EFFECT OF ACUTE AND CHRONIC EXERCISE ON IMMUNOENDOCRINE RESPONSES IN PROFESSIONAL RUGBY UNION

by

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This research programme was carried out in collaboration with Wales Rugby Union and University of Wales Institute Cardiff

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Statement of Originality

This work has not been submitted for any degree or doctoral candidate at any university. To the best of my knowledge and belief, the dissertation contains no material previously written or published by another person except where due reference is made in the thesis itself.

Signature: 

Brian Cunniffe
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ABSTRACT

Prolonged and intense exercise is known to modulate and suppress certain aspects of the immunoendocrine system. Such effects are thought to be largely mediated by the release of stress hormones and regulatory cytokines which originate from a variety of stress related paradigms in sport. These include acute physical exertion, chronic and repetitive exercise as well as other psychological and psychosocial aspects of training and competing in an elite environment. It may be of particular interest to study the effects of regular competition and training on immunoendocrine markers in rugby union players. At the professional level, rugby is an intense and physically demanding game where a significant amount of tissue trauma occurs as a result of the many game collisions. The aims of the studies outlined in this thesis were to determine the effects of acute, repeated and chronic exercise exposure on immunoendocrine markers and illness incidence in professional rugby union. Additional case studies were also undertaken to supplement main study findings.

The first part of the thesis documented the effects of acute and repeated exercise on immunoendocrine markers in a cohort of international rugby union players. Data in study 1 showed that large disturbances in immunoendocrine and hormone levels occur in players (n = 10) following game play. The magnitude of this response appeared dependent on game physicality (number of rucks/mauls, tackles) and the number of collisions players received during match play. Findings also showed suppression in host immunity, and in particular, innate immune function (neutrophil degranulation) which was not resolved 38 h (-29%) into the recovery period. In study 2, bloods were taken from players (n = 8) across a 21-day international rugby series. Data revealed that players entered the international camp with residual muscle damage (creatine kinase; CK) and inflammation (hs-CRP) following previous club involvement in European cup rugby. Further increases in stress related markers (cortisol, IL-6, CK, CRP) were not evident throughout the players time at the international training base. Conversely, a progressive increase in anabolic-catabolic balance (T/C ratio) was observed across time. In comparison to values on camp-entry (day 1), increases in T/C ratio were evident on day-5 (9.8%), day-7 (13%), day-19 (35%) and day-21 (45%) (P < 0.05). This data is suggestive of improved physiological recovery and was commensurate with team fitness goals (reduced volume + maintenance of training intensity) for that time. Findings suggest that monitoring of player club activities and training load preceding international duty is pertinent if they are required to represent their country inside 7 days.

The second part of the thesis evaluated the stress induced effects of chronic rugby exposure in professional club players. Questionnaire data analysed from study 3 showed that players (n = 65) perceived current season length as being ‘too long’ (55%), ‘poorly structured’ (56%) and that game demands are increasing with time (52%). Furthermore, the majority of players (80%) felt that time ‘away’ from rugby was not sufficiently long enough and were in favour of a mid-season break (2 wks in duration).

Investigation into the effects of chronic exercise on illness incidence, immunological and psychological state was carried out in a squad of club players (n = 30) over a competitive season (n = 48 wks) in studies 4-6. Findings revealed that specific periods in a rugby season resulted in disturbances to hormonal and immune status. These periods occurred following breaks in club game fixtures [November international and Six-nations period: February/early March], times of increased training intensity and increased ratio of conditioning/rugby activity. Peaks in number of upper respiratory illnesses (URIs) and disturbances in psychometric variables also occurred during these time periods. In 23% of all URIs, players reported that the presence of the illness either reduced activity (14.4%) or felt the need to go to bed (8.6%). Positional differences in variables were also observed. A higher incidence of URIs (3.4 vs 4.3) and lower concentrations of resting immune markers [salivary lysozyme: s-Lys (-31%); immunoglobulin A: s-IgA (-27%)] was observed in ‘backs’ (vs forwards) over the season. Higher mid-season cortisol levels was also observed in backs (P < 0.05) while conversely, greater concentrations of plasma CK and CRP were found in forwards throughout the season. These findings indicate positional specific differences in response to exercise load and point to the role of group specific recovery at certain times during the season. Data from study 6 showed that the number of training related complaints decreased across the season, findings
which closely resembled corresponding decreases in plasma CRP values. This data is suggestive of a ‘repeated-bout’ effect or ‘contact adaptation’ in rugby union.

