia	2.9 ± 1.5	9.5 ± 3.4#	5.3 ± 1.9#†		1.0 ± 1.0	3.0 ± 1.4#	2.2 ± 1.3#	

Data are mean ± SD. #significantly different from pre-exercise (*P*<0.01). †significantly different from post-exercise (*P*<0.01).

**Table 7.3: Lymphocyte subsets before, immediately after and 2 h after exercise in normoxic (N) and hypoxic (H) conditions.**

		Absolute (x10^9 cells/L)			Relative* (% total leukocytes / lymphocytes)		
		PRE	POST	2h POST	PRE	POST	2h POST
Lymphocytes	N	1.54 ± 0.53	1.59 ± 0.51	1.30 ± 0.46 †	32 ± 9	23 ± 6 #	15 ± 7 #†
	H	1.49 ± 0.47	1.75 ± 0.35	1.02 ± 0.27 †	32 ± 9	23 ± 6 #	12 ± 7 #†
T	N	0.79 ± 0.28	0.74 ± 0.24	0.53 ± 0.16 †	52 ± 10	48 ± 13	43 ± 15
(CD3+)	H	0.75 ± 0.31	0.81 ± 0.31	0.52 ± 0.12 †	50 ± 13	46 ± 12	52 ± 14
T helper	N	0.46 ± 0.12	0.39 ± 0.10	0.32 ± 0.10 #†	30 ± 5	26 ± 6	27± 11
(CD3+CD4+)	H	0.43 ± 0.15	0.39 ± 0.11	0.31± 0.06 #†	29 ± 8	23 ± 7	32 ± 9
T suppressor	N	0.32 ± 0.18	0.32 ± 0.14	0.20 ± 0.08 †	20 ± 8	20 ± 8	16 ± 4
(CD3+CD8+)	H	0.30 ± 0.18	0.38 ± 0.22	0.20 ± 0.08 †	19 ± 8	21 ± 9	19 ± 7
T regulatory	N	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	2.0 ± 0.6	1.7 ± 0.7	1.2 ± 0.5
(CD4+CD25+CD127-)	H	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	2.0 ± 0.5	1.6 ± 0.8	1.8 ± 1.1

Data are mean ± SD. #significantly different from pre-exercise (*P*<0.05). †significantly different from post-exercise (*P*<0.05). n=8. *Relative numbers of lymphocytes are presented as percentage of total leukocytes, while lymphocyte subsets are presented as percentage of total lymphocytes.
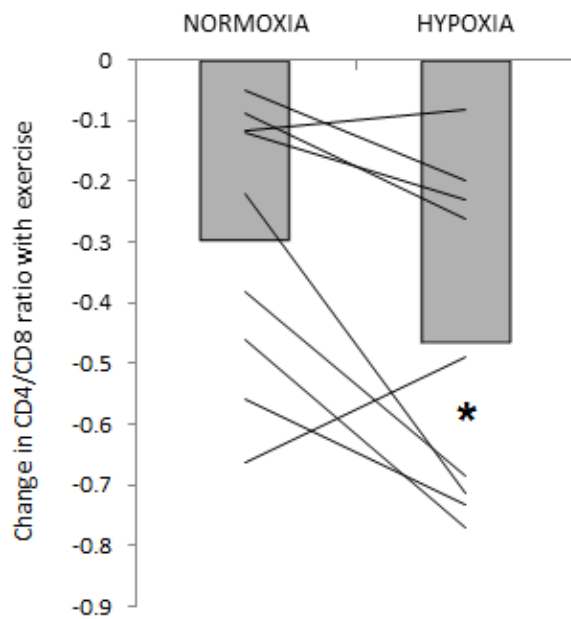
Figure 7.4: Change in CD4/CD8 ratio from pre- to post-exercise. Bars represent group mean, lines represent individual changes. *significant difference from normoxia (*P*<0.05).

7.5 DISCUSSION

The main finding of the current study is that, despite a somewhat augmented stress response as indicated by higher post-exercise plasma cortisol, an acute exercise bout in hypoxic conditions equivalent to 2000 masl does not appear to pose any meaningful additional threat to immune function compared to exercising at the same relative intensity in normoxia. However, previous studies have found that hypoxic exercise at the same absolute exercise intensity can exacerbate immune disturbances, highlighting the importance of accurately controlling exercise intensity at altitude. Lundby & Steensberg (2004) found that there was no difference in post-exercise plasma IL-6 concentration with either acute or chronic hypoxic exposure when participants cycled at the same relative exercise intensity, whilst IL-6 release was augmented when exercising at the same absolute intensity. This is in agreement with the current study where there was no effect of hypoxia on post-exercise plasma concentrations of

IL-6, or any of the other measured cytokines when cycling at 70% of altitude specific $\dot{V}O_{2max}$. The comparatively modest difference in post-exercise cortisol of 134 nmol/L may not be of sufficient magnitude to influence cytokine production or meaningfully impair host defence, particularly since this difference was no longer present following 2 h recovery.

It is well established that exercise causes changes in circulating lymphocytes subsets, including a transient decrease in CD4:CD8 ratio (Fry et al., 1992, Nieman et al., 1991). The results of the current study suggest that this exercise-induced decrease is more pronounced when exercising in hypoxic conditions, even when relative exercise intensity is the same. Although changes in CD4+ and CD8+ T lymphocytes did not reach statistical significance when analysed separately, it appears that both hypoxic and normoxic exercise may similarly decrease CD4+ cell counts, while only in the hypoxic trial was there also a concurrent increase in CD8+ cells. Although plasma catecholamine concentrations were not measured in the current study, it is possible to speculate that the greater reduction in CD4:CD8 ratio observed in the hypoxic trial may have been due to higher levels of circulating adrenaline. Compared to CD4+ cells, CD8+ lymphocytes have more β-adrenergic receptors (Landmann et al., 1984, Van Tits et al., 1990) which likely explains the greater mobilization of CD8+ cells observed with exercise. Although only speculation, it has been previously shown that the catecholamine response in trained individuals is augmented when exercising in hypoxic conditions at the same absolute intensity, and to a lesser degree also at the same relative intensity (Kjaer et al., 1988). This is supported by the higher post-exercise cortisol observed in the hypoxic trial in the current study, since the magnitude of the cortisol response typically reflects the magnitude of the catecholamine response. Whatever the underlying mechanism, the greater reduction in CD4:CD8 ratio in the hypoxic trial is unlikely to have meaningful negative implications for host defence since it appears to be primarily driven by a greater increase in circulating CD8+ cells rather than a reduction in CD4+ cells.

The observed reductions in TNF-α and IL-1 release in response to antigen challenge 2 h post-exercise in both trials correspond with previous studies reporting a temporal suppression of mitogen- or LPS-stimulated production of type 1 cytokines following prolonged exercise (Baum et al., 1997, Drenth et al., 1995, Lewicki et al., 1988, Starkie et al., 2001). Conversely, while IL-4 production was also significantly reduced in the current study, previous research has found that acute endurance exercise has little or no effect on IL-4 release in response to mitogen stimulation (Lancaster et al., 2004, Lancaster et al., 2005b). However, in the current study these changes likely primarily reflect changes in the number of responder cells in the culture (i.e. changes in circulating leukocyte subsets). This is a weakness of the method used, since it does not allow identification of which cells in the culture are producing specific cytokines. On the other hand, using viral and/or bacterial antigens to stimulate cytokine production in whole blood culture probably most closely represents the natural environment, avoiding artefacts from cell isolation and preparation and enabling normal interactions between antigens and immune components within the natural hormonal milieu. Furthermore, it gives a measure of the total capacity to respond to an immune challenge at that specific time-point. The results of the current study indicate that the post-exercise reduction in cytokine production is not exacerbated when exercise of the same relative intensity is performed in hypoxic conditions.

Previous research has found that a period of "live high, train low" altitude training impairs mucosal immunity (Tiollier et al., 2005). Pilardeau and colleagues (1990) found that saliva flow rate was significantly increased when exercising at 4350 masl compared to at sea level, while alpha-amylase concentration was unaffected. However, we are not aware of any previous studies that have investigated the effects of acute exercise and hypoxia on salivary IgA, lysozyme and lactoferrin, which are postulated to play an important role in mucosal immunity and first-line defence against invading microbials (Durr et al., 2006, Fahlman and

Engels, 2005, Neville et al., 2008, Tenovuo, 1989). Exercise-induced changes in salivary S-IgA and lactoferrin do not appear to be affected by moderate hypoxia when relative exercise intensity is the same. The significant trial effect observed for salivary lysozyme can likely be largely explained by higher lysozyme concentration and secretion rate already at baseline in the normoxic trial, rather than an effect of hypoxia. Hence, it does not appear that cycling for 75 min at 70% $\dot{V}O_{2max}$ negatively influences mucosal immunity, regardless of whether exercise is performed in normoxic or hypoxic conditions. Indeed, both concentration and secretion rates of lysozyme and lactoferrin were increased post exercise in both conditions. This corresponds with previous studies which have reported similarly elevated concentrations of salivary lysozyme and lactoferrin following intense endurance exercise at sea-level (Allgrove et al., 2008, West et al., 2010).

7.6 CONCLUSION

Despite somewhat higher plasma cortisol and lower CD4:CD8 ratio immediately post-exercise in the hypoxic trial, these differences are unlikely to be of sufficient magnitude to meaningfully impair host defence, particularly since they appear transient in nature and since the other measured markers of immunity were unaffected by hypoxia. Hence, exercising at the same relative intensity in moderate hypoxia does not appear to exacerbate the exercise-induced immune disturbances typically seen with endurance exercise at sea level. Since previous studies have, however, shown that exercising in hypoxia at the same absolute intensity can augment immune disturbances and potentially increase the risk of opportunistic infection post exercise, this highlights the importance of athletes adjusting and accurately monitoring exercise intensity when training and competing at altitude.

CHAPTER 8: CHANGES IN MUCOSAL IMMUNITY AND SLEEP DURING ALTITUDE TRAINING IN ELITE CROSS-COUNTRY SKIERS

Ida S. Svendsen[1], Erlend Hem[2], Espen Tønnessen[2] & Michael Gleeson[1]

1) School of Sport, Exercise and Health Sciences, Loughborough University, UK.
2) Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway

8.1 ABSTRACT

PURPOSE: To describe changes in mucosal immunity and sleep in nine world-class male cross-country skiers during a 10-day altitude training camp compared to sea-level (SL).

METHODS: Unstimulated morning saliva samples were collected at SL and on day 1, 3 and 10 at 2000 m above sea-level for determination of salivary AMPs, cortisol, testosterone and osmolality. Sleep was analysed via wristwatch actigraphy. **RESULTS**: Low-intensity endurance training increased by 87% at altitude compared to SL, while high-intensity training and resistance training were reduced. Salivary cortisol concentration significantly ($P<0.05$) decreased on day 1 at altitude compared to SL (11 (95% CI 8-13) vs. 18 (13-23) nmol/l. Salivary immunoglobulin-A (S-IgA) secretion increased on day 3 compared to SL (157 (92-238) µg/min vs. 81 (52-118) µg/min) as did LL-37 (cathelicidin) secretion (5.6 (2.5-8.6) vs. 2.2 (1.2-3.2) ng/min. While at altitude, two athletes experienced upper-respiratory illness (URI). These were the only two participants to have a reduction in S-IgA concentration on days 1 and 3 at altitude compared to SL. Assumed sleep time was significantly reduced by 9% at altitude compared to SL, as was sleep efficiency (79.9 ± 4.9% vs. 83.7 ± 7.0%). Day-to-day changes in sleep variables did not correlate with training load. **CONCLUSION**: A 10-day camp at moderate altitude with a high training load does not result in depression of mucosal immunity in elite endurance athletes, although both sleep quantity and quality are reduced. Monitoring individual changes in S-IgA concentration during the first 3 days at altitude could be a useful predictor of URI during altitude training.

8.2 INTRODUCTION

Altitude training is a key component of the training process of many of today's elite endurance athletes, used to acclimatise athletes prior to competition at altitude (Fulco et al., 2000) or to augment red blood cell volume and enhance oxygen transport capacity in an attempt to improve endurance performance at sea level (Stray-Gundersen et al., 2001). For winter sports athletes, natural altitude also provides the opportunity to train on snow at times of year when this is not possible at sea level. Consequently, these athletes typically spend between 60 and 100 training days per year at moderate altitude (2000-3000 masl) (Tonnessen et al., 2014).

Although the results of Chapter 7 suggest that a single, acute bout of exercise in hypobaric hypoxia equivalent to 2000 masl has limited negative effect on immune function when relative exercise intensity is matched, the consequences of a prolonged training camp at moderate altitude on immunity are still not fully understood. Some previous evidence suggests that altitude training can compromise immunity in well-trained endurance athletes, including inhibition of cytokine production and lymphocyte proliferative responses (Pyne et al., 2000), and reduced levels of S-IgA (Tiollier et al., 2005). However, no study to date has examined the effect of altitude training on other salivary AMPs such as lysozyme and LL-37 (cathelicidin), which have also been postulated to play an important role in mucosal immunity (Bosch et al., 2002, Doss et al., 2010). Furthermore, rather than a "live high, train low" protocol, winter sports athletes typically use a "live high, train high, train low" model (Saunders et al., 2009), both sleeping and training at elevations of ≥2000 masl. It is possible that this might further compound immune disturbances.

As well as compromised immunity, sleep is adversely affected at altitude (Reite et al., 1975). With many key metabolic, immunologic and restorative physiological processes adversely

affected by sleep disturbance (Samuels, 2009), reduced sleep quality in athletes while at altitude could result in impaired daytime functioning (Van Dongen et al., 2003) and inadequate recovery between training sessions, as well as potentially exacerbating immunological disturbances (Irwin, 2002). Previous studies have found that the sleep of young, elite footballers is disrupted at 3600 masl (Roach et al., 2013, Sargent et al., 2013). However, this elevation is more extreme than endurance athletes are exposed to during training camps. The effect of a training camp at moderate altitude on the sleep of elite endurance athletes has not been fully elucidated, and it is not known whether changes in immune variables at altitude might be related to reduced sleep quality.

While it is seldom feasible to conduct intervention studies with elite athletes, whose careers depend on marginal performance gains, descriptive studies provide a viable alternative by which to further our understanding of this population. The aims of the current study were therefore to describe changes in sleep and mucosal immunity in a cohort of world-class cross-country skiers during an altitude training camp with a high training load. We also wished to determine whether any of these variables might be useful in predicting which athletes contract an upper respiratory illness (URI) while at altitude.

8.3 METHODS

8.3.1 Participants

Nine elite male cross-country skiers (age: 23 ± 3 years, $\dot{V}O_{2max}$: 82.7 ± 3.9 ml/kg/min, body mass: 74.1 ± 3.6 kg, annual training volume: 781 ± 97 h) volunteered their written, informed consent to participate in the study, which was approved by the Loughborough University ethical review committee. All of the athletes were members of the Norwegian senior or U23 team for cross-country skiing. All athletes were sea level natives. Two athletes had spent 14 days at 2000 masl seven weeks prior to the start of the study. None of the other athletes had

been exposed to natural or simulated altitude for at least three months prior to the start of the study.

8.3.2 Training at altitude

All of the athletes attended an 11-day altitude training camp. Athletes resided at 2000 masl while typically training two sessions per day; the first session as skiing on the glacier at 2800-3000 masl, and the second session as dry-land training (roller-skiing or running) at 1000–2000 masl. Subsequently, each day athletes spent approximately 4 h at 2800-3000 masl, 1-3 h at 1000-2000 masl and the remaining 17-19 h at 2000 masl. Athletes wore heart-rate monitors during every training session and blood lactate concentration was monitored 2-3 times per session during approximately half of the training sessions. Athletes recorded all of their training daily in an online training diary, as previously described. Training Impulse (TRIMP) (Banister and Calvert, 1980) was calculated using the following formula: TRIMP = Training time (min)*Intensity zone. Living and sleeping conditions at altitude were as similar as possible to sea level, with all athletes housed in single or twin rooms.

8.3.3 Illness incidence

Any illness before, during or after the altitude training camp was also recorded in training diaries. Athletes were instructed that even minor symptoms experienced while at altitude should be reported to the team physician, to verify whether these were due to infection.

8.3.4 Mucosal immunity

Saliva samples were collected from each athlete at sea level four days prior to departure (SL), and on day 1 (following the first night at 2000 masl; A1), day 3 (A3) and day 10 (A10) of the altitude training camp for determination of saliva flow rate, osmolality, salivary

concentrations of cortisol and testosterone and salivary concentrations and secretion rates of S-IgA, Lysozyme and LL-37 (Cathelicidin) as previously described.

8.3.5 Sleep

Sleep quantity and quality were assessed via wristwatch actigraphy. Each athlete wore a wrist-mounted digital tri-axial accelerometer (Motionwatch 8, CamNtech) for 5 nights prior to departure (SL) and every night while at altitude. The actigraphs were configured to sum and store data in 1-min epochs with high sensitivity (0.01 g) and a sampling rate of 60 Hz. Sleep measurements included: assumed sleep, sleep efficiency (SE) defined as actual sleep time as a percentage of time in bed, sleep onset latency and Fragmentation Index defined as the sum of the mobile time (%) and the immobile bouts <=1min (%). In addition, each athlete completed a sleep diary each morning and evening where they recorded lights out and wake up time, nap times and subjective sleep quality on a scale of 1-10. Averaging data over several nights provides a better representation of typical sleep patterns (Ancoli-Israel, 2005). Hence, sleep data were averaged for the 5 nights at sea level and for the 10 nights at altitude. The final night at sea level and the final night at altitude were not included in analyses, due to early morning departure. Sleep variables were also examined day-to-day to determine whether there were differences during the initial days at altitude compared to later in the training camp and whether sleep quality/quantity was related to daily training load.

8.3.6 Statistical analysis

Data are presented as mean ± SD unless otherwise stated. All statistical analyses were performed using IBM SPSS Statistics 22.0. The Shapiro–Wilk test was used to determine if data were normally distributed. A one-way repeated measures ANOVA was used to determine if there were changes in markers of mucosal immunity at altitude (A1, A3 and A10) compared to SL, with a post hoc test with Bonferroni stepwise correction for multiple

comparisons used to determine the location of variance. A paired T-Test was used to determine whether there were significant differences in sleep variables between SL and altitude. Variables that were found to be significantly non-normal were transformed to a normal distribution using a log transformation prior to analysis and are presented as back-transformed mean with 95% CIs. Statistical significance was accepted as $P<0.05$. To examine the magnitude of differences, effect sizes (ES) were computed using Cohen's *d*, where 0.2 was considered a small effect, 0.5 a medium effect, and 0.8 a large effect.

8.4 RESULTS

8.4.1 Training load

Table 8.1 shows training load variables at sea level and at altitude. Total training volume was significantly increased by 43% at altitude compared to SL. This was driven by an increase in low-intensity endurance training (I-zone 1), while resistance training and moderate- and high-intensity endurance training were significantly reduced. Only $4 \pm 2\%$ of endurance training at altitude was performed at moderate-high intensity (I-zone 3-5), compared to $13 \pm 3\%$ at SL. However, due to the increase in low-intensity training, TRIMP was significantly higher at altitude compared to SL.

8.4.2 Salivary cortisol and testosterone

Table 8.2 presents salivary concentrations of testosterone and cortisol and testosterone/cortisol ratio at sea level and at altitude. There was no significant change in salivary testosterone at any time point. However, salivary cortisol concentration was significantly decreased on day 1 at altitude compared to SL (**Figure 8.1**). Testosterone/cortisol ratio was significantly increased on day 1 and day 3 at altitude compared to SL.

Table 8.1: Training load variables for two weeks at sea level and 10 days at altitude.

	SEA LEVEL	ALTITUDE
Total training volume (min/day)	128 ± 18	182 ± 34*
Resistance training (min/day)	13 ± 6	7 ± 3#
I-zone 1 (min/day)	92 ± 12	172 ± 33*
I-zone 2 (min/day)	4 ± 6	4 ± 3
I-zone 3 (min/day)	8 ± 2	8 ± 4
I-zone 4 (min/day)	3 ± 2	0 ± 0#
I-zone 5 (min/day)	3 ± 2	0 ± 0#
% HIT	13 ± 3	4 ± 2#
TRIMP	177 ± 22	217 ± 42*

Data are presented as mean ± SD. *significantly different from sea level (P<0.001). #significantly different from sea level (P<0.01). %HIT = percentage of endurance training performed in I-zone 3-5; TRIMP = Training Impulse

Table 8.2: Resting salivary cortisol and testosterone for nine elite cross-country skiers at sea level and at altitude

	SEA LEVEL	ALTITUDE Day 1	ALTITUDE Day 3	ALTITUDE Day 10
Testosterone (pmol/l)	540 (404-676)	485 (340-630)	593 (439-746)	511 (370-653)
Cortisol (nmol/l)	18 (13-23)	11 (8-13)*	13 (10-17)	13 (9-17)
Testosterone/cortisol	31 (23-40)	47 (33-68)*	47 (31-69)*	41 (29-59)

Data are presented as mean (95% CI). *indicates significant difference from sea level (*P*<0.05).

8.4.3 Salivary AMPs

Table 4 presents salivary concentrations and secretion rates of AMPs at sea level and at altitude. Salivary S-IgA secretion rate was significantly increased on day 3 at altitude (**Figure 8.2**). There were no significant changes in S-IgA concentration. Saliva LL-37 secretion was significantly increased on day 3 and day 10 at altitude. There were no significant changes in lysozyme concentration or secretion rate.

8.4.4 Hydration and energy balance

There was no significant change in body mass, saliva osmolality or saliva flow rate during the altitude training camp (**Table 8.3**).

8.4.5 Illness incidence

During the 10-day period at altitude, two of the athletes experienced symptoms of URI. The first athlete on day 5 at altitude, and the second on day 10. In both cases, duration of symptoms was three days. These two paticipants were also the only athletes that had a reduction from sea level in S-IgA concentration on day 1 and day 3 day at altitude, with reductions of $-30 \pm 27\%$ on day 1 and $-15 \pm 14\%$ on day 3 observed for these two athletes.

Figure 8.1: Percentage change from sea level (SL) in salivary cortisol and testosterone concentration for nine elite cross-country skiers on day 1, 3 and 10 at altitude.

Bars= group mean; Markers= individual values; Line= sea level result (100%)
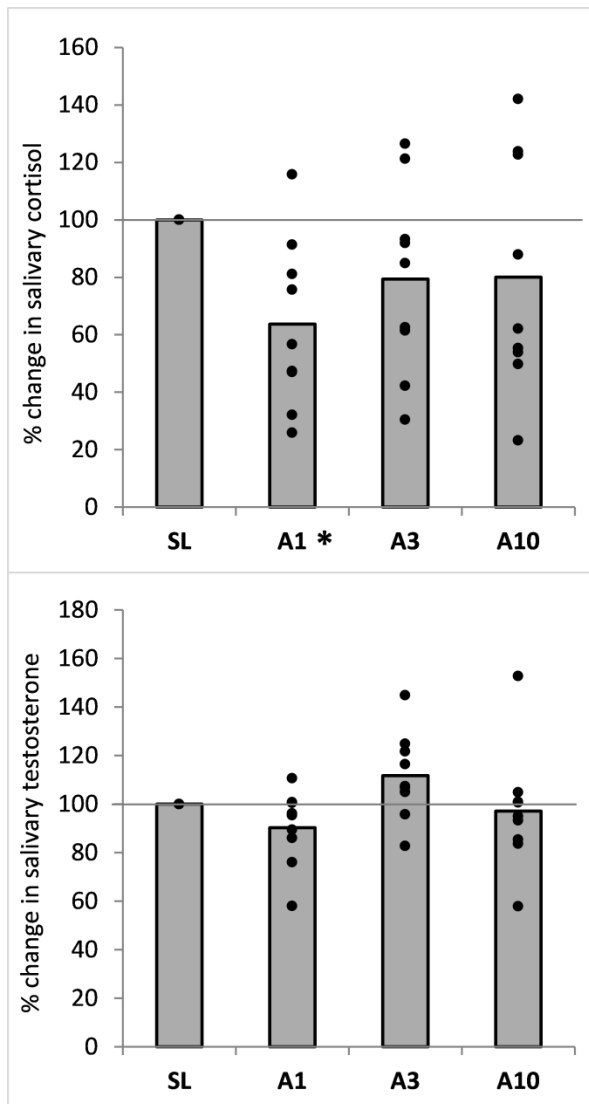
*indicates significant difference from SL (*P*<0.05)
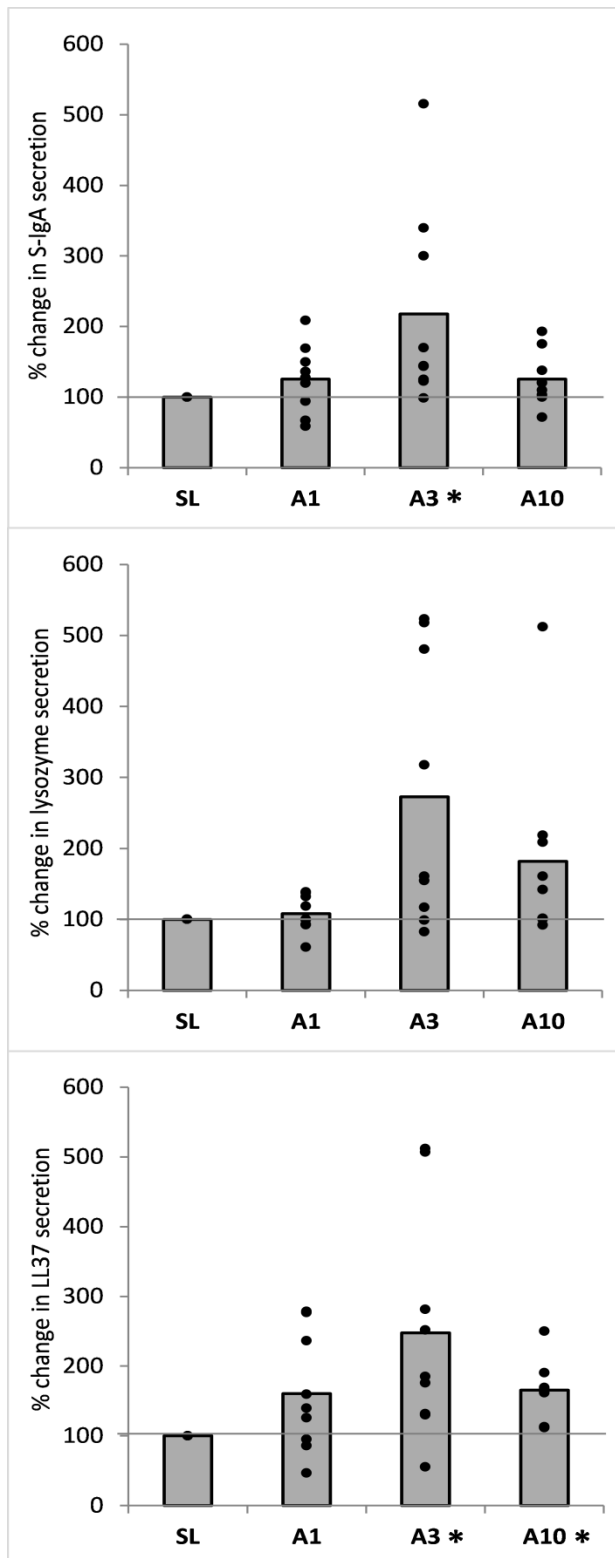
Figure 8.2: Percentage change from sea level (SL) in salivary S-IgA, lysozyme and LL-37 secretion for nine elite cross-country skiers on day 1, 3 and 10 at altitude.

Bars= group mean; Markers= individual values; Line= sea level result (100%)

*indicates significant difference from SL (*P*<0.05)

Table 8.3: Salivary AMPs, flow rate and osmolality for nine elite cross-country skiers at sea level and at altitude

	SEA LEVEL	ALTITUDE Day 1	ALTITUDE Day 3	ALTITUDE Day 10
S-IgA				
Concentration (mg/l)	346 (157-536)	329 (227-432)	554 (298-810)	376 (173-580)
Secretion rate (µg/min)	81 (52-118)	90 (69-115)	157 (92-238)*	96 (65-133)
LL-37				
Concentration (µg/l)	7.6 (4.7-10.5)	11.1 (5.8-16.4)	13.5 (8.6-18.4)	10.7 (6.5-14.9)
Secretion rate (ng/min)	2.2 (1.2-3.2)	3.4 (2.1-6.2)	5.6 (2.5-8.6)*	3.8 (1.9-5.7)*
Lysozyme				
Concentration (µg/l)	793 (551-1080)	815 (509-1192)	1629 (807-2736)	1195 (610-1974)
Secretion rate (ng/min)	208 (144-301)	219 (143-335)	448 (268-749)	322 (191-542)
Saliva flow rate and osmolality				
Flow rate (ml/min)	0.30 (0.24-0.36)	0.33 (0.23-0.42)	0.37 (0.27-0.47)	0.36 (0.24-0.47)
Osmolality (mosm/kg)	51 (42-59)	50 (42-58)	59 (50-68)	52 (42-63)

Data are presented as mean (95% CI). *indicates significant difference from sea level ($P<0.05$).

8.4.6 Sleep

Table 8.4 presents sleep variables at sea level and at altitude. Assumed sleep time was significantly reduced by 9% at altitude compared to SL. This was primarily due to athletes waking earlier to train, and was to some degree compensated for by increased nap time during the day. SE was significantly reduced at altitude compared to SL. **Figure 8.3** shows mean SE and training load for each day at sea level and at altitude. Followng arival at altitude, SE fell progressively reaching its lowest point on the sixth night, after which it recovered somewhat. Changes in SE did not correlate with changes in training load. There were no significant changes in subjective sleep quality, sleep onset latency or Fragmentation Index.

Table 8.4: Sleep variables for nine elite cross-country skiers at sea level and at altitude.

	SEA LEVEL	ALTITUDE
Assumed nighttime sleep (min)	506 ± 29	459 ± 14*
Sleep efficiency (%)	83.7 ± 7.0	79.9 ± 4.9#
Fragmentation index	31.2 ± 8.5	31.8 ± 4.4
Sleep latency (min)	11 ± 5	17 ± 7
Subjective sleep quality (1-10)	7 ± 1	6 ± 1
Nap time (min/day)	21 ± 26	60 ± 22*

Data are presented as mean ± SD. *indicates significant difference from sea level (P<0.01) #indicates significant difference from sea level (P<0.05).

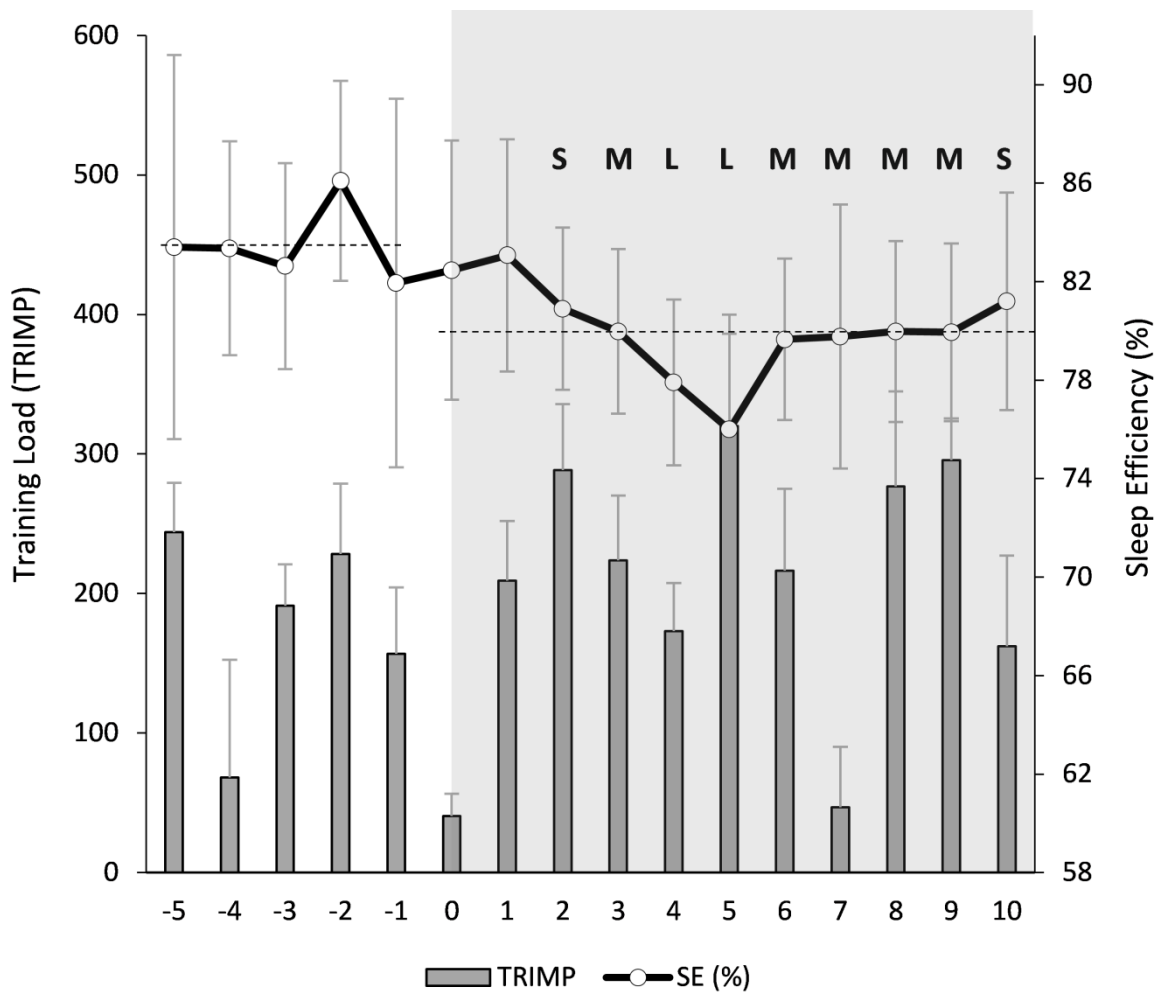**Figure 8.3: Sleep efficiency (line) and training load (bars) for five days at sea level (-5 to -1) and 10 days at altitude (1-10).**

Values are mean ± 95% CI.

------ mean for sea level/altitude.

S = small effect size vs. mean sleep efficiency at sea level.

M = medium effect size vs. mean sleep efficiency at sea level.

L = large effect size vs. mean sleep efficiency at sea level.

8.5 DISCUSSION

The main findings of the current study are that a 10-day altitude training camp with a high training load does not compromise mucosal immunity in a cohort of world-class endurance athletes, despite modest reductions in sleep quality and quantity. However, the two athletes who did experience URI during the altitude training camp were the only two participants that had a reduction from sea level in salivary S-IgA concentration on day 1 and day 3 at altitude.

8.5.1 Mucosal Immunity

A 10-day altitude training camp resulted in elevated salivary AMP (S-IgA and LL-37) secretion rates. S-IgA secretion has been postulated to be one of the most useful clinical biomarkers to predict URI incidence in athletes (Fahlman and Engels, 2005), and a number of previous studies on athletes emphasize the importance of S-IgA in the protection from infections of the upper respiratory tract (Fahlman and Engels, 2005, Neville et al., 2008). This is supported by the finding that the two athletes in the current study who subsequently experienced URI were also the only two participants to have a reduction in S-IgA concentration during the first three days at altitude. LL-37 is also secreted at the mucosal surfaces and exhibits a broad range of antimicrobial activity (Durr et al., 2006). Increased secretion rates of these two salivary AMPs may therefore provide enhanced protection against invading microbes. These results are not in agreement with the findings of Tiollier et al. (2005) who reported reduced S-IgA concentrations in elite cross-country skiers during altitude training. In this study, athletes spent 11 h/day at 2500-3500 masl, with the remainder of the day and all training at 1200 masl. In contrast, athletes in the current study spent a minimum of 21 h/day at ≥2000 masl. Furthermore, Tiollier and colleagues did not report training load at sea level, and since the control group, living and training at 1200 masl,

2

showed similar reductions in S-IgA after 6 and 12 days, it is possible that some of these changes may be attributable to alterations in training load as well as to hypoxia.