Finally, comparison of methods used in the recording of illness data revealed that players were more honest when disclosing the existence of banal infections to a web-based training diary and under-reported infections to medical staff.

**Key words:** Rugby, stress hormones, immunoendocrine, inflammation, overreaching, illness, saliva, immunoglobulin A, muscle damage, recovery, immune suppression.
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PUBLICATIONS

The following is a list of publications and communications arising from the material presented in this thesis thus far.


COMMUNICATIONS


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ABBREVIATIONS

ACTH: Adrenocorticotropic hormone
ADP: Adenosine monophosphate
ANOVA: Analysis of variance
APR: Acute phase response
ATP: Adenosine triphosphate
BSA: Bovine serum albumin.
BV: Blood volume
C: Cortisol
CBG: Cortisol binding protein
CD: Cluster of differentiation
CI: Confidence interval
CK: Creatine kinase
CNS: Central nervous system
CO₂: Carbon dioxide
CRP: C reactive protein
CV: Coefficient of variation
Da: Dalton (molecular weight unit)
EDTA: Ethylenediaminetetraacetic acid
ELISA: Enzyme linked immunosorbent assay
FITC: Fluorescein isothiocyanate
Gln: Glutamine
Glutamate
Hb: Haemoglobin
Hct: Haematocrit
HPA: Hypothalamic-pituitary-adrenal axis
HPG: Hypothalamic-pituitary-gonadal axis
HTML: Hypertext Mark-up Language
Ig: Immunoglobulin
IL: Interleukin
IRB: International rugby board
ITP: Intensified training period
LPS: Lipopolysaccharide
mAbs: Monoclonal antibodies
Mb: Myoglobin

MFI: Mean fluorescence Intensity
MHC: Major histocompatibility complex
N: Nitrogen
NK: natural killer
O₂: Oxygen
PBS: Phosphate buffer saline
PE: Phycoerythrin
PE-Cy: Phycoerythrin Cyanin
PHP: personalised home page.
pIgA: Polymeric immunoglobulin A
PMN: Polymorphonuclear leukocyte
PRA: Professional rugby players association
PV: Plasma volume
RER: Respiratory exchange ratio
RFU: Rugby Football Union
RPE: Rating of perceived exertion
RH: Relative humidity
RT: Room temperature
SD: Standard deviation
SEM: Standard error of mean
s-IgA: salivary immunoglobulin A
s-Lys: salivary lysozyme
T: Testosterone
Th: Helper T cell
TMB: Tetramethylbezidine
URI: Upper respiratory illness
URTI: Upper respiratory tract infection
VO₂ max: Maximum oxygen consumption
VO₂peak: Peak oxygen consumption
WBC: White blood cells
WRU: Wales Rugby Union
Chapter 1

General introduction

1.1 INTRODUCTION

Rugby union is considered one of the most intense and physically demanding field games in the world (Mashiko et al., 2004). Since its emergence as a professional sport (1995), the game appears to have become a faster, ruck dominated game that contains more phases of play (Eaves and Hughes, 2003). Players have become bigger and faster and are involved in more physical contact and collisions during game play (Duthie et al, 2003; Quarrie and Hopkins, 2007). Changes in game demands have been mirrored by issues such as increasing injury incidence (Garraway et al., 2000; Brooks and Kemp, 2008), player burnout (Cresswell and Eklund, 2005) and player unrest (Ipsos-MORI, 2006). Such issues ultimately present great challenges to game administrators and affiliated fitness staff. Implementation of recovery programmes, periodisation of training and management of daily stresses arising from heavy training routines are arguably more important for the contemporary player than ever before. It is feasible that with appropriate management of these stressors, the negative outcomes of the training process may be ameliorated. This may play an important process in overall player welfare and longevity of the game.