It is well documented that heavy training loads can reduce S-IgA levels (Tharp and Barnes, 1990, Gleeson et al., 1999), and reduced relative training intensity is one possible explanation for the positive changes observed in the current study. In accordance with typical practice, the volume of high-intensity training was reduced while at altitude. Despite an increase in training volume and hence TRIMP, athletes only performed two moderate-intensity interval sessions in I-zone 3 while at altitude, with the remainder composed of low-intensity training in I-zone 1. Furthermore, due to lower partial pressure of oxygen and consequent reduction in $\dot{V}O_{2max}$, skiing/running speed is lower at altitude even when training at the same heart rate and/or blood lactate concentration. Hence, the muscular load is also reduced, which may influence hormonal and immunological responses, although this was not supported by the findings in Chapter 7 which found that plasma cortisol was higher post-exercise in hypoxia, even at a lower absolute workload.

As well as changes in training, it is likely that changes in AMP secretion were also driven directly by hypoxia. Acute, intense exercise has been found to transiently increase secretion rates of salivary AMPs (Allgrove et al., 2008), likely via increased sympathetic nervous system activity and circulating catecholamines up-regulating the expression and/or mobilisation of the polymeric Ig receptor, thereby increasing transcytosis of S-IgA (Walsh et al., 2011b). Since hypoxia similarly increases sympathetic activity (Mazzeo et al., 1998) via both chemoreceptor- and baroreceptor-mediated excitation (Hainsworth et al., 2007), this may stimulate AMP secretion and temporarily enhance mucosal immunity. However, the altitude training period in the current study was relatively short, and we cannot eliminate the possibility that negative changes in these immune markers might occur with a more prolonged altitude sojourn, or upon return to sea level.

It appears that negative changes in S-IgA concentration during the initial days at altitude could be a useful predictor of subsequent illness. Since all of the athletes in the current study lived and trained together during the altitude training camp, exposure to pathogens is likely to have been similar between individuals, but the reduction in S-IgA observed in these two athletes may have made them somewhat more susceptible to URI. However, the reason why these athletes had reduced concentrations of S-IgA is not clear, since there were no marked differences in the other measured variables compared to the athletes who did not become ill.

The maintenance of body mass, saliva osmolality and saliva flow rate suggests that fluid and energy intake during the altitude training camp were sufficient to prevent severe hypohydration or weight loss, despite both fluid loss and energy expenditure being elevated at altitude (Meyer et al., 2011).

8.5.2 Sleep

The results of the current investigation add to previous studies which have reported reduced sleep quality in non-elite athletes sleeping at simulated moderate altitude (Hoshikawa et al., 2007) and young elite football players during a training camp at 3600 masl (Roach et al., 2013, Sargent et al., 2013). However, contrary to previous findings, the current study found that SE progressively declined following arrival at altitude, with the most severe disturbances observed on the 5th and 6th night, before recovering somewhat towards the end of the training camp. Intuitively, sleep disturbances would be expected to be greatest before the athletes begin to acclimatise. Indeed, Roach and colleagues (2013) found that sleep disturbances were most severe during the initial days at altitude. However, these athletes underwent a 10 h time zone shift prior to the start of the study, and it is possible that improvements were due to acclimatization to the new time zone, as well as to the altitude itself. However, why meaningful reductions in SE in the current study should arise only after the first two nights at

altitude is not easily explainable. Although sleep variables did not directly correlate with training load, it is possible to speculate that maintained SE during the first two nights at altitude might be related to athletes being well rested on arrival, following 2-3 easy training days. The accumulated effect of consecutive high-volume training days and hypoxia might then lead to a progressive reduction in sleep quality, until the athletes begin to acclimatise to the altitude, at which point SE begins to recover. However, further study is necessary to corroborate these findings. It is worth noting that the athletes in the current study had low SE, even at baseline. These values are similar to those reported by Leeder et al. (2012), who found poorer sleep quality in elite athletes compared to age and sex-matched controls, suggesting that heavy training loads may detrimentally influence sleep.

Sleep is important for both cognitive and physical functioning (Reite et al., 1975, Samuels, 2009). Deep sleep is believed to be particularly important since this is the time when growth hormone is predominantly released (Takahashi et al., 1968), with sleep deprivation diminishing this release (Sassin et al., 1969). Since growth hormone is involved in muscle growth, repair and recovery (Kraemer, 1988) disrupted or insufficient sleep may have implications for subsequent exercise performance and training adaptation. However, whether the modest reductions observed in the current study are of clinical relevance is not clear. Strenuous exercise combined with sleep deprivation and calorie deficit has been found to suppress immunity and decrease plasma immunoglobulin levels (Boyum et al., 1996). However, despite high training loads, athletes in the current study remained weight-stable and the observed sleep disturbances do not appear to be of sufficient magnitude to negatively influence the measured immune variables.

The athletes in the current study were world-class cross-country skiers with exceptionally high aerobic capacity, in the setting of a typical training camp. Consequently, the applicability and generalizability of these results to elite endurance athlete populations is

5

high. However, the results would undeniably have been strengthened by the inclusion of a control group training at sea level, and/or non-exercising controls at altitude. Because these were professional athletes during an important preparation phase for the upcoming season, it was unfortunately not feasible for any of the athletes to forgo the training camp to act as controls.

Since there were significant differences in training variables, it is not clear whether altitude, changes in training, or both, contributed to the observed changes in mucosal immunity and sleep. However, elite athletes do not typically implement the same intensity and volume of training at altitude as they do at sea level. The training of elite cross-country skiers is characterized by high training volumes of ~800 h/year, with a large proportion of endurance training performed at low intensity (91% in zone 1 and 2) (Tonnessen et al., 2014). When athletes are at altitude, this high volume, low intensity model is exaggerated, with 96% of endurance training performed in I-zone 1 and 2. Matching training between the two conditions would therefore make the results less relevant to this population. While tightly controlled laboratory studies are crucial in elucidating mechanistic connections, they may not always accurately reflect the real-world practices of elite athletes. Applied field studies can therefore play an important part in bridging the gap between science and practice.

8.6 CONCLUSION

Despite previous evidence indicating a negative effect of hypoxic training on immunity, it appears that it is possible for elite endurance athletes to sleep and train at moderate altitude without compromising mucosal immunity. Reducing high-intensity training while at altitude, as well as maintaining hydration and energy balance, may have a role to play in ameliorating immunological disturbances. Monitoring individual changes in S-IgA levels upon arrival at altitude could provide coaches and practitioners with a useful predictor of URI. While both

sleep quality and quantity are reduced at altitude, these reductions do not appear to be severe and could likely be compensated for via, for example, making provision for daytime naps and modest adjustments in training and meal times to allow athletes more time in bed.

CHAPTER 9: GENERAL DISCUSSION

9.1 OVERVIEW OF EXPERIMENTAL STUDIES

CHAPTER 2: Risk factors for illness in elite endurance athletes
• Season, air travel, competition and large fluctuations in training load = risk factors
• <u>Frequency</u>: 3-4 episodes per year. <u>Duration of symptoms</u>: ~5 days
• Higher performance level = fewer days of illness

CHAPTER 3: Influence of repeated competition on illness and performance
• Participating in a stage race = 3x greater risk of illness
• Male athletes more prone to illness and see a drop in subsequent race performance → Due to lower training load before and after the event?

CHAPTER 4: Influence of overreaching & CHO supplementation on immunity
• 8-days intensified training = symptoms of OR in highly trained cyclists.
• Immunological and performance changes relatively modest.
• Increasing CHO from 20 g/h to 60 g/h during training does not better maintain performance, or alleviate physiological/immunological disturbances.

CHAPTER 5: Influence of hydration status & endurance exercise on immunity
• No effect of moderate hypohydration (~4% body mass loss) on cortisol or immune responses to exercise
• Prolonged exercise = augmented anti-inflammatory cytokine response to antigen challenge

CHAPTER 6: Influence of acute hypoxia & endurance exercise on immunity
• Plasma cortisol and exercise-induced decrease in CD4:CD8 ratio greater when exercising at same relative intensity in hypoxia vs. normoxia
• Other immune markers unaffected
• Differences unlikely to meaningfully impair host defence, particularly as they are transient in nature.

CHAPTER 7: Influence of altitude training on mucosal immunity & sleep
• 10-days at moderate altitude does not result in depression of mucosal immunity in elite endurance athletes
• Sleep quantity and quality reduced
• Changes in S-IgA during initial days at altitude = useful predictor of URI?

HYPOTHESIS 1: Heavy training loads impair immunity and increase infection risk in endurance athletes

This hypothesis was not supported by our findings in Chapter 3. Neither between-person nor within-person differences in training load were found to be significant predictors of illness risk. In Chapter 4, an intensified period of training did result in some immunological disturbances. However, these immune changes were relatively modest, and whether such changes translate into meaningful increases in infection risk is not clear.

HYPOTHESIS 2: Taking part in competitions increases an athlete's risk of infection

This hypothesis was supported by the findings of Chapter 3 and 4, which indicate that competing, both in single events and multi-day stage races, approximately triples an athlete's risk of illness.

HYPOTHESIS 3: International air travel increases an athlete's risk of infection

This hypothesis was supported by the results of Chapter 3, which found that aircraft travel makes an athlete five times more likely to experience illness within the next three days.

HYPOTHESIS 4: Exposure to natural or simulated altitude exacerbates immune disturbances associated with prolonged exercise, and increases an athlete's infection risk

This hypothesis was not supported by the findings in this thesis. In Chapter 3 we found that, when accounting for air travel to and from the training camp, altitude was not a significant predictor of illness. Although Chapter 7 found that there was an augmented cortisol response to exercise at a simulated altitude of 2,000 masl, this did not appear to translate to greater immune disturbances when exercise was performed at the same relative intensity. Similarly,

mucosal immunity was not impaired in cross-country skiers during a 10-day altitude training camp in Chapter 8.

HYPOTHESIS 5: Increasing CHO intake before, during and after training sessions helps prevent OR and alleviates immunological disturbances associated with intensified training

This hypothesis was not supported by the findings in Chapter 5. In terms of preventing immune disturbances associated with intensified training, a high CHO intake (60g/h) during exercise provided no additional benefit compared to a lower CHO dose (20g/h) in cyclists consuming an otherwise self-selected diet.

HYPOTHESIS 5: Moderate hypohydration, due to inadequate fluid intake before and during exercise, exacerbates exercise-induced immune disturbances and increases an athlete's risk of infection

This hypothesis was not supported by the findings in Chapter 6. Twenty-four hour fluid restriction, resulting in hypohydration equivalent to ~4 % body mass loss, did not influence plasma cortisol concentrations or exacerbate immune disturbances associated with prolonged cycling exercise in temperate conditions.

9.2 HOW FREQUENTLY DO ATHLETES BECOME ILL?

Despite a perception amongst athletes and coaches that endurance athletes experience more URS episodes than the general population, no scientific studies to date have been able to conclusively verify this. The findings in Chapter 3 indicate that elite winter endurance athletes experience three to four episodes of respiratory and/or gastrointestinal illness per year, with symptoms typically lasting five days. Only a small number of previous studies have examined illness rates in athletes for periods of more than a few weeks or months. Hellard and colleagues (2015) reported a similar frequency of infectious illness for elite swimmers who experienced, on average, four episodes of respiratory, gastrointestinal and/or urogenital infection per person-year. This suggests that elite endurance athletes have broadly similar illness rates, regardless of whether their competition season falls during the winter or summer months. Although we unfortunately do not have equivalent data for a sedentary or recreationally active control group in Norway during the same time period, these illness rates do not appear to differ markedly from those of adults in the general population, who typically experience approximately two to four episodes of respiratory infection per year (Monto, 2002, Heikkinen and Jarvinen, 2003).

Spence and colleagues (2007) reported that elite triathletes experienced, on average, one URS episodes over a 5 month period, significantly more than sedentary controls or recreational athletes. However, there was no significant difference in the number of URS episodes with an identified pathogen. Hence, the difference may not be due to higher infection rates in athletes but, rather, down to more frequent reporting of non-infectious URS. Although the mechanisms underlying symptomatic but non-infectious URS are not clear, they may be due to inflammation from mechanical damage to airways caused by breathing large volumes of air during strenuous exercise, or migration of inflammatory cytokines released during exercise and as a result of muscle damage. Another possibility is that elite athletes over-

11

report URS compared to the general population. This is perhaps not surprising, since minor symptoms which do not noticeably impair daily function in a sedentary individual may become much more apparent when attempting to perform strenuous physical activity.

Although total illness rates appear to be broadly similar between summer and winter endurance athletes, it is important to note that there appears to be high inter-individual variability in infection susceptibility. Even within a homogenous group of elite athletes, the frequency and duration of URS and other minor infectious illness appears to vary considerably. Amongst 37 male and female athletes on the Norwegian national cross-country skiing team, mean annual illness rates varied from 1-7 events per year between athletes, despite little difference in training load, travel and competition schedule or training environment. Indeed, since these were athletes from the same team, many would have spent substantial portions of the year traveling, competing and training together in the same locations. These between-person variations can likely be explained, at least in part, by genetic differences. For example, Cox et al. (2010) found that cytokine gene polymorphisms may partly account for differences in URS risk in highly-trained athletes. Correspondingly, in Chapters 4, 5 and 6 we observed high inter-individual differences in cytokine production. However, behavioural and lifestyle differences outside of training, such as diet, sleep behaviours, hygiene and domestic circumstances, almost certainly also contribute.

Higher performance level appears to be associated with a lower number of annual illness days. In Chapter 3 we found that having won at least one Olympic or World Championship medal was a negative predictor of illness duration compared with athletes that had not medalled in a major championship. This is in accordance with Hellard et al. (2015) who found that international level swimmers experienced fewer episodes of illness than national level swimmers. It also lends some support to the theory of Malm (2006) that the relationship between infection and training load follows an S-curve, where high training loads may

negatively influence host defence, but successful elite athletes must have a robust immune system capable of withstanding and recovering quickly from infection, even during periods of high physiological stress. However, personal communications with support staff and coaches suggests that the most successful athletes are often also those who are the most meticulous in terms of hygiene, sleep, nutrition and hydration. It is possible that, as well as potential genetic/physiological differences, such behaviours may contribute towards disparities in infection rates.

9.3 WHEN DO ENDURANCE ATHLETES BECOME ILL?

9.3.1 Season

As previously mentioned, URS episodes in endurance athletes appear to cluster around periods of intense training and competition. As such, it might be expected that athletes from winter and summer sports experience different annual patterns of illness, since they compete and undertake heavy training periods at different times of the year. However, Chapter 3 shows that peak illness rates for cross-country skiers occur during the winter months, similar to patterns of illness observed for athletes from summer sports (Hellard et al., 2015) and mirroring normal seasonal trends for respiratory infections in the general population (Monto, 2002). Cross-country skiers are twice as likely to experience illness during the winter and spring compared to summer, even when correcting for other factors such as differences in the number of competitions and flights.

9.3.2 Periods of competition

Previous studies have found that athletes have an increased risk of URS following competition (Nieman et al., 1990, Nieman et al., 2006a). This is supported by the findings in Chapter 3, which show that competition is a potent risk factor for illness, making an athlete

three times more likely to experience URS or gastrointestinal illness within the subsequent three days. Not surprisingly, therefore, Chapter 4 found that taking part in a stage race, where athletes compete on multiple consecutive days, similarly increases the risk of illness. Additionally, and perhaps most importantly, such an event can impact detrimentally on performance for at least six weeks, particularly in male athletes for whom the competition load is greater. Competitions are characterised by extreme physical exertion, but may also be associated with psychological stress and environmental extremes of temperature and altitude, as well as typically exposing athletes to crowds of spectators and media. All of the above factors may contribute to the observed increase in illness risk.

9.3.3 Periods of heavy training

Similar to competing, previous studies suggest that heavy training negatively impacts on immunity and makes athletes more susceptible to infection (Gleeson et al., 1995, Gleeson et al., 1999, Gleeson et al., 2011a, Heath et al., 1991, Spence et al., 2007). This was not supported by our data in Chapter 3, which failed to find a significant relationship between illness incidence and training load. However, we did find that athletes whose training incorporated more extreme day-to-day fluctuations in training load were somewhat more susceptible to illness. Interestingly, Hellard and colleagues (2015) found that the risk of illness in swimmers was 50% lower during competition periods compared with intensive training periods, whereas our data show that risk of illness in cross-country skiers is significantly increased following competition, but not significantly related to intensive training. Unlike swimmers, however, cross-country skiers undertake their most intensive training periods during the summer (Tonnessen et al., 2014). Perhaps, then, both competition and intensive training can negatively influence host defence, but this only manifests as significantly higher infection rates during the winter months when URS frequency in the general population peak, and pathogen exposure is therefore higher. Training and/or

competing in the winter may also be associated with increased airway irritation due to breathing high volumes of very cold air, which may mimic the sore throat symptoms of an upper-respiratory infection and result in athletes reporting more frequent URS at this time.

High training loads are an inevitable part of elite sport. However, when high training loads are combined with insufficient recovery, a state of non-functional OR or OT may occur, and this appears to be coupled with an increased frequency of infection (Meeusen et al., 2013, McKenzie, 1999). Chapter 5 shows that eight days of intensified training results in a number of changes consistent with the symptoms of short-term overreaching in highly trained cyclists, but that the immunological changes are relatively modest. Although determining the clinical relevance of such changes is difficult, they are unlikely to be of sufficient magnitude to have any profound influence on host defence. Small, transient immune impairments are likely outweighed by the longer-term performance benefits that an athlete would expect to reap as a consequence of such a training period. As such, these data do not provide grounds on which to advise highly trained athletes against incorporating short-term periods (≤8 days) of intensified training into their training programmes. However, athletes and coaches should be aware that there may be a small increase in infection risk as a result of functional OR during these periods. Non-functional OR and OT can, on the other hand, result in long-term detriments to immunity and performance which can persist for up to several years (Meeusen et al., 2013). It is therefore of critical importance that athletes respect the balance between training and recovery. Since the effects of more prolonged periods of intensified training have not been investigated, and since the potential negative consequences can be substantial, intensified training periods lasting much in excess of one week are not advised, and such training should not be completed without adequate nutritional support, or in the presence of other life stressors.

Although the prevalence of OT in elite athlete populations is high (Morgan et al., 1987, Matos et al., 2011), its aetiology remains poorly understood. A number of explanations have been proposed, including elevations in circulating pro-inflammatory cytokines (Smith, 2000), glycogen depletion (Snyder, 1998) and endocrine disturbances (Meeusen et al., 2013). The most effective marker to diagnose OT remains a negative change in performance, despite maintained or increased training load, combined with mood disturbances (Meeusen et al., 2013). A number of studies have attempted to identify novel markers of OR, in order to facilitate earlier detection of an athlete who is at risk of becoming overtrained, before any long-term detriment to health and/or performance occurs. However, the majority of biochemistry measures investigated to date likely only provide a general indication of training stress, and are not able to consistently distinguish between overreached and non-overreached athletes (Coutts et al., 2007). Given that an increase in infection susceptibility is cited as a common symptom of OT, investigating immune system changes presents an attractive starting point in the search for novel markers. Although a number of variables in Chapter 5 were significantly altered by the intensified training period, none correlated significantly with performance changes. As such, it is unlikely that any of these markers can be used to accurately differentiate between overreached and non-overreached athletes. Indeed, many of the changes observed, particularly in circulating leukocyte numbers, are likely primarily a reflection of incomplete recovery from the previous exercise bout. Furthermore, the immune changes observed are unlikely to be of sufficient magnitude to explain the increase in infection incidence commonly reported in overtrained athletes. The changes in resting and post-exercise plasma cortisol and ACTH following the intensified training period do, however, provide some support for the theory that changes in the sensitivity of the Hypothalamic-Pituitary-Adrenal axis may play a role in the aetiology of OT (Meeusen et al., 2013).

Clearly, it is not ethical to intentionally induce a true state of OT in study participants, and it is possible that some of the modest changes observed in Chapter 5 may manifest as more severe immune disturbances if intensified training was sustained for a longer period resulting in non-functional OR or OT. Indeed, some of the participants in the current study showed very little impairment in performance, suggesting that we were not successful in inducing a state of OR in these individuals. This is interesting, since previous studies report successfully OR their participants using a similar, or even slightly shorter, period of intensified training (Halson et al., 2003, Achten et al., 2004, Halson et al., 2002). However, our participants had a substantially higher aerobic capacity than athletes in these studies, and it is possible to speculate that they may therefore also have had a higher tolerance for the intensified training.

9.3.4 Periods of travel

Chapter 3 indicates that air travel is a potent risk factor for illness amongst athletes. This corresponds with data from non-athletes which has found that individuals are more likely to experience URS following a flight (Zitter et al., 2002) and that cabin staff are at a greater risk of URS compared to the general population (Whelan et al., 2003). Since elite athletes have to travel frequently between competition venues and training camps, exploring strategies to effectively minimise infection risk during air travel is a worthwhile area for future research.

9.3.5 Exposure to environmental extremes

Previous studies examining the effects of high altitude (>3,000 masl) on immune function indicate that exposure to hypoxia can result in immune disturbances and potentially increase infection risk (Mazzeo, 2005, Oliver et al., 2013, Pyne et al., 2000, Facco et al., 2005, Hartmann et al., 2000). However, the results of Chapter 7 and 8 suggest that, when absolute workload is reduced so that relative exercise intensity is the same as at sea-level, acute or short-term (up to 10 days) training at moderate altitude (≤3,000 masl) does not appear to

17

result in any meaningful impairment to the measured immune markers, despite a somewhat augmented stress hormone response to acute exercise. However, correctly adjusting exercise intensity when training at altitude can be challenging, particularly during the initial days of exposure, and an athlete's perception of effort may require "recalibrating". The use of blood lactate and heart rate measures can be a useful tool to provide objective feedback to athletes to ensure that they exercise at the desired workload.

In accordance with previous studies with recreational athletes and at more extreme elevations (Roach et al., 2013, Hoshikawa et al., 2007), both sleep quality and quantity are reduced in elite endurance athletes while training at moderate altitude. These reductions do not appear to negatively influence immunity, but may have implications for recovery and adaptation, particularly since training volume during such training camps is typically high. Changes in mealtime and training schedules to allow athletes more time in bed, and making provision for daytime napping can likely offset these relatively modest reductions in nocturnal sleep quality. Indeed, a recent study by Faraut and colleagues (2015) found that two 30 min daytime naps normalized the catecholamine and immune perturbations associated with sleep restriction.

9.3.6 Summary

Elite endurance athletes typically experience three to four episodes of URS and/or gastrointestinal illness per year, with the highest illness rates observed during the winter months. As such, annual illness rates in these athletes do not appear to differ markedly from those of adults in the general population, and these data do not support the theory that endurance athletes are more prone to opportunistic infections. Furthermore, the timing of these episodes does not appear to deviate markedly from normal seasonal trends, although illness events do typically cluster around periods of competition and travel. However, even

within a homogenous athlete population, large individual differences exist in illness susceptibility that cannot be explained by differences in training, competition, travel or environmental conditions. Since fewer annual illness days appears to be associated with success in elite endurance sport, exploring novel strategies to minimise illness risk in athletes remains an important area of research.

9.4 POSSIBLE MECHANISMS FOR INCREASED INFECTION RISK

It is clear that both acute and chronic endurance exercise influences a number of aspects of immune function. However, the immune system is characterised by a large amount of redundancy, so that it is not easy to determine what magnitude of change is necessary to meaningfully impact on host disease susceptibility.

Chapter 6 provides further evidence of a temporal shift towards a more type 2 dominant cytokine response to antigen challenge following prolonged exercise, perhaps indicative of compromised host-defence to viral infection. This is in accordance with a number of previous studies that have used mitogen as stimulant (Lancaster et al., 2004, Lancaster et al., 2005b, Steensberg et al., 2001). The ability of cells to produce cytokines in response to an immune challenge has consequences for the functional capacity of the immune system as a whole, and is likely reflective of an individual's ability to defend against invading pathogens. As such, these changes may contribute towards explaining the "open-window" of infection observed during the short-term recovery from endurance exercise.

A number of previous studies have shown an association between salivary S-IgA and infection susceptibility in athletes (Gleeson et al., 1999, Gleeson et al., 2012), and Chapter 7 provides further support for this relationship since the two athletes who contracted a URS were also the only two participants to have a drop in salivary S-IgA concentration upon ascent to altitude. To date, S-IgA concentrations and secretion rates remain the only immune

markers to show a consistent relationship with URS risk in athletes (Walsh et al., 2011b). Furthermore, the collection of saliva is simple and non-invasive, making it an attractive and viable tool for measuring immune status of athletes in the field. However, since S-IgA secretion rates are highly variable both within and between individuals (Neville et al., 2008), defining a normal range is difficult. It is therefore important that individualised baseline/healthy values be established for each athlete.

9.5 WHAT CAN BE DONE TO MINIMISE INFECTION RISK?

9.5.1 Training

It is generally accepted that there exists a dose-response relationship between training and performance. Asking athletes to reduce their total training load is therefore not a feasible means of lowering infection risk. However, it is possible that the same total training dose can be organised and administered in a slightly different way in order to minimise immune disturbances. Current guidance suggests that, in order to maintain immune health, athletes should employ gradual and periodised increases in training loads, avoid excessively high training loads, included non-specific cross-training, ensure sufficient rest and recovery and add variety to limit training monotony (Foster, 1998, Walsh et al., 2011a). However, our data in Chapter 3 indicate that Training Monotony (mean daily training load divided by the standard deviation) was actually inversely related to illness incidence, while none of the other measures of training were significantly related to illness incidence. It is possible to question whether the calculation originally proposed by Foster (1998), and used in Chapter 3, is a true measure of training monotony. Since it reflects only day-to-day fluctuations in total training load, the expression "Training Monotony" would perhaps be better replaced with a term such as "Training Load Volatility". In order to truly measure training monotony, a formula that also accounts for activity type is necessary; however, this was unfortunately beyond the scope

of the current thesis. It is somewhat unexpected that none of the other training variables significantly predicted illness. However, descriptive studies suggest that the training of elite cross-country skiers typically already conforms to the above-mentioned guidelines (Tonnessen et al., 2014) and, in terms of reducing immune disturbances, there may therefore be limited scope to further optimise training practices of most elite endurance athletes.

9.5.2 Nutrition

Much research has been dedicated to finding effective strategies to ameliorate exercise and training-induced immune disturbances. However, the majority appear to have only a limited effect on post-exercise immune function. Regardless of whether an athlete is in energy balance, euhydrated, nutritionally supplemented and well-rested, prolonged and/or intense physical exercise will inevitably result in some magnitude of immune disturbances. However, some strategies do appear to be beneficial in ameliorating these perturbations. In terms of nutritional strategies, consuming a high CHO diet and supplementing with CHO during exercise appear to be the most effective strategies to alleviate exercise-related immunodepression. Studies have found that ingesting a high dose of CHO is beneficial in reducing immune disturbances following acute, prolonged exercise (Nieman et al., 2003, Henson et al., 1998, Nieman et al., 2006b, Bishop et al., 2003) and consuming a high CHO diet appears to better maintain some aspects of immunity during periods of intensified training (Costa et al., 2005). Indeed, a review by Gunzer et al. (2012) suggested that, in terms of macronutrients, the single most effective strategy for maintaining immune function in athletes is to consume a ≥6% CHO beverage during prolonged exercise.

However, the effect of acute CHO supplementation immediately before, during and after exercise during intensified training while consuming an otherwise self-selected diet had not been previously explored. Many athletes do not meet the current ACSM and IOC guidelines

of 30-60 g CHO per hour during endurance training, but nor do they typically perform prolonged exercise while ingesting zero CHO. In Chapter 5 we therefore wished to determine whether ingesting the upper end of the ACSM recommended dose of CHO during an intensified training period akin to a training camp, has any additional benefit for performance and/or immunity above that of a more modest CHO intake (20 g per hour). Supplementation with the low CHO dose was found to be equally effective in maintaining performance and immunocompetence during short-term intensified training. This may be particularly relevant for individuals who experience gastrointestinal distress when ingesting higher CHO doses, or for athletes who wish to moderate their calorie intake. However, it should be noted that both the low and high CHO supplemented groups had a high total dietary CHO intake outside of training sessions. As such, even if only a modest dose is ingested in and around the exercise sessions themselves, based on previous studies and the findings in Chapter 5, total daily CHO intake during intensified training should not be less than ~7 g/kg bm.

9.5.3 Hydration

Chapter 6 indicates that moderate hypohydration (≤4% body mass loss post-exercise) does not influence plasma cortisol or systemic immunological responses to exercise performed in temperate conditions. Nor does this level of hypohydration appear to detrimentally effect mucosal immunity (Killer et al., 2015). However, the effect of more severe hypohydration on such immune markers remains unclear, and for performance and health general recommendations are that athletes should aim to drink enough to maintain euhydration, limiting body mass loss to <2% (Rodriguez et al., 2009). Maintaining euhydration becomes a particular concern when athletes are exercising in the heat or at altitude, when fluid losses, both during and outside of exercise, are exacerbated. Indeed, Mitchell et al. (2002) found that hypohydration resulted in an augmented cortisol response when exercise was performed in the heat, but had no effect when exercising in temperate ambient conditions.

9.5.4 Competition and travel schedule

Based on the findings from Chapters 3 and 4, it is clear that athletes and coaches should carefully consider competition and travel schedules. This becomes particularly key in the lead up to important events, due to the potential negative consequences for both health and performance. Competitions are an important part of the training process and can be an important source of motivation, as well as often providing financial and sponsorship incentives. Athletes may therefore feel pressure to, or wish to, take part in as many competitions as possible. However, it is clear that an excessive competition schedule can markedly increase infection risk and potentially impair performance for a number of weeks. The number of competitions in which an athlete participates will to a large degree, be influenced by the characteristics and structure of the sport in which they partake. For example, cross-country skiers will compete most weekends during the winter, while a marathon runner may only take part in a handful of races each year. In some sports, most notably road cycling, many athletes compete in stage races requiring heavy exertion over multiple consecutive days. When deciding whether or not an athlete should take part in such a competition in the same season that they are also aiming to attain peak performance for Olympic Games or World Championships, coaches and athletes should reflect carefully on the potential impact this may have on the rest of the competition season. If the decision is made to participate, careful consideration should be given regarding how to optimise training in the weeks and days before and after the race, in order to prevent maladaptive outcomes and instead turn the event into a beneficial component of the athlete's championship preparation.

9.6 LIMITATIONS AND FUTURE DIRECTIONS

As with any body of work, there are a number of limitations to this thesis which should be acknowledged. Firstly, Chapters 3 and 4 are based on self-reported training and illness data. Although elite endurance athletes appear to self-report their training with high fidelity (Sylta et al., 2014), the same level of accuracy has not been confirmed for illness. The results of these studies would undoubtedly have been strengthened had every episode of illness been verified by a physician and/or pathology analyses. However, achieving this with a cohort of 38 elite athletes over a period of many years would not only have required a huge amount of resources, but also that athletes committed to reporting to the laboratory and providing a sample whenever they experienced symptoms. This was not feasible with this particular population. For similar reasons, Chapter 8 is descriptive in nature as it was not viable for any of the athletes to forgo the altitude training camp to act as controls. It is possible to question how much can really be learned from such descriptive studies, since the lack of a control group limits interpretation of the data. However, this type of design often provides one of the only means by which to study elite athlete populations, who are rarely willing to participate in intervention studies. Applied field studies are characterised by high external validity, and the results are likely of more relevance to elite athlete populations than many tightly controlled laboratory studies conducted with recreational athletes or university student populations. As such, although Chapter 8 provides little new information about the underlying mechanisms of how altitude trainings influences immune function, it does deliver an important take-home message that current altitude training practice allows elite athletes to train at moderate altitude without compromising mucosal immunity. However, there is certainly a need for future well-controlled studies exploring the mechanisms and effects of altitude training on other aspects of immunity, and to determine whether strategies related to

training, nutrition and recovery can be effective in abolishing immune perturbations associated with hypoxic training.

Host defence is the end product of a very large number of cells and responses, with the immune system displaying a large amount of redundancy. Hence, determining the clinical relevance of changes in a single immune marker is difficult. The question therefore remains as to how to measure immune function in a meaningful way. In this thesis, we chose to look at a number or immune variables in combination, in an effort to determine the effects of the interventions on a number of aspects of host defence. Since previous research has shown that an elevated anti-inflammatory cytokine production is associated with both training load and increased infection risk in athletes (Gleeson et al., 2012, Gleeson et al., 2013a) this was chosen as a primary outcome measure in Chapters 5, 6 and 7. Compared to mitogen-stimulation, the cytokine response to the multi-antigen vaccine used in these chapters is closer to the natural response to the types of opportunistic infections that might typically be encountered during recovery from exercise. However, measuring cytokine production from whole blood culture may be confounded by increased cell death in cell culture (Green and Rowbottom, 2003) and, more critically, by changes in numbers of circulating lymphocytes and monocytes with exercise. As such, although measuring resting antigen-stimulated cytokine production by whole-blood culture is a promising method of exploring cellular immune responses and changes in infection susceptibility with long-term training, it is perhaps less useful when attempting to determine changes to host defence with acute exercise. On the other hand, changes in absolute cytokine production are likely to have implications for infection susceptibility, regardless of the underlying mechanism. Measuring cytokine production from isolated peripheral blood mononuclear cells overcomes the issue of exercise-induced shifts in leukocyte populations, but removes the cells from the normal

hormonal milieu. This presents a limitation, given the interactive and context-dependent nature of cytokines.

In chapters 5, 6 and 7 we observed large inter-individual variability in cytokine responses. This somewhat limited our ability to detect significant effects as a result of the interventions, since differences between individuals were often greater than the magnitudes of change observed following exercise/training. This large inter-individual variability in cytokine production is an interesting finding in itself, and may contribute towards explaining why some individuals are more illness-prone than others. Indeed, data from Cox et al. (2010) suggests that cytokine gene polymorphisms may partly account for differences in infection risk in highly-trained athletes. However, as a result, future studies examining changes in antigen-stimulated cytokine production following exercise/training may benefit from larger sample sizes in order to improve statistical power. Given that we observed large inter-individual differences, and that results are heavily influenced by exercise-induced changes in the circulating number of responder cells, the value of antigen-stimulated cytokine production by whole blood culture as an immune measure may primarily lie in cross-sectional studies, where resting samples can be collected from a larger cohort of individuals. Other markers of immune function may be more appropriate for acute exercise intervention studies, which are typically also characterized by more modest sample sizes.

Although *in vitro* and *ex vivo* measures, such as those used in the current study, offer a valuable insight into the effect of an intervention on immunity, they often lack clinical relevance since the extent to which such changes translates to increased infection risk is unclear. A small number of recent studies within the field of exercise immunology have therefore utilized cutaneous measures of *in vivo* immunity, such as delayed-type hypersensitivity responses to intradermal injection of antigens, or contact hypersensitivity responses to epicutaneous application of antigens (e.g. Davison et al., 2015, Diment et al.,

2015, Harper Smith et al., 2011). Assessing responses to a whole-body immune challenge has advantages over *ex vivo* and *in vitro* immune measures, since these methods avoid isolating immune cells from the integrated neural and hormonal influences within the normal tissue environment. Being relatively non-invasive, these cutaneous measures of *in vivo* immunity provide an attractive avenue by which to further explore the effects of exercise stress on immunity in humans. There is also a need for a greater number of longitudinal studies that include measures of both immune function AND infection incidence in athletes. This can assist in determining the clinical relevance of training-induced changes in commonly measured immune markers.

Chapters 3 and 4 included no measures of immune function, and purely examined illness incidence. Ultimately, whether or not an athlete contracts an infection might be regarded as the gold-standard outcome measure. However, determining whether or not infection susceptibility is increased requires follow-up over a more prolonged period of time because, although a factor may impact negatively on host defence, this will not manifest as an infection unless the individual is in fact exposed to a pathogen. Furthermore, a large proportion of symptomatic URS are not caused by an infectious agent, and it is possible that differences in illness rates between non-athletes and athletes may be primarily due to athletes experiencing a greater number of unidentified/non-infectious URS episodes (Spence et al., 2007). If this is the case, then mechanisms other than impaired host defence might underlie the increased illness incidence associated with heavy training, although it should be noted that the study by Spence and colleagues was conducted during the summer months, a time period when low incidence of infectious URI would be expected. Since URS appear to be associated with reduced athletic performance regardless of whether or not they are associated with an identifiable pathogen (Walsh et al., 2011b), a better understanding of the aetiology of

symptomatic but non-infectious URS in athletes would improve our ability to prevent and treat them.