Anecdotal evidence would suggest that elite rugby union players experience an increasing demand on both quality and quantity of training in order to improve performance during competition. Despite this, there exists limited research on game demands and associated player welfare. Few detailed studies have examined the role of daily exercise stress at the elite level. The ability to manage large training and competition loads requires a fine balance between exercise stress and concomitant recovery. Monitoring this balance and detecting signs of maladaptation to the training process may represent an important part in overall player management. Routine training programmes of elite rugby players may consist of several bouts of intensive exercise in a given day, predominantly in the form of rugby training, weights and conditioning. It is feasible that these demands could place considerable stress upon players. This is important since exercise stress is capable of modifying immune function and immune suppression is implicated in the development of illness (Nieman et al., 1990). Furthermore,
heavy training has been associated with decreased mood state, decreased performance, endocrine disturbances, overreaching and potentially overtraining (Kreider et al., 1998; Kuipers and Keizer, 1988). It is feasible that daily training demands coupled with acute stress arising from participation in the game itself, may place this athlete group at increased predisposition to illness and overreaching. Tissue damage and associated inflammation from body contact and muscle trauma (Takarada, 2006) may act to augment the observed immune response. This is significant, since the immune and endocrine systems are modified both by chronic stress and by inflammation (Northoff et al., 1995; Smith, 2000). In particular, innate immune responses play an important role in prevention of illness and infection (West et al., 2006). Because innate immunity is a key regulator of inflammatory processes and muscle damage is a key architect in the initiation of the inflammatory response, the effect of regular participation in a collision sport like rugby union offers an intriguing model in exercise immunology and endocrinology.

1.2 REASONS FOR INVESTIGATION: CURRENT STATE OF PLAY

Limited research

Although research in other field sports such as soccer has received considerable attention in the literature, there is currently a limited amount of scientific investigation within rugby union. This dearth of information is more evident at the elite level, which is surprising considering the intense nature of the sport and changes in the game since the onset of professionalism in the early 1990’s. While previous studies have attempted to investigate game demands indirectly through injury analysis (Brooks et al., 2005), time-motion analysis (Deutsch et al., 2007) and psychological burnout (Cresswell and Eklund, 2006), few studies, have explored the role of game stress on non-injury related matters such as immune function, hormonal status, illness potential and well-being. Traditionally research has attempted to document rugby related stress through injury incidence arising from games (Fuller et al., 2007) or prospective injury recording (Garraway et al., 2000; Alsop et al., 2000; 2005; Takemura et al., 2007) with few studies providing specific details of preceding training loads or phases (Brooks et al., 2008). Consequently, it is not known with certainty what facets of the game (e.g. contact or non-contact training phases) or which times of the season (e.g. pre-season, mid-season) have the most profound effect on observed stress responses. With respect to immune function, this is important since increased training loads over and above that of individuals coping response have been associated with increased susceptibility to colds and infections (Nieman and Pedersen, 1999; Fry et al., 1991).
Outside of rugby, few studies have attempted to investigate biochemical and hormonal responses to athletic stress within real life competitive environments at the elite level. This, perhaps is not surprising given the administrative issues and previously documented challenges in collecting quality data in team sports (Mujika, 2007). Notwithstanding the above, research in rugby union presents an intriguing and challenging area for many of the following reasons.

**Contemporary demands**

Currently, the professional rugby union season (northern hemisphere) runs between the months of August and May in a non-world cup year, with teams normally beginning pre-season training during the month of July. In addition to this, players who represent their country at international level are often required to play during the summer months; a time period which would be normally set aside as recovery from the previous season. In Wales, a professional player may be required to participate in up to 36 games across a 49 week season. This game number is dependent on individual player form, injury status and overall team performance. These games are usually played between the months of September in one year to May of the following year. During this time, breaks in seasonal fixture are normally used by coaches to focus on certain aspects of fitness outside of training loads normally undertaken between games within the season.

“One of the reasons I retired from international rugby was that I had played a total of 71 matches in the last two years—that’s roughly one every 10 days”


While it is known that high energy collisions between players place a large contact load on the body from an injury perspective (Quarrie and Hopkins, 2007), it is not known what effect this high contact load has on immune function at an acute level. Furthermore, it is not known what effect, if any, repeated daily stress in the form of rugby union has on chronic immuno-endocrine function and well-being. It is feasible that if temporary alterations in immuno-endocrine function occur with acute exercise load i.e. game/heavy training, repeated exercise without sufficient recovery may exacerbate alterations over a competitive season. Documentation of contemporary daily training loads and stress levels in elite rugby union is therefore worthy of investigation. In addition to this, investigation is needed in relation to the effects of high level competition on player well-being (mood state, illness potential).
“I think the issue of injury and, dare I say it, burnout, is a growing concern for the entire global game because of the sheer collision nature of the sport now”

Damian Hopley, Chief executive Professional Rugby players Association (RFU), BBC sport 26th Sept 2006.