Even within a homogenous athlete group there are large inter-individual variations in illness susceptibility which cannot be explained by competition- or training-related factors. Much of this difference is likely to be genetic. However, cross-sectional studies exploring behavioural or lifestyle differences between illness-prone and less illness-prone athletes, could be useful in determining whether there are feasible changes that athletes can implement in order to reduce their risk of illness. This is particularly important since there appears to be an association between athletic success and predisposition to illness, although the causality of this relationship remains unclear. The results from Chapter 3, and from previous studies of swimmers, provide typical annual illness rates for elite athletes, and allow practitioners and researchers to identify athletes who deviate markedly from the norm.

Research pertaining to infection risk associated with prolonged, strenuous exercise and high training loads is primarily of relevance to highly trained and elite athlete populations. Although recreational athletes may also undertake strenuous exercise and relatively large volumes of training, the repercussions of minor illnesses such as upper-respiratory tract infections for these individuals are small compared to an elite athlete whose career depends upon being in optimal physical condition. Despite this, the vast majority of studies in the area of exercise immunology have been conducted with moderately trained individuals. The extent to which these findings are generalizable to elite athletes is questionable, and prescribing accurate guidelines for this population based on results from moderately trained or untrained individuals is problematic. As such, although conducting studies with elite athletes is challenging, there is a need for more research in this population.

9.7 PRACTICAL GUIDELINES AND APPLICATION IN THE FIELD

1. **FINDING**: Illness risk amongst athletes is particularly high in winter months and during periods of competition, intensified training periods and international travel.

   GUIDELINE: Additional precautions should be taken at these times (e.g. minimising exposure to crowds and infected individuals and optimising nutrition, recovery and hygiene).

2. **FINDING**: There is high inter-individual variation in illness incidence. While the average elite endurance athlete experiences 3-4 upper-respiratory and/or gastrointestinal illnesses per year, 16% of athletes experience 6 or more episodes per year and can be categorised as "illness prone".

   GUIDELINE: Coaches/support staff should identify illness prone athletes, as they may benefit from additional support (e.g. to correct potential nutrient or energy deficiencies or implement lifestyle changes) in order to normalise illness rates.

3. **FINDING**: Highly-trained endurance athletes ($\dot{V}O_{2max}$ >65 ml/kg/min) are reasonably robust to short-term periods of intensified training (≤8 days)

   GUIDELINE: Intensified training periods can be incorporated into the training of these athletes without substantial impairments to immunity or performance, but these should not be so prolonged or severe as to induce a state of non-functional OR.

4. **FINDING**: In athletes undertaking intensified training and consuming an otherwise self-selected diet, ingesting 60 g of CHO per hour during exercise is no better than 20 g of CHO per hour in terms of maintaining immunity and performance.

   GUIDELINE: A moderate CHO intake (~20 g/hour) during exercise is sufficient to maintain immunity and performance during intensified training, provided total daily CHO intake is not less than 7 g/kg bm.

5. **FINDING**: Changes in immune markers do not correlate with the magnitude of performance decrement following intensified training, and are therefore not able to differentiate between overreached and non-overreached athletes.

   GUIDELINE: To-date, no biochemical marker is able to consistently diagnose OT, and regular and standardised performance testing of athletes is therefore important in order to monitor for OR.

6. **FINDING**: Moderate hypoxia equivalent to 2,000 masl does not meaningfully exacerbate immune disturbances when exercise is performed at the same relative intensity.

   GUIDELINE: Training load should be carefully monitored and controlled while at altitude. Absolute workload should be reduced so that relative exercise intensity is not higher than at sea-level. For this, heart rate and blood lactate measures may be useful to help athletes "recalibrate" their perception of effort at altitude.

7. **FINDING**: Athletes who subsequently contract a URS have a reduction in salivary S-IgA concentration on day 1 and 3 at altitude.

   GUIDELINE: Monitoring individual changes in salivary S-IgA upon ascent to altitude can be a useful and non-invasive predictor of infection risk in athletes.

8. **FINDING**: Athletes experience reduced sleep quality and quantity while residing and training at altitude.

   GUIDELINE: Training and mealtime schedules during altitude training should allow athletes adequate time in bed. Daytime naps can help athletes compensate for reductions in nocturnal sleep quality.

9. **FINDING**: Traveling on aircraft makes an athlete five times more likely to contract an illness.

   GUIDELINE: In the final days and weeks leading up to a major championship, air travel should be minimised where possible. When traveling on aircraft, limit unnecessary stress and take precautions to minimise pathogen exposure.

10. **FINDING**: Taking part in a competition triples an athlete's risk of illness.

    GUIDELINE: Although competitions are an important part of the training and peaking process, it is advised that the competition schedule be moderated leading up to important events in order to reduce the risk of illness**.**

11. FINDING: Taking part in periods of repeated competition not only increases infection risk, but can also impair race performance for many weeks.

GUIDELINE: Participation in multi-day stage races is not advised in the lead up to major championships.

12. FINDING: Elite endurance athletes with greater training load volatility have higher illness rates.

GUIDELINE: Large day-to-day fluctuations in training/competition load should be avoided.

13. FINDING: Male cross-country skiers have lower training loads but higher competition loads than their female counterparts, and also experience greater illness rates and performance decrements following a stage-race.

GUIDELINE: Higher habitual training loads, achieved via a gradual, periodised increase, can allow athletes to better tolerate and recover from the physical demands associated with an intense period of competition or training.

9.8 CONCLUSION

Although athletes are not clinically immune deficient, multiple small changes to immune function as a result of endurance exercise and training have been suggested to compromise resistance to URS and other minor infections.

The results of this thesis, however, indicate that elite endurance athletes typically experience 3-4 upper-respiratory and/or gastrointestinal illnesses per year. As such, this does not support the assertion that endurance athletes have abnormally high infection rates compared to the general population. That being said, illness incidence varies considerably between individuals, with 16% of athletes experiencing ≥6 episodes (≥30 days) of illness per year. The loss of thirty training days every season can clearly have significant negative implications for performance and for an athlete's career, particularly if they fall during critical periods of training or competition. Indeed, we have found that ultimate athletic success (medalling at Olympic Games or World Championships) is associated with significantly fewer annual illness days. Understanding the factors that influence an athlete's infection risk therefore remains an important line of enquiry.

The results of the present thesis indicate that two of the most potent risk factors for infection in endurance athletes are air travel and competition; respectively tripling and quintupling an athlete's risk of illness. Other factors, such as inadequate CHO intake, hypohydration, and hypoxic exposure, may influence infection risk at the extremes. However, the majority of endurance athletes are unlikely to severely restrict CHO or fluid intake, or be exposed to extremes of altitude (>3,000 masl). Within the moderate ranges that elite athletes would expect to encounter them, and which were consequently explored in this thesis, these factors appear to have negligible influence on immunity and infection risk.

REFERENCES

ABBASI, A., FEHRENBACH, E., HAUTH, M., WALTER, M., HUDEMANN, J., WANK, V., NIESS, A. M. & NORTHOFF, H. 2013. Changes in spontaneous and LPS-induced ex vivo cytokine production and mRNA expression in male and female athletes following prolonged exhaustive exercise. *Exerc Immunol Rev,* 19.

ACHTEN, J., HALSON, S. L., MOSELEY, L., RAYSON, M. P., CASEY, A. & JEUKENDRUP, A. E. 2004. Higher dietary carbohydrate content during intensified running training results in better maintenance of performance and mood state. *J Appl Physiol,* 96.

ALLGROVE, J. E., GOMES, E., HOUGH, J. & GLEESON, M. 2008. Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. *J Sports Sci,* 26.

ANCOLI-ISRAEL, S. 2005. Chapter 124 - Actigraphy. *In:* DEMENT, M. H. K. R. C. (ed.) *Principles and Practice of Sleep Medicine (Fourth Edition).* Philadelphia: W.B. Saunders.

AUPHAN, N., DIDONATO, J. A., ROSETTE, C., HELMBERG, A. & KARIN, M. 1995. Immunosuppression by glucocorticoids - inhibition of nf-kappa-b activity through induction of i-kappa-b synthesis. *Science,* 270.

BAILEY, D. M. & DAVIES, B. 1997. Physiological implications of altitude training for endurance performance at sea level: a review. *Br J Sports Med,* 31.

BAILEY, D. M., DAVIES, B., ROMER, L., CASTELL, L., NEWSHOLME, E. & GANDY, G. 1998. Implications of moderate altitude training for sea-level endurance in elite distance runners. *Eur J Appl Physiol,* 78.

BAJ, Z., KANTORSKI, J., MAJEWSKA, E., ZEMAN, K., POKOCA, L., FORNALCZYK, E., TCHORZEWSKI, H., SULOWSKA, Z. & LEWICKI, R. 1994a. Immunological status of competitive cyclists before and after the training season. *Int J Sports Med,* 15.

BANISTER, E. W. & CALVERT, T. W. 1980. Planning for future performance: implications for long term training. *Can J Appl Sport Sci,* 5.

BAUM, M., MULLERSTEINHARDT, M., LIESEN, H. & KIRCHNER, H. 1997. Moderate and exhaustive endurance exercise influences the interferon-gamma levels in whole-blood culture supernatants. *Eur J Appl Physiol,* 76.

BEIS, L. Y., WRIGHT-WHYTE, M., FUDGE, B., NOAKES, T. & PITSILADIS, Y. P. 2012. Drinking Behaviors of Elite Male Runners During Marathon Competition. *Clin J Sports Med,* 22.

BILLAT, V., LEPRETRE, P. M., HEUGAS, A. M., LAURENCE, M. H., SALIM, D. & KORALSZTEIN, J. P. 2003. Training and bioenergetic characteristics in elite male and female Kenyan runners. *Med Sci Sports Exerc,* 35.

BILLAT, V. L., DEMARLE, A., SLAWINSKI, J., PAIVA, M. & KORALSZTEIN, J. P. 2001. Physical and training characteristics of top-class marathon runners. *Med Sci Sports Exerc,* 33.

BIRON, C. A., NGUYEN, K. B., PIEN, G. C., COUSENS, L. P. & SALAZAR-MATHER, T. P. 1999. Natural killer cells in antiviral defense: Function and regulation by innate cytokines. *Annual Rev Immunol,* 17.

BISHOP, N. C., BLANNIN, A. K., ARMSTRONG, E., RICKMAN, M. & GLEESON, M. 2000. Carbohydrate and fluid intake affect the saliva flow rate and IgA response to cycling. *Med Sci Sports Exerc,* 32.

BISHOP, N. C., BLANNIN, A. K., WALSH, N. P. & GLEESON, M. 2001a. Carbohydrate beverage ingestion and neutrophil degranulation responses following cycling to fatigue at 75% VO2 max. *Int J Sports Med,* 22.

BISHOP, N. C., BLANNIN, A. K., WALSH, N. P., ROBSON, P. J. & GLEESON, M. 1999. Nutritional aspects of immunosuppression in athletes. *Sports Med,* 28.

BISHOP, N. C., SCANLON, G. A., WALSH, N. P., MCCALLUM, L. J. & WALKER, G. J. 2004. No effect of fluid intake on neutrophil responses to prolonged cycling. *J Sports Sci,* 22.

BISHOP, N. C., WALKER, G. J., BOWLEY, L. A., EVANS, K. F., MOLYNEUX, K., WALLACE, F. A. & SMITH, A. C. 2005. Lymphocyte responses to influenza and tetanus toxoid in vitro following intensive exercise and carbohydrate ingestion on consecutive days. *J Appl Physiol,* 99.

BISHOP, N. C., WALSH, N. & SCANLON, G. A. 2003. Effect of prolonged exercise and carbohydrate on total neutrophil elastase content. *Med Sci Sports Exerc,* 35.

BISHOP, N. C., WALSH, N. P., HAINES, D. L., RICHARDS, E. E. & GLEESON, M. 2001b. Pre-exercise carbohydrate status and immune responses to prolonged cycling: I. Effect on neutrophil degranulation. *Int J Sport Nutr Exerc Metab,* 11.

BISHOP, N. C., WALSH, N. P., HAINES, D. L., RICHARDS, E. E. & GLEESON, M. 2001c. Pre-exercise carbohydrate status and immune responses to prolonged cycling: II. Effect on plasma cytokine concentration. *Int J Sport Nutr Exerc Metab,* 11.

BLANNIN, A. K., ROBSON, P. J., WALSH, N. P., CLARK, A. M., GLENNON, L. & GLEESON, M. 1998. The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. *Int J Sports Med,* 19.

BLEGEN, M., CHEATHAM, C., CAINE-BISH, N., WOOLVERTON, C., MARCINKIEWICZ, J. & GLICKMAN, E. 2008. The immunological and metabolic responses to exercise of varying intensities in normoxic and hypoxic environments. *J Strength Cond Res,* 22.

BOSCH, J. A., RING, C., DE GEUS, E. J., VEERMAN, E. C. & AMERONGEN, A. V. 2002. Stress and secretory immunity. *Int Rev Neurobiol,* 52.

BOULET, L.-P. 2012. Cough and upper airway disorders in elite athletes: a critical review. *Br J Sports Med,* 46.

BOX, G. E. P. & COX, D. R. 1964. An analysis of transformations. *J R Stat Soc Series B Stat Methodol***,** 26.

BOYUM, A., WIIK, P., GUSTAVSSON, E., VEIBY, O. P., RESELAND, J., HAUGEN, A. H. & OPSTAD, P. K. 1996. The effect of strenuous exercise, calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines. *Scand J Immunol,* 43.

BROUWER, E. 1957. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. *Acta Physiol Pharmacol Neerl,* 6.

BURKE, L. M., HAWLEY, J. A., WONG, S. H. & JEUKENDRUP, A. E. 2011. Carbohydrates for training and competition. *J Sports Sci,* 29.

CALDER, P. C. & JACKSON, A. A. 2000. Undernutrition, infection and immune function. *Nutr Res Rev,* 13.

CASTELL, L. M. & NEWSHOLME, E. A. 1997. The effects of oral glutamine supplementation on athletes after prolonged, exhaustive exercise. *Nutrition,* 13.

CLOW, A. & HUCKLEBRIDGE, F. 2001. The impact of psychological stress on immune function in the athletic population. *Exerc Immunol Rev,* 7.

COHEN, S., DOYLE, W. J., ALPER, C. M., JANICKI-DEVERTS, D. & TURNER, R. B. 2009. Sleep habits and susceptibility to the common cold. *Arch Intern Med,* 169.

COLE, A. S. & EASTOE, J. E. 2014. *Biochemistry and oral biology*, Butterworth-Heinemann.

CONNORS, M., GIESE, N. A., KULKARNI, A. B., FIRESTONE, C. Y., MORSE, H. C. & MURPHY, B. R. 1994. Enhanced pulmonary histopathology induced by respiratory syncytial virus (rsv) challenge of formalin-inactivated rsv-immunized balb/c mice is abrogated by depletion of interleukin-4 (IL-4) and IL-10. *J Virol,* 68.

CONVERTINO, V. A. 1991. Blood volume: its adaptation to endurance training. *Med Sci Sports Exerc,* 23.

COSTA, R. J., FORTES, M. B., RICHARDSON, K., BILZON, J. L. & WALSH, N. P. 2012. The effects of postexercise feeding on saliva antimicrobial proteins. *Int J Sport Nutr Exerc Metab,* 22.

COSTA, R. J., WALTERS, R., BILZON, J. L. & WALSH, N. P. 2011. Effects of immediate postexercise carbohydrate ingestion with and without protein on neutrophil degranulation. *Int J Sport Nutr Exerc Metab,* 21.

COSTA, R. J. S., JONES, G. E., LAMB, K. L., COLEMAN, R. & WILLIAMS, J. H. H. 2005. The effects of a high carbohydrate diet on cortisol and salivary immunoglobulin A (s-IgA) during a period of increase exercise workload amongst olympic and ironman triathletes. *Int J Sports Med,* 26.

COUTTS, A. J., REABURN, P., PIVA, T. J. & ROWSELL, G. J. 2007a. Monitoring for overreaching in rugby league players. *European J Appl Physiol,* 99.

COX, A. J., GLEESON, M., PYNE, D. B., CALLISTER, R., FRICKER, P. A. & SCOTT, R. J. 2010. Cytokine gene polymorphisms and risk for upper respiratory symptoms in highly-trained athletes. *Exerc Immunol Rev,* 16.

COX, A. J., GLEESON, M., PYNE, D. B., CALLISTER, R., HOPKINS, W. G. & FRICKER, P. A. 2008. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. *Clin J Sport Med,* 18.

CROSS, A., ASHER, L., SEGUIN, M., YUAN, L., KELLY, N., HAMMACK, C., SADOFF, J. & GERNSKI, P. 1995. The importance of a lipopolysaccharide-initiated, cytokine-mediated host-defense mechanism in mice against extraintestinally invasive escherichia-coli. *J Clin Invest,* 96.

CROSS, M. C., RADOMSKI, M. W., VANHELDER, W. P., RHIND, S. G. & SHEPHARD, R. J. 1996. Endurance exercise with and without a thermal clamp: effects on leukocytes and leukocyte subsets. *J Appl Physiol,* 81.

DA SILVA, R. P., MUENDEL, T., NATALI, A. J., BARA FILHO, M. G., ALFENAS, R. C. G., LIMA, J. R. P., BELFORT, F. G., LOPES, P. R. N. R. & MARINS, J. C. B. 2012. Pre-game hydration status, sweat loss, and fluid intake in elite Brazilian young male soccer players during competition. *J Sports Sci,* 30.

DAVIS, J. M., KOHUT, M. L., COLBERT, L. H., JACKSON, D. A., GHAFFAR, A. & MAYER, E. P. 1997. Exercise, alveolar macrophage function, and susceptibility to respiratory infection. *J Appl Physiol,* 83.

DAVISON, G., KEHAYA, C., DIMENT, B. C. & WALSH, N. P. 2015. Carbohydrate supplementation does not blunt the prolonged exercise-induced reduction of in vivo immunity. *Eur J Nutr, 11*.

DERIJK, R., MICHELSON, D., KARP, B., PETRIDES, J., GALLIVEN, E., DEUSTER, P., PACIOTTI, G., GOLD, P. W. & STERNBERG, E. M. 1997. Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 beta (IL-1 beta), IL-6, and tumor necrosis factor-alpha (TNF alpha) production in humans: High sensitivity of TNF alpha and resistance of IL-6. *J Clin Endocrinol Metab,* 82.

DILL, D. B. & COSTILL, D. L. 1974. Calculation of percentage changes in volumes of blood, plasma, and red-cells in dehydration. *J Appl Physiol,* 37.

DIMENT, B. C., FORTES, M. B., EDWARDS, J. P., HANSTOCK, H. G., WARD, M. D., DUNSTALL, H. M., FRIEDMANN, P. S. & WALSH, N. P. 2015. Exercise Intensity and Duration Effects on In Vivo Immunity. *Med Sci Sports Exerc,* 47.

DOSS, M., WHITE, M. R., TECLE, T. & HARTSHORN, K. L. 2010. Human defensins and LL-37 in mucosal immunity. *J Leukoc Biol,* 87.

DRENTH, J. P. H., VANUUM, S. H. M., VANDEUREN, M., PESMAN, G. J., VANDERVENJONGEKRIJG, J. & VANDERMEER, J. W. M. 1995. Endurance run increases circulating il-6 and il-1ra but down-regulates ex-vivo tnf-alpha and il-1-beta production. *J Appl Physiol,* 79.

DURR, U. H., SUDHEENDRA, U. S. & RAMAMOORTHY, A. 2006. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. *Biochim Biophys Acta,* 1758.

EDWARDS, J. E., LINDEMAN, A. K., MIKESKY, A. E. & STAGER, J. M. 1993. Energy balance in highly trained female endurance runners. *Med Sci Sports Exerc,* 25.

EKBLOM, B., EKBLOM, O. & MALM, C. 2006. Infectious episodes before and after a marathon race. *Scand J Med Sci Sports,* 16.

ELENKOV, I. J. & CHROUSOS, G. P. 2002. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Neuroendocrine Immune Basis of the Rheumatic Diseases Ii, Proceedings,* 966.

ENGEBRETSEN, L., SOLIGARD, T., STEFFEN, K., ALONSO, J. M., AUBRY, M., BUDGETT, R., DVORAK, J., JEGATHESAN, M., MEEUWISSE, W. H., MOUNTJOY, M., PALMER-GREEN, D., VANHEGAN, I. & RENSTROM, P. A. 2013. Sports injuries and illnesses during the London Summer Olympic Games 2012. *Br J Sports Med,* 47.

ENGEBRETSEN, L., STEFFEN, K., ALONSO, J. M., AUBRY, M., DVORAK, J., JUNGE, A., MEEUWISSE, W., MOUNTJOY, M., RENSTROM, P. & WILKINSON, M. 2010. Sports injuries and illnesses during the Winter Olympic Games 2010. *Br J Sports Med.* 47.

ENGLER, R. J., NELSON, M. R., KLOTE, M. M., VANRADEN, M. J., HUANG, C. Y., COX, N. J., KLIMOV, A., KEITEL, W. A., NICHOL, K. L., CARR, W. W. & TREANOR, J. J. 2008. Half- vs full-dose trivalent inactivated influenza vaccine (2004-2005): age, dose, and sex effects on immune responses. *Arch Intern Med,* 168.

EVANS, P., DER, G., FORD, G., HUCKLEBRIDGE, F., HUNT, K. & LAMBERT, S. 2000. Social class, sex, and age differences in mucosal immunity in a large community sample. *Brain Behav Immun,* 14.

FABBRI, M., SMART, C. & PARDI, R. 2003. T lymphocytes. *Int J Biochem Cell Biol,* 35.

FACCO, M., ZILLI, C., SIVIERO, M., ERMOLAO, A., TRAVAIN, G., BAESSO, I., BONAMICO, S., CABRELLE, A., ZACCARIA, M. & AGOSTINI, C. 2005. Modulation of immune response by the acute and chronic exposure to high altitude. *Med Sci Sports Exerc,* 37.

FAHLMAN, M. M. & ENGELS, H. J. 2005. Mucosal IgA and URTI in American college football players: A year longitudinal study. *Med Sci Sports Exer,* 37.

FALAGAS, M. E., MOURTZOUKOU, E. G. & VARDAKAS, K. Z. 2007. Sex differences in the incidence and severity of respiratory tract infections. *Respir Med,* 168.

FARAUT, B., NAKIB, S., DROGOU, C., ELBAZ, M., SAUVET, F., DE BANDT, J. P. & LEGER, D. 2015. Napping reverses the salivary interleukin-6 and urinary norepinephrine changes induced by sleep restriction. *J Clin Endocrinol Metab,* 100.

FERNANDO ARAGON-VARGAS, L., MONCADA-JIMENEZ, J., HERNANDEZ-ELIZONDO, J., BARRENECHEA, A. & MONGE-ALVARADO, M. 2009.

Evaluation of pre-game hydration status, heat stress, and fluid balance during professional soccer competition in the heat. *Eur J Sports Sci,* 9.

FERRETTI, G., MOIA, C., THOMET, J. M. & KAYSER, B. 1997. The decrease of maximal oxygen consumption during hypoxia in man: a mirror image of the oxygen equilibrium curve. *J Physiol,* 498.

FISKERSTRAND, A. & SEILER, K. S. 2004. Training and performance characteristics among Norwegian International Rowers 1970-2001. *Scand J Med Sci Sports ,* 14.

FORTES, M. B., DIMENT, B. C., DI FELICE, U. & WALSH, N. P. 2012. Dehydration decreases saliva antimicrobial proteins important for mucosal immunity. *Appl Physiol Nutr Metab,* 37.

FOSTER, C. 1998. Monitoring training in athletes with reference to overtraining syndrome. *Med Sci Sports Exer,* 30.

FRICKER, P. A., GLEESON, M., FLANAGAN, A., PYNE, D. R., MCDONALD, W. A. & CLANCY, R. L. 2000. A Clinical Snapshot: Do Elite Swimmers Experience More Upper Respiratory Illness Than Nonathletes? *Clin Ex Physiol,* 2.

FRICKER, P. A., PYNE, D. B., SAUNDERS, P. U., COX, A. J., GLEESON, M. & TELFORD, R. D. 2005. Influence of training loads on patterns of illness in elite distance runners. *Clin J Sports Med,* 15.

FRY, R. W., MORTON, A. R., GARCIAWEBB, P., CRAWFORD, G. P. M. & KEAST, D. 1992. Biological responses to overload training in endurance sports. *Eur J Appl Physiol,* 64.

FULCO, C. S., ROCK, P. B. & CYMERMAN, A. 2000. Improving athletic performance: is altitude residence or altitude training helpful? *Aviat Space Environ Med,* 71.

FULLAGAR, H. H., DUFFIELD, R., SKORSKI, S., WHITE, D., BLOOMFIELD, J., KOLLING, S. & MEYER, T. 2015. Sleep, Travel and Recovery Responses of National Footballers During and Following Long-Haul International Air Travel. *Int J Sports Physiol Perform,* 11.

FURMAN, D., HEJBLUM, B. P., SIMON, N., JOJIC, V., DEKKER, C. L., THIEBAUT, R., TIBSHIRANI, R. J. & DAVIS, M. M. 2014. Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proc Natl Acad Sci,* 111.

GILLUM, T. L., KUENNEN, M. R., CASTILLO, M. N., WILLIAMS, N. L. & JORDAN-PATTERSON, A. T. 2015. Exercise, but not acute sleep loss, increases salivary antimicrobial protein secretion. *J Strength Cond Res,* 29.

GLEESON, M. 2008. Dosing and efficacy of glutamine supplementation in human exercise and sport training. *J Nutr,* 138.

GLEESON, M., BISHOP, N., OLIVEIRA, M., MCCAULEY, T., TAULER, P. & MUHAMAD, A. S. 2012. Respiratory infection risk in athletes: association with antigen-stimulated IL-10 production and salivary IgA secretion. *Scand J Med Sci Sports ,* 22.

GLEESON, M., BISHOP, N., OLIVEIRA, M. & TAULER, P. 2013a. Influence of training load on upper respiratory tract infection incidence and antigen-stimulated cytokine production. *Scand J Med Sci Sports,* 23.

GLEESON, M., BISHOP, N. & WALSH, N. 2013b. *Exercise immunology*, Routledge.

GLEESON, M. & BISHOP, N. C. 2000. Special feature for the Olympics: effects of exercise on the immune system: modification of immune responses to exercise by carbohydrate, glutamine and anti-oxidant supplements. *Immunol Cell Biol,* 78.

GLEESON, M. & BISHOP, N. C. 2005. The T cell and NK cell immune response to exercise. *Ann Transplant,* 10.

GLEESON, M., BISHOP, N. C., STENSEL, D. J., LINDLEY, M. R., MASTANA, S. S. & NIMMO, M. A. 2011b. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nature Rev Immunol,* 11.

GLEESON, M., BLANNIN, A. K., WALSH, N. P., BISHOP, N. C. & CLARK, A. M. 1998. Effect of low- and high-carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. *Int J Sport Nutr,* 8.

GLEESON, M., MCDONALD, W. A., CRIPPS, A. W., PYNE, D. B., CLANCY, R. L. & FRICKER, P. A. 1995. The effect on immunity of long-term intensive training in elite swimmers. *Clin Exp Immunol,* 102.

GLEESON, M., MCDONALD, W. A., PYNE, D. B., CRIPPS, A. W., FRANCIS, J. L., FRICKER, P. A. & CLANCY, R. L. 1999. Salivary IgA levels and infection risk in elite swimmers. *Med Sci Sports Exer,* 31.

GLEESON, M., NIEMAN, D. C. & PEDERSEN, B. K. 2004. Exercise, nutrition and immune function. *J Sports Sci,* 22.

GLEESON, M., PYNE, D. B., AUSTIN, J. P., LYNN FRANCIS, J., CLANCY, R. L., MCDONALD, W. A. & FRICKER, P. A. 2002. Epstein-Barr virus reactivation and upper-respiratory illness in elite swimmers. *Med Sci Sports Exerc,* 34.

GOMEZ-CABRERA, M. C., DOMENECH, E., ROMAGNOLI, M., ARDUINI, A., BORRAS, C., PALLARDO, F. V., SASTRE, J. & VINA, J. 2008. Oral

administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *Am J Clin Nutr,* 87.

GREEN, K. J. & ROWBOTTOM, D. G. 2003. Exercise-induced changes to in vitro T-lymphocyte mitogen responses using CFSE. *J Appl Physiol,* 95.

GUNZER, W., KONRAD, M. & PAIL, E. 2012. Exercise-induced immunodepression in endurance athletes and nutritional intervention with carbohydrate, protein and fat-what is possible, what is not? *Nutrients,* 4.

HAAHR, P. M., PEDERSEN, B. K., FOMSGAARD, A., TVEDE, N., DIAMANT, M., KLARLUND, K., HALKJAERKRISTENSEN, J. & BENDTZEN, K. 1991. Effect of physical exercise on invitro production of interleukin-1, interleukin-6, tumor-necrosis-factor-alpha, interleukin-2 and interferon-gamma. *Int J Sports Med,* 12.

HAINSWORTH, R., DRINKHILL, M. J. & RIVERA-CHIRA, M. 2007. The autonomic nervous system at high altitude. *Clin Auton Res,* 17.

HALSON, S. L., BRIDGE, M. W., MEEUSEN, R., BUSSCHAERT, B., GLEESON, M., JONES, D. A. & JEUKENDRUP, A. E. 2002. Time course of performance changes and fatigue markers during intensified training in trained cyclists. *J Appl Physiol,* 93.

HALSON, S. L., LANCASTER, G. I., ACHTEN, J., GLEESON, M. & JEUKENDRUP, A. E. 2004. Effects of carbohydrate supplementation on performance and carbohydrate oxidation after intensified cycling training. *J Appl Physiol,* 97.

HALSON, S. L., LANCASTER, G. I., JEUKENDRUP, A. E. & GLEESON, M. 2003. Immunological responses to overreaching in cyclists. *Med Sci Sports Exer,* 35.

HANDZLIK, M. K., SHAW, A. J., DUNGEY, M., BISHOP, N. C. & GLEESON, M. 2013. The influence of exercise training status on antigen-stimulated IL-10 production in whole blood culture and numbers of circulating regulatory T cells. *European J Appl Physiol,* 113.

HARPER SMITH, A. D., COAKLEY, S. L., WARD, M. D., MACFARLANE, A. W., FRIEDMANN, P. S. & WALSH, N. P. 2011. Exercise-induced stress inhibits both the induction and elicitation phases of in vivo T-cell-mediated immune responses in humans. *Brain Behav Immun,* 25.

HARTMANN, G., TSCHOP, M., FISCHER, R., BIDLINGMAIER, C., RIEPL, R., TSCHOP, K., HAUTMANN, H., ENDRES, S. & TOEPFER, M. 2000. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. *Cytokine,* 12.

HAUSSWIRTH, C., LOUIS, J., AUBRY, A., BONNET, G., DUFFIELD, R. & Y, L. E. M. 2014. Evidence of disturbed sleep and increased illness in overreached endurance athletes. *Med Sci Sports Exerc,* 46.

HE, C. S., BISHOP, N. C., HANDZLIK, M. K., MUHAMAD, A. S. & GLEESON, M. 2014. Sex differences in upper respiratory symptoms prevalence and oral-respiratory mucosal immunity in endurance athletes. *Exerc Immunol Rev,* 20.

HE, C. S., FRASER, W. D., TANG, J., BROWN, K., RENWICK, S., RUDLAND-THOMAS, J., TEAH, J., TANQUERAY, E. & GLEESON, M. 2015. The effect of 14 weeks of vitamin D supplementation on antimicrobial peptides and proteins in athletes. *J Sports Sci***,** 34.

HE, C. S., HANDZLIK, M., FRASER, W. D., MUHAMAD, A., PRESTON, H., RICHARDSON, A. & 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.

HEATH, G. W., FORD, E. S., CRAVEN, T. E., MACERA, C. A., JACKSON, K. L. & PATE, R. R. 1991. Exercise and the incidence of upper respiratory tract infections. *Med Sci Sports Exerc,* 23.

HEIKKINEN, T. & JARVINEN, A. 2003. The common cold. *Lancet,* 361.

HELLARD, P., AVALOS, M., GUIMARAES, F., TOUSSAINT, J. F. & PYNE, D. B. 2015. Training-related risk of common illnesses in elite swimmers over a 4-yr period. *Med Sci Sports Exerc,* 47.

HENSON, D. A., NIEMAN, D. C., PARKER, J. C., RAINWATER, M. K., BUTTERWORTH, D. E., WARREN, B. J., UTTER, A., DAVIS, J. M., FAGOAGA, O. R. & NEHLSEN-CANNARELLA, S. L. 1998. Carbohydrate supplementation and the lymphocyte proliferative response to long endurance running. *Int J Sports Med,* 19.

HENSON, D. A., NIEMAN, D. C., PISTILLI, E. E., SCHILLING, B., COLACINO, A., UTTER, A. C., FAGOAGA, O. R., VINCI, D. M. & NEHLSEN-CANNARELLA, S. L. 2004. Influence of carbohydrate and age on lymphocyte function following a marathon. *Int J Sport Nutr Exerc Metab,* 14.

HOOPER, S. L., MACKINNON, L. T., GORDON, R. D. & BACHMANN, A. W. 1993. Hormonal responses of elite swimmers to overtraining. *Med Sci Sports Exerc,* 25.

HOOPER, S. L., MACKINNON, L. T., HOWARD, A., GORDON, R. D. & BACHMANN, A. W. 1995. Markers for monitoring overtraining and recovery. *Med Sci Sports Exerc,* 27.

HOSHIKAWA, M., UCHIDA, S., SUGO, T., KUMAI, Y., HANAI, Y. & KAWAHARA, T. 2007. Changes in sleep quality of athletes under normobaric hypoxia equivalent to 2,000-m altitude: a polysomnographic study. *J Appl Physiol,* 103.

HOX, J. 2010. *Multilevel analysis: Techniques and applications*, Routledge.

IBFELT, T., PETERSEN, E. W., BRUUNSGAARD, H., SANDMAND, M. & PEDERSEN, B. K. 2002. Exercise-induced change in type 1 cytokine-producing CD8(+) T cells is related to a decrease in memory T cells. *J Appl Physiol,* 93.

IRWIN, M. 2002. Effects of sleep and sleep loss on immunity and cytokines. *Brain Behav Immun*, 16.

IRWIN, M., MCCLINTICK, J., COSTLOW, C., FORTNER, M., WHITE, J. & GILLIN, J. C. 1996. Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. *Faseb J,* 10.

KANALEY, J. A., WELTMAN, J. Y., PIEPER, K. S., WELTMAN, A. & HARTMAN, M. L. 2001. Cortisol and growth hormone responses to exercise at different times of day. *J Clin Endocrinol Metab,* 86.

KAPPEL, M., TVEDE, N., GALBO, H., HAAHR, P. M., KJAER, M., LINSTOW, M., KLARLUND, K. & PEDERSEN, B. K. 1991. Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. *J Appl Physiol,* 70.

KILLER, S. C., SVENDSEN, I. S., CARTER, J. M., RANDELL, R. K., POOL, S. P., SANDFORD, G. N., JEUKENDRUP, A. E. & GLEESON, M. 2014. Impact Of Short-term Intensified Training On Sleep Quality And Mood State In Well-trained Cyclists. *Med Sci Sports Exerc,* 46

KILLER, S. C., SVENDSEN, I. S. & GLEESON, M. 2015. The influence of hydration status during prolonged endurance exercise on salivary antimicrobial proteins. *Eur J Appl Physiol*, 115.

KJAER, M., BANGSBO, J., LORTIE, G. & GALBO, H. 1988. Hormonal response to exercise in humans: influence of hypoxia and physical training. *Am J Physiol,* 254.

KLEIN, S. L. 2000. The effects of hormones on sex differences in infection: from genes to behavior. *Neurosci Biobehav Rev,* 24.

KLENTROU, P., CIESLAK, T., MACNEIL, M., VINTINNER, A. & PLYLEY, M. 2002. Effect of moderate exercise on salivary immunoglobulin A and infection risk in humans. *Eur J Appl Physiol,* 87.

KLOKKER, M., KJAER, M., SECHER, N. H., HANEL, B., WORM, L., KAPPEL, M. & PEDERSEN, B. K. 1995. Natural killer cell response to exercise in humans: effect of hypoxia and epidural anesthesia. *J Appl Physiol,* 78.

KOHUT, M. L., BOEHM, G. W. & MOYNIHAN, J. A. 2001. Prolonged exercise suppresses antigen-specific cytokine response to upper respiratory infection. *J Appl Physiol,* 90.