Anecdotal evidence and existing findings
The issue of excessive game demands has been subject of recent attention and highlighted in an RFU commissioned study on player welfare (Ipsos MORI, 2006). In this study, it was found that the majority of players thought that they played ‘too much rugby’, the season is ‘too long’ and the severity of injuries is increasing. Research was based on initial findings showing that players within the professional rugby structure in England exhibited two to three times the level of exhaustion compared to their New Zealand counterparts (Cresswell, 2005). This was substantiated in a more recent study leading into the autumn internationals (2006) where half of the England squad showed signs of mental and physical exhaustion (Ipsos MORI, 2006). Although worthy of consideration, care should be taken when interpreting these findings since conclusions were made from questionnaire data and no physiological indicators of overtraining, burnout or performance decrement were measured. The issue of player burnout was a hot media topic at the time and so conclusions should be made with this in mind.

Failed implementation of prior recommendations
On commencement of this investigation, IRB recommendations (IRB player welfare conference, Dublin, 2002) for player protection consisted of the following:

- That players have 4 weeks continuous holiday / leave / active rest per year.
- That the pre-season (non-game) period which should follow holiday/leave be 8-10 weeks.
- That normally there should be no more than 1 game per week.
- That for the elite professional player, monitoring be incorporated so that the welfare of the player be proactively managed.
- Appropriate active rest periods to be considered 1-2 times per year to best maintain the health of the athlete.

It was recognised that certain factors would impact on the fulfilment of these aspirations such as a lack of definitive research, the circumstances of individual unions, current commercial arrangements and competition structures. Most, if not all, of the above recommendations were not adhered to in domestic professional rugby on commencement of this investigation. It is possible that difficulties in implementation may arise since domestic rugby within the British
Isles does not seem to have a clear seasonal structure. Furthermore, at present there is no clear consensus on the amount of games and recovery time necessary for elite players from the sports governing body, International Rugby Board (IRB).

“Something has to give – unfortunately it always tends to be the players bodies”

Damian Hopley, Chief executive Professional Rugby players Association (RFU), BBC sport 26th Sept 2006

**Domestic Issues**

Enforcement of the above recommendations has been left to the individual home unions, and unless the players are centrally contracted to the national union, left up to the individual clubs/regions. Within Wales, players are not centrally contracted and so regulation of game number, training periodisation and time away from rugby is at the discretion of its regions. This is in cooperation with their umbrella organisation, the Wales Rugby Union (WRU). On commencing this research, wide coverage of player welfare issues was evident within England rugby. Intense debate between the player’s representatives union (PRA) and RFU lead to threats of a player strike over key issues such as time away from rugby, excessive player demands and possibility of player burnout. A three year project on player burnout was also commissioned in 2005, with preliminary results showing elite players in England to be more susceptible to burnout than their counterparts in New-Zealand (Ipsos-MORI, 2006). However, the study assessed player attitudes only, without any biochemical or physiological markers pertaining to player fatigue and burnout. Globally, ambiguity exists within the professional game as to the definition of ‘time off’ within rugby. Anecdotally, a considerable amount of ‘time off’ in Wales seems to be in the form of controlled formalised activity and not active rest away from team environments *per se*. These active rest periods differ considerably between home unions and are usually influenced by external forces e.g. television deals, domestic competitions, effectiveness of domestic player associations and cultural considerations. Despite recommendations by the IRB that there should not be more than one game played per week, introduction of a newly developed Anglo-Welsh cup has recently been introduced by the Welsh and English rugby unions. This competition, played between professional teams in England and Wales, will ultimately lead to mid-week games. With the above in mind, it was of interest to present investigators to find out current attitudes and physiological/biochemical responses to elite game demands in Wales.
Summary

Previous research carried out in England (Ipsos MORI, 2006) appears to provide anecdotal evidence that the length of the season may be a bigger issue currently then the total number of games. Thus, documentation of training volume and intensity as well as duration between games needs particular focus in this investigation. There is a paucity of available evidence, anecdotally or scientific on the amount of recovery time undertaken by elite squads. Inadequate recovery both in an acute setting (recovery duration) or longitudinally (incorporation of recovery weeks, mid-season breaks) may be more important then the nature or magnitude of the exercise stress itself. Hence, documenting current training loads and volume as well as recovery need to be considered so that conclusions can be made regarding the causative effects. Current trends and player opinions need to be substantiated from objective measures of well-being as viewed though changes in immune function, hormonal and illness data.

1.3 INTENDED PROCESS OF INVESTIGATION

With the above in mind, the current thesis will be divided into two main sections. The first section will attempt to investigate changes in immunoendocrine markers