KOPF, M., BAUMANN, H., FREER, G., FREUDENBERG, M., LAMERS, M., KISHIMOTO, T., ZINKERNAGEL, R., BLUETHMANN, H. & KOHLER, G. 1994. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature,* 368.

KRAEMER, W. J. 1988. Endocrine responses to resistance exercise. *Med Sci Sports Exerc,* 20.

KUNZ, H., BISHOP, N. C., SPIELMANN, G., PISTILLO, M., REED, J., OGRAJSEK, T., PARK, Y., MEHTA, S. K., PIERSON, D. L. & SIMPSON, R. J. 2015. Fitness level impacts salivary antimicrobial protein responses to a single bout of cycling exercise. *Eur J Appl Physiol,* 115.

KVERNMO, H., OLSEN, J. O. & OSTERUD, B. 1992. Changes in blood-cell response following strenuous physical exercise. *Eur J Appl Physiol,* 64.

LAING, S. J., BLACKWELL, J., GWYNNE, D., WALTERS, R. & WALSH, N. P. 2005a. Neutrophil degranulation response to 2 hours of exercise in a 30 degrees C environment. *Aviat Space Environ Med,* 76.

LAING, S. J., GWYNNE, D., BLACKWELL, J., WILLIAMS, M., WALTERS, R. & WALSH, N. P. 2005b. Salivary IgA response to prolonged exercise in a hot environment in trained cyclists. *Eur J Appl Physiol,* 93.

LAING, S. J., JACKSON, A. R., WALTERS, R., LLOYD-JONES, E., WHITHAM, M., MAASSEN, N. & WALSH, N. P. 2008. Human blood neutrophil responses to prolonged exercise with and without a thermal clamp. *J Appl Physiol,* 104.

LANCASTER, G. I., HALSON, S. L., KHAN, Q., DRYSDALE, P., WALLACE, F., JEUKENDRUP, A. E., DRAYSON, M. T. & GLEESON, M. 2004. Effects of acute exhaustive exercise and chronic exercise training on type 1 and type 2 T lymphocytes. *Exercise Immunology Review,* 10.

LANCASTER, G. I., KHAN, Q., DRYSDALE, P., WALLACE, F., JEUKENDRUP, A. E., DRAYSON, M. T. & GLEESON, M. 2005a. The physiological regulation of toll-like receptor expression and function in humans. *J Physiol,* 563.

LANCASTER, G. I., KHAN, Q., DRYSDALE, P. T., WALLACE, F., JEUKENDRUP, A. E., DRAYSON, M. T. & GLEESON, M. 2005b. Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. *J Appl Physiol,* 98.

LANDMANN, R. M., BURGISSER, E., WESP, M. & BUHLER, F. R. 1984. Beta-adrenergic receptors are different in subpopulations of human circulating lymphocytes. *J Recept Res,* 4.

LAVOY, E. C., BOSCH, J. A., LOWDER, T. W. & SIMPSON, R. J. 2013. Acute aerobic exercise in humans increases cytokine expression in CD27(-) but not CD27(+) CD8(+) T-cells. *Brain Behav Immun,* 27.

LEEDER, J., GLAISTER, M., PIZZOFERRO, K., DAWSON, J. & PEDLAR, C. 2012. Sleep duration and quality in elite athletes measured using wristwatch actigraphy. *J Sports Sci,* 30.

LEHMANN, M., FOSTER, C., DICKHUTH, H. H. & GASTMANN, U. 1998. Autonomic imbalance hypothesis and overtraining syndrome. *Med Sci Sports Exer,* 30.

LEVINE, B. D. & STRAYGUNDERSEN, J. 1997. "Living high training low": Effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol,* 83.

LEWICKI, R., TCHORZEWSKI, H., MAJEWSKA, E., NOWAK, Z. & BAJ, Z. 1988. Effect of maximal physical exercise on lymphocyte-t subpopulations and on interleukin-1 (il 1) and interleukin-2 (il 2) production invitro. *Int J Sports Med,* 9.

LI, T. L. & GLEESON, M. 2005. The effects of carbohydrate supplementation during repeated bouts of prolonged exercise on saliva flow rate and immunoglobulin A. *J Sports Sci,* 23.

LUCEY, D. R., CLERICI, M. & SHEARER, G. M. 1996. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. *Clin Microbiol Rev,* 9.

LUNDBY, C. & STEENSBERG, A. 2004. Interleukin-6 response to exercise during acute and chronic hypoxia. *Eur J Appl Physiol,* 91.

MACKINNON, L. T. & HOOPER, S. 1994. Mucosal (secretory) immune-system responses to exercise of varying intensity and during overtraining. *Int J Sports Med,* 15.

MACKINNON, L. T., HOOPER, S. L., JONES, S., GORDON, R. D. & BACHMANN, A. W. 1997. Hormonal, immunological, and hematological responses to intensified training in elite swimmers. *Med Sci Sports Exer,* 29.

MALM, C. 2006. Susceptibility to infections in elite athletes: the S-curve. *Scand J Med Sci Sports,* 16.

MARESH, C. M., WHITTLESEY, M. J., ARMSTRONG, L. E., YAMAMOTO, L. M., JUDELSON, D. A., FISH, K. E., CASA, D. J., KAVOURAS, S. A. & CASTRACANE, V. D. 2006. Effect of hydration state on testosterone and cortisol responses to training-intensity exercise in collegiate runners. *Int J Sports Med,* 27.

MATOS, N. F., WINSLEY, R. J. & WILLIAMS, C. A. 2011. Prevalence of Nonfunctional Overreaching/Overtraining in Young English Athletes. *Med Sci Sports Exer,* 43.

MATTHEWS, A., PYNE, D., SAUNDERS, P., FALLON, K. & FRICKER, P. 2010. A self-reported questionnaire for quantifying illness symptoms in elite athletes. *Open Access J Sports Med,* 1.

MAUGHAN, R. J., MERSON, S. J., BROAD, N. P. & SHIRREFFS, S. M. 2004. Fluid and electrolyte intake and loss in elite soccer players during training. *Int J Sport Nutr and Exercise Metabolism,* 14.

MAUGHAN, R. J., SHIRREFFS, S. M., MERSON, S. J. & HORSWILL, C. A. 2005. Fluid and electrolyte balance in elite male football (soccer) players training in a cool environment. *J Sports Sci,* 23.

MAXWELL, S. E. & DELANEY, H. D. 2004. *Designing experiments and analyzing data: A model comparison perspective*, Psychology Press.

MAZZEO, R. S. 2005. Altitude, exercise and immune function. *Exer Immunol Rev,* 11.

MAZZEO, R. S., CHILD, A., BUTTERFIELD, G. E., MAWSON, J. T., ZAMUDIO, S. & MOORE, L. G. 1998. Catecholamine response during 12 days of high-altitude exposure (4, 300 m) in women. *J Appl Physiol,* 84.

MAZZEO, R. S., DONOVAN, D., FLESHNER, M., BUTTERFIELD, G. E., ZAMUDIO, S., WOLFEL, E. E. & MOORE, L. G. 2001. Interleukin-6 response to exercise and high-altitude exposure: influence of alpha-adrenergic blockade. *J Appl Physiol,* 91.

MCFARLIN, B. K. & MITCHELL, J. B. 2003. Exercise in hot and cold environments: differential effects on leukocyte number and NK cell activity. *Aviat Space Environ Med,* 74.

MCKENZIE, D. C. 1999. Markers of excessive exercise. *Can J Appl Physiol,* 24.

MEEUSEN, R., DUCLOS, M., FOSTER, C., FRY, A., GLEESON, M., NIEMAN, D., RAGLIN, J., RIETJENS, G., STEINACKER, J. & URHAUSEN, A. 2013. Prevention, diagnosis and treatment of the overtraining syndrome: Joint consensus statement of the European College of Sport Science (ECSS) and the American College of Sports Medicine (ACSM). *Eur J Sports Sci,* 13.

MEYER, N. L., MANORE, M. M. & HELLE, C. 2011. Nutrition for winter sports. *J Sports Sci,* 29.

MEYER, T., FAUDE, O., URHAUSEN, A., SCHARHAG, J. & KINDERMANN, W. 2004. Different effects of two regeneration regimens on immunological parameters in cyclists. *Med Sci Sports Exer,* 36.

MITCHELL, J. B., DUGAS, J. P., MCFARLIN, B. K. & NELSON, M. J. 2002. Effect of exercise, heat stress, and hydration on immune cell number and function. *Med Sci Sports Exer,* 34.

MITCHELL, J. B., PIZZA, F. X., PAQUET, A., DAVIS, B. J., FORREST, M. B. & BRAUN, W. A. 1998. Influence of carbohydrate status on immune responses before and after endurance exercise. *J Appl Physiol,* 84.

MONTO, A. S. 2002. Epidemiology of viral respiratory infections. *Am J Med,* 112.

MOREIRA, A., DELGADO, L., MOREIRA, P. & HAAHTELA, T. 2009. Does exercise increase the risk of upper respiratory tract infections? *Br Med Bull,* 90.

MORGADO, J. M., RAMA, L., SILVA, I., INACIO, M. D. J., HENRIQUES, A., LARANJEIRA, P., PEDREIRO, S., ROSADO, F., ALVES, F., GLEESON, M., PAIS, M. L., PAIVA, A. & TEIXEIRA, A. M. 2012. Cytokine production by monocytes, neutrophils, and dendritic cells is hampered by long-term intensive training in elite swimmers. *Europ J Appl Physiol,* 112.

MORGAN, W. P., OCONNOR, P. J., SPARLING, P. B. & PATE, R. R. 1987. Psychological characterization of the elite female distance runner. *Int J Sports Med,* 8.

MOSMANN, T. R. & COFFMAN, R. L. 1989. Th1-cell and th2-cell - different patterns of lymphokine secretion lead to different functional-properties. *Annual Rev Immunol,* 7.

MOUGIN, F., SIMON-RIGAUD, M. L., DAVENNE, D., RENAUD, A., GARNIER, A., KANTELIP, J. P. & MAGNIN, P. 1991. Effects of sleep disturbances on subsequent physical performance. *Eur J Appl Physiol Occup Physiol,* 63.

MUJIKA, I., CHATARD, J. C., BUSSO, T., GEYSSANT, A., BARALE, F. & LACOSTE, L. 1995. Effects of training on performance in competitive swimming. *Canadian J Appl Physiol,* 20.

MUNS, G. 1994. Effect of long-distance running on polymorphonuclear neutrophil phagocytic function of the upper airways. *Int J Sports Med,* 15.

MURPHY, E. A., DAVIS, J. M., CARMICHAEL, M. D., GANGEMI, J. D., GHAFFAR, A. & MAYER, E. P. 2008. Exercise stress increases susceptibility to influenza infection. *Brain Behav Immun,* 22.

NEAL, C. M., HUNTER, A. M. & GALLOWAY, S. D. 2011. A 6-month analysis of training-intensity distribution and physiological adaptation in Ironman triathletes. *J Sports Sci,* 29.

NEVILLE, V., GLEESON, M. & FOLLAND, J. P. 2008. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Med Sci Sports Exerc,* 40.

NIEMAN, D. C. 1994. Exercise, upper respiratory tract infection, and the immune system. *Med Sci Sports Exerc,* 26.

NIEMAN, D. C. 2000. Is infection risk linked to exercise workload? *Med Sci Sports Exer,* 32.

NIEMAN, D. C. 2007. Marathon training and immune function. *Sports Med,* 37.

NIEMAN, D. C., DAVIS, J. M., HENSON, D. A., WALBERG-RANKIN, J., SHUTE, M., DUMKE, C. L., UTTER, A. C., VINCI, D. M., CARSON, J. A., BROWN, A., LEE, W. J., MCANULTY, S. R. & MCANULTY, L. S. 2003. Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. *J Appl Physiol,* 94.

NIEMAN, D. C., FAGOAGA, O. R., BUTTERWORTH, D. E., WARREN, B. J., UTTER, A., DAVIS, J. M., HENSON, D. A. & NEHLSEN-CANNARELLA, S. L. 1997a. Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h or running. *Am J Clin Nutr,* 66.

NIEMAN, D. C., HENSON, D. A., DUMKE, C. L., LIND, R. H., SHOOTER, L. R. & GROSS, S. J. 2006a. Relationship between salivary IgA secretion and upper respiratory tract infection following a 160-km race. *J Sports Med Phys Fitness,* 46.

NIEMAN, D. C., HENSON, D. A., GARNER, E. B., BUTTERWORTH, D. E., WARREN, B. J., UTTER, A., DAVIS, J. M., FAGOAGA, O. R. & NEHLSEN-CANNARELLA, S. L. 1997b. Carbohydrate affects natural killer cell redistribution but not activity after running. *Med Sci Sports Exerc,* 29.

NIEMAN, D. C., HENSON, D. A., GOJANOVICH, G., DAVIS, J. M., MURPHY, E. A., MAYER, E. P., PEARCE, S., DUMKE, C. L., UTTER, A. C., MCANULTY, S. R. &

MCANULTY, L. S. 2006b. Influence of carbohydrate on immune function following 2 h cycling. *Res Sports Med,* 14.

NIEMAN, D. C., HENSON, D. A., MCANULTY, S. R., MCANULTY, L., SWICK, N. S., UTTER, A. C., VINCI, D. M., OPIELA, S. J. & MORROW, J. D. 2002. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *J Appl Physiol,* 92.

NIEMAN, D. C., JOHANSSEN, L. M., LEE, J. W. & ARABATZIS, K. 1990. Infectious episodes in runners before and after the Los Angeles Marathon. *J Sports Med Phys Fitness,* 30.

NIEMAN, D. C., MILLER, A. R., HENSON, D. A., WARREN, B. J., GUSEWITCH, G., JOHNSON, R. L., DAVIS, J. M., BUTTERWORTH, D. E. & NEHLSEN-CANNARELLA, S. L. 1993. Effects of high- vs moderate-intensity exercise on natural killer cell activity. *Med Sci Sports Exerc,* 25.

NIEMAN, D. C., NEHLSENCANNARELLA, S. L., DONOHUE, K. M., CHRITTON, D. B. W., HADDOCK, B. L., STOUT, R. W. & LEE, J. W. 1991. The effects of acute moderate exercise on leukocyte and lymphocyte subpopulations. *Med Sci Sports Exer,* 23.

NIEMAN, D. C. & PEDERSEN, B. K. 1999. Exercise and immune function. Recent developments. *Sports Med,* 27.

NIEMAN, D. C., SIMANDLE, S., HENSON, D. A., WARREN, B. J., SUTTLES, J., DAVIS, J. M., BUCKLEY, K. S., AHLE, J. C., BUTTERWORTH, D. E., FAGOAGA, O. R. & ET AL. 1995. Lymphocyte proliferative response to 2.5 hours of running. *Int J Sports Med,* 16.

OLIVER, S. J., COSTA, R. J., LAING, S. J., BILZON, J. L. & WALSH, N. P. 2009. One night of sleep deprivation decreases treadmill endurance performance. *Eur J Appl Physiol,* 107.

OLIVER, S. J., MACDONALD, J. H., HARPER SMITH, A. D., LAWLEY, J. S., GALLAGHER, C. A., DI FELICE, U. & WALSH, N. P. 2013. High altitude impairs in vivo immunity in humans. *High Alt Med Biol,* 14.

OSTROWSKI, K., ROHDE, T., ASP, S., SCHJERLING, P. & PEDERSEN, B. K. 1999. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol,* 515.

OSTROWSKI, K., ROHDE, T., ZACHO, M., ASP, S. & PEDERSEN, B. K. 1998. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol,* 508.

PARRYBILLINGS, M., BUDGETT, R., KOUTEDAKIS, Y., BLOMSTRAND, E., BROOKS, S., WILLIAMS, C., CALDER, P. C., PILLING, S., BAIGRIE, R. & NEWSHOLME, E. A. 1992. Plasma amino-acid-concentrations in the overtraining syndrome - possible effects on the immune-system. *Med Sci Sports Exer,* 24.

PEAKE, J., PEIFFER, J. J., ABBISS, C. R., NOSAKA, K., LAURSEN, P. B. & SUZUKI, K. 2008. Carbohydrate gel ingestion and immunoendocrine responses to cycling in temperate and hot conditions. *Int J Sport Nutr Exerc Metab,* 18.

PEAKE, J. M. 2002. Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. *Exerc Immunol Rev,* 8.

PEDERSEN, B. K., HELGE, J. W., RICHTER, E. A., ROHDE, T. & KIENS, B. 2000. Training and natural immunity: effects of diets rich in fat or carbohydrate. *Eur J Appl Physiol,* 82.

PEDERSEN, B. K. & HOFFMAN-GOETZ, L. 2000. Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev,* 80.

PEDERSEN, B. K. & TOFT, A. D. 2000. Effects of exercise on lymphocytes and cytokines. *Br J Sports Med,* 34.

PETERS, E. M., GOETZSCHE, J. M., GROBBELAAR, B. & NOAKES, T. D. 1993. Vitamin C supplementation reduces the incidence of postrace symptoms of upper-respiratory-tract infection in ultramarathon runners. *Am J Clin Nutr,* 57.

PETROVSKY, N., MCNAIR, P. & HARRISON, L. C. 1998. Diurnal rhythms of pro-inflammatory cytokines: Regulation by plasma cortisol and therapeutic implications. *Cytokine,* 10.

PILARDEAU, P., RICHALET, J. P., BOUISSOU, P., VAYSSE, J., LARMIGNAT, P. & BOOM, A. 1990. Saliva flow and composition in humans exposed to acute altitude hypoxia. *Eur J Appl Physiol Occup Physiol,* 59.

PITKARANTA, A., NOKSO-KOIVISTO, J., JANTTI, V., TAKALA, A., KILPI, T. & HOVI, T. 1999. Lowered yields of virus-induced interferon production in leukocyte cultures and risk of recurrent respiratory infections in children. *J Clin Virol,* 14.

PLYLEY, M. J., SHEPHARD, R. J., DAVIS, G. M. & GOODE, R. C. 1987. Sleep deprivation and cardiorespiratory function. Influence of intermittent submaximal exercise. *Eur J Appl Physiol Occup Physiol,* 56.

PYNE, D. B. & GLEESON, M. 1998. Effects of intensive exercise training on immunity in athletes. *Int J Sports Med,* 19..

PYNE, D. B., MCDONALD, W. A., GLEESON, M., FLANAGAN, A., CLANCY, R. L. & FRICKER, P. A. 2001. Mucosal immunity, respiratory illness, and competitive performance in elite swimmers. *Med Sci Sports Exerc,* 33.

PYNE, D. V., MCDONALD, W. A., MORTON, D. S., SWIGGET, J. P., FOSTER, M., SONNENFELD, G. & SMITH, J. A. 2000. Inhibition of interferon, cytokine, and lymphocyte proliferative responses in elite swimmers with altitude exposure. *J Interferon Cytokine Res,* 20.

REDDY, M., EIRIKIS, E., DAVIS, C., DAVIS, H. M. & PRABHAKAR, U. 2004. Comparative analysis of lymphocyte activation marker expression and cytokine secretion profile in stimulated human peripheral blood mononuclear cell cultures: an in vitro model to monitor cellular immune function. *J Immunol Methods,* 293.

REESER, J. C., WILLICK, S. & ELSTAD, M. 2003. Medical services provided at the Olympic Village polyclinic during the 2002 Salt Lake City Winter Games. *WMJ,* 102.

REITE, M., JACKSON, D., CAHOON, R. L. & WEIL, J. V. 1975. Sleep physiology at high altitude. *Electroencephalogr Clin Neurophysiol,* 38.

RHIND, S. G., GANNON, G. A., SHEK, P. N., BRENNER, I. K., SEVERS, Y., ZAMECNIK, J., BUGUET, A., NATALE, V. M., SHEPHARD, R. J. & RADOMSKI, M. W. 1999. Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. *J Appl Physiol,* 87.

RHIND, S. G., GANNON, G. A., SHEPHARD, R. J., BUGUET, A., SHEK, P. N. & RADOMSKI, M. W. 2004. Cytokine induction during exertional hyperthermia is abolished by core temperature clamping: neuroendocrine regulatory mechanisms. *Int J Hyperthermia,* 20.

RICARDO, J. S., CARTNER, L., OLIVER, S. J., LAING, S. J., WALTERS, R., BILZON, J. L. & 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.

RICKEN, K. H., RIEDER, T., HAUCK, G. & KINDERMANN, W. 1990. CHANGES IN LYMPHOCYTE SUBPOPULATIONS AFTER PROLONGED EXERCISE. *Int J Sports Med,* 11.

ROACH, G. D., SCHMIDT, W. F., AUGHEY, R. J., BOURDON, P. C., SORIA, R., CLAROS, J. C., GARVICAN-LEWIS, L. A., BUCHHEIT, M., SIMPSON, B. M.,

HAMMOND, K., KLEY, M., WACHSMUTH, N., GORE, C. J. & SARGENT, C. 2013. The sleep of elite athletes at sea level and high altitude: a comparison of sea-level natives and high-altitude natives (ISA3600). *Br J Sports Med,* 47.

ROBSON, P. J., BLANNIN, A. K., WALSH, N. P., CASTELL, L. M. & GLEESON, M. 1999. Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. *Int J Sports Med,* 20.

ROBSON-ANSLEY, P. J., BLANNIN, A. & GLEESON, M. 2007. Elevated plasma interleukin-6 levels in trained male triathletes following an acute period of intense interval training. *European J Appl Physiol,* 99.

RODRIGUEZ, N. R., DI MARCO, N. M. & LANGLEY, S. 2009. American College of Sports Medicine position stand. Nutrition and athletic performance. *Med Sci Sports Exerc,* 41.

ROHDE, T., MACLEAN, D. A. & PEDERSEN, B. K. 1998. Effect of glutamine supplementation on changes in the immune system induced by repeated exercise. *Med Sci Sports Exerc,* 30.

ROWBOTTOM, D. G., KEAST, D., GOODMAN, C. & MORTON, A. R. 1995. THE hematological, biochemical and immunological profile of athletes suffering from the overtraining syndrome. *Eur J Appl Physiol,* 70.

SALTIN, B. & KARLSSON, J. 1971. Muscle glycogen utilization during work of different intensities. *Muscle metabolism during exercise.* Springer.

SAMUEL, C. E. 2001. Antiviral actions of interferons. *Clin Microbiol Rev,* 14.

SAMUELS, C. 2009. Sleep, recovery, and performance: the new frontier in high-performance athletics. *Phys Med Rehabil Clin N Am,* 26.

SARGENT, C., SCHMIDT, W. F., AUGHEY, R. J., BOURDON, P. C., SORIA, R., CLAROS, J. C., GARVICAN-LEWIS, L. A., BUCHHEIT, M., SIMPSON, B. M., HAMMOND, K., KLEY, M., WACHSMUTH, N., GORE, C. J. & ROACH, G. D. 2013. The impact of altitude on the sleep of young elite soccer players (ISA3600). *Br J Sports Med,* 47.

SASSIN, J. F., PARKER, D. C., JOHNSON, L. C., ROSSMAN, L. G., MACE, J. W. & GOTLIN, R. W. 1969. Effects of slow wave sleep deprivation on human growth hormone release in sleep: preliminary study. *Life Sci,* 8.

SAUNDERS, P. U., TELFORD, R. D., PYNE, D. D., GORE, C. J. & HAHN, A. G. 2009. Improved race performance in elite middle-distance runners after cumulative altitude exposure. *Int J Sports Physiol Perform,* 4.

SEILER, K. S. & KJERLAND, G. O. 2006. Quantifying training intensity distribution in elite endurance athletes: is there evidence for an "optimal" distribution? *Scand J Med Sci Sports,* 16.

SHEK, P. N., SABISTON, B. H., BUGUET, A. & RADOMSKI, M. W. 1995. Strenuous exercise and immunological changes - a multiple-time-point analysis of leukocyte subsets, cd4/cd8 ratio, immunoglobulin production and nk cell response. *Int J Sports Med,* 16.

SHEPHARD, R. J. 2003. Adhesion molecules, catecholamines and leucocyte redistribution during and following exercise. *Sports Med,* 33.

SIMPSON, R. J., MCFARLIN, B. K., MCSPORRAN, C., SPIELMANN, G., O HARTAIGH, B. & GUY, K. 2009. Toll-like receptor expression on classic and pro-inflammatory blood monocytes after acute exercise in humans. *Brain Behav Immun,* 23.

SLIVKA, D. R., HAILES, W. S., CUDDY, J. S. & RUBY, B. C. 2010. Effects of 21 days of intensified training on markers of overtraining. *J Strength Cond Res,* 24.

SMITH, J. A. 1995. Exercise, training and red blood cell turnover. *Sports medicine,* 19**,** 9-31.

SMITH, L. L. 2000. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exer,* 32.

SMITS, H. H., GRUNBERG, K., DERIJK, R. H., STERK, P. J. & HIEMSTRA, P. S. 1998. Cytokine release and its modulation by dexamethasone in whole blood following exercise. *Clin Exp Immunol,* 111.

SNYDER, A. C. 1998. Overtraining and glycogen depletion hypothesis. *Med Sci Sports Exerc,* 30.

SNYDER, A. C., KUIPERS, H., CHENG, B., SERVAIS, R. & FRANSEN, E. 1995. Overtraining following intensified training with normal muscle glycogen. *Med Sci Sports Exer,* 27.

SOLIGARD, T., STEFFEN, K., PALMER-GREEN, D., AUBRY, M., GRANT, M. E., MEEUWISSE, W., MOUNTJOY, M., BUDGETT, R. & ENGEBRETSEN, L. 2015. Sports injuries and illnesses in the Sochi 2014 Olympic Winter Games. *Br J Sports Med,* 49.

SORRELLS, S. F. & SAPOLSKY, R. M. 2007. An inflammatory review of glucocorticoid actions in the CNS. *Brain Behav Immun,* 21.

SPENCE, L., BROWN, W. J., PYNE, D. B., NISSEN, M. D., SLOOTS, T. P., MCCORMACK, J. G., LOCKE, A. S. & FRICKER, P. A. 2007. Incidence, etiology,

and symptomatology of upper respiratory illness in elite athletes. *Med Sci Sports Exerc,* 39.

STARKIE, R. L., ANGUS, D. J., ROLLAND, J., HARGREAVES, M. & FEBBRAIO, M. A. 2000. Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. *J Physiol,* 528.

STARKIE, R. L., HARGREAVES, M., ROLLAND, J. & FEBBRAIO, M. A. 2005. Heat stress, cytokines, and the immune response to exercise. *Brain Behav Immun,* 19.

STARKIE, R. L., ROLLAND, J. & FEBBRAIO, M. A. 2001. Effect of adrenergic blockade on lymphocyte cytokine production at rest and during exercise. *Amer J Physiol-Cell Physiol,* 281.

STEENSBERG, A., TOFT, A. D., BRUUNSGAARD, H., SANDMAND, M., HALKJAER-KRISTENSEN, J. & PEDERSEN, B. K. 2001. Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *J Appl Physiol,* 91.

STEENSBERG, A., VAN HALL, G., OSADA, T., SACCHETTI, M., SALTIN, B. & PEDERSEN, B. K. 2000. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol,* 529**.**

STEERENBERG, P. A., VANASPEREN, I. A., AMERONGEN, A. V., BIEWENGA, J., MOL, D. & MEDEMA, G. 1997. Salivary levels of immunoglobulin A in triathletes. *Europ J Oral Sci,* 105.

STRAY-GUNDERSEN, J., CHAPMAN, R. F. & LEVINE, B. D. 2001. "Living high-training low" altitude training improves sea level performance in male and female elite runners. *J Appl Physiol,* 91.

STREET, N. E. & MOSMANN, T. R. 1991. Functional diversity of lymphocyte-t due to secretion of different cytokine patterns. *Faseb J,* 5.

SVENDSEN, I. S., GLEESON, M., HAUGEN, T. A. & TONNESSEN, E. 2015. Effect of an intense period of competition on race performance and self-reported illness in elite cross-country skiers. *Scand J Med Sci Sports*, 25.

SYLTA, O., TONNESSEN, E. & SEILER, S. 2014. Do elite endurance athletes report their training accurately? *Int J Sports Physiol Perform,* 9.

TAKAHASHI, Y., KIPNIS, D. M. & DAUGHADAY, W. H. 1968. Growth hormone secretion during sleep. *J Clin Invest,* 47.

TANG, Y. W. & GRAHAM, B. S. 1994. Anti-il-4 treatment at immunization modulates cytokine expression, reduces illness, and increases cytotoxic t-lymphocyte activity in mice challenged with respiratory syncytial virus. *J Clin Invest,* 94.

TENOVUO, J. 1989. Nonimmunoglobulin defense factors in human saliva. *Human saliva: Clin Chem Microbiol,* 2.

THARP, G. D. & BARNES, M. W. 1990. Reduction of saliva immunoglobulin levels by swim training. *Eur J Appl Physiol Occup Physiol,* 60.

TIOLLIER, E., SCHMITT, L., BURNAT, P., FOUILLOT, J. P., ROBACH, P., FILAIRE, E., GUEZENNEC, C. & RICHALET, J. P. 2005. Living high-training low altitude training: effects on mucosal immunity. *Eur J Appl Physiol,* 94.

TOMASI, T. B., TRUDEAU, F. B., CZERWINSKI, D. & ERREDGE, S. 1982a. Immune parameters in athletes before and after strenuous exercise. *J Clin Immunol,* 2**,** 173-8.

TONNESSEN, E., SVENDSEN, I. S., RONNESTAD, B. R., HISDAL, J., HAUGEN, T. A. & SEILER, S. 2015. The annual training periodization of 8 world champions in orienteering. *Int J Sports Physiol Perform,* 10.

TONNESSEN, E., SYLTA, O., HAUGEN, T. A., HEM, E., SVENDSEN, I. S. & SEILER, S. 2014. The road to gold: training and peaking characteristics in the year prior to a gold medal endurance performance. *PLoS One,* 9.

TRINCHIERI, G. 1997. Cytokines acting on or secreted by macrophages during intracellular infection (IL-10, IL-12, IFN-gamma). *Current Opin Immunol,* 9.

TSINTZAS, O. K., WILLIAMS, C., WILSON, W. & BURRIN, J. 1996. Influence of carbohydrate supplementation early in exercise on endurance running capacity. *Med Sci Sports Exer,* 28.

URHAUSEN, A., GABRIEL, H. & KINDERMANN, W. 1995. Blood hormones as markers of training stress and overtraining. *Sports Med,* 20.

URHAUSEN, A., GABRIEL, H. H. W. & KINDERMANN, W. 1998. Impaired pituitary hormonal response to exhaustive exercise in overtrained endurance athletes. *Med Sci Sports Exer,* 30.

VAN DER POLL, T., COYLE, S. M., BARBOSA, K., BRAXTON, C. C. & LOWRY, S. F. 1996. Epinephrine inhibits tumor necrosis factor-alpha and potentiates interleukin 10 production during human endotoxemia. *J Clin Invest,* 97.

VAN DONGEN, H. P., MAISLIN, G., MULLINGTON, J. M. & DINGES, D. F. 2003. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral

functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep,* 26.

VAN ERP-BAART, A. M., SARIS, W. H., BINKHORST, R. A., VOS, J. A. & ELVERS, J. W. 1989. Nationwide survey on nutritional habits in elite athletes. Part I. Energy, carbohydrate, protein, and fat intake. *Int J Sports Med,* 10.

VAN TITS, L. J., MICHEL, M. C., GROSSE-WILDE, H., HAPPEL, M., EIGLER, F. W., SOLIMAN, A. & BRODDE, O. E. 1990. Catecholamines increase lymphocyte beta 2-adrenergic receptors via a beta 2-adrenergic, spleen-dependent process. *Am J Physiol,* 258.

VENKATRAMAN, J. T., FENG, X. & PENDERGAST, D. 2001. Effects of dietary fat and endurance exercise on plasma cortisol, prostaglandin E2, interferon-gamma and lipid peroxides in runners. *J Am Coll Nutr,* 20.

VENKATRAMAN, J. T. & PENDERGAST, D. R. 2002. Effect of dietary intake on immune function in athletes. *Sports Med,* 32.

VERDE, T., THOMAS, S. & SHEPHARD, R. J. 1992. Potential markers of heavy training in highly trained distance runners. *Br J Sports Med,* 26.

VERTHELYI, D. 2001. Sex hormones as immunomodulators in health and disease. *Int Immunopharmacol,* 1.

VOLPE, S. L., POULE, K. A. & BLAND, E. G. 2009. Estimation of prepractice hydration status of National Collegiate Athletic Association Division I athletes. *J Athl Train,* 44.

WALSH, N. P., BISHOP, N. C., BLACKWELL, J., WIERZBICKI, S. G. & MONTAGUE, J. C. 2002. Salivary IgA response to prolonged exercise in a cold environment in trained cyclists. *Med Sci Sports Exerc,* 34.

WALSH, N. P., BLANNIN, A. K., BISHOP, N. C., ROBSON, P. J. & GLEESON, M. 2000. Effect of oral glutamine supplementation on human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise. *Int J Sport Nutr Exerc Metab,* 10.

WALSH, N. P., BLANNIN, A. K., ROBSON, P. J. & GLEESON, M. 1998. Glutamine, exercise and immune function. Links and possible mechanisms. *Sports Med,* 26.

WALSH, N. P., GLEESON, M., PYNE, D. B., NIEMAN, D. C., DHABHAR, F. S., SHEPHARD, R. J., OLIVER, S. J., BERMON, S. & KAJENIENE, A. 2011a. Position statement. Part two: Maintaining immune health. *Exerc Immunol Rev,* 17.

WALSH, N. P., GLEESON, M., SHEPHARD, R. J., WOODS, J. A., BISHOP, N. C., FLESHNER, M., GREEN, C., PEDERSEN, B. K., HOFFMAN-GOETZ, L.,

ROGERS, C. J., NORTHOFF, H., ABBASI, A. & SIMON, P. 2011b. Position statement. Part one: Immune function and exercise. *Exerc Immunol Rev,* 17.

WALSH, N. P. & WHITHAM, M. 2006. Exercising in environmental extremes : a greater threat to immune function? *Sports Med,* 36.

WATERHOUSE, J., REILLY, T., ATKINSON, G. & EDWARDS, B. 2007. Jet lag: trends and coping strategies. *Lancet,* 369.

WEINSTOCK, C., KONIG, D., HARNISCHMACHER, R., KEUL, J., BERG, A. & NORTHOFF, H. 1997. Effect of exhaustive exercise stress on the cytokine response. *Med Sci Sports Exer,* 29.

WEST, N. P., PYNE, D. B., KYD, J. M., RENSHAW, G. M., FRICKER, P. A. & CRIPPS, A. W. 2010. The effect of exercise on innate mucosal immunity. *Br J Sports Med,* 44.

WHELAN, E. A., LAWSON, C. C., GRAJEWSKI, B., PETERSEN, M. R., PINKERTON, L. E., WARD, E. M. & SCHNORR, T. M. 2003. Prevalence of respiratory symptoms among female flight attendants and teachers. *Occup Environ Med,* 60.

WILDER-SMITH, A., MUSTAFA, F. B., PENG, C. M., EARNEST, A., KOH, D., LIN, G., HOSSAIN, I. & MACARY, P. A. 2012. Transient immune impairment after a simulated long-haul flight. *Aviat Space Environ Med,* 83.

WITARD, O. C., TURNER, J. E., JACKMAN, S. R., TIPTON, K. D., JEUKENDRUP, A. E., KIES, A. K. & BOSCH, J. A. 2012. High-Intensity Training Reduces CD8(+) T-cell Redistribution in Response to Exercise. *Med Sci Sports Exer,* 44.

XING, Z., GAULDIE, J., COX, G., BAUMANN, H., JORDANA, M., LEI, X. F. & ACHONG, M. K. 1998. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest,* 101.

ZALDIVAR, F., WANG-RODRIGUEZ, J., NEMET, D., SCHWINDT, C., GALASSETTI, P., MILLS, P. J., WILSON, L. D. & COOPER, D. M. 2006. Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. *J Appl Physiol,* 100.

ZAPICO, A. G., CALDERON, F. J., BENITO, P. J., GONZALEZ, C. B., PARISI, A., PIGOZZI, F. & DI SALVO, V. 2007. Evolution of physiological and haematological parameters with training load in elite male road cyclists: a longitudinal study. *J Sports Med Phys Fitness,* 47.

ZITTER, J. N., MAZONSON, P. D., MILLER, D. P., HULLEY, S. B. & BALMES, J. R. 2002. Aircraft cabin air recirculation and symptoms of the common cold. *JAMA,* 288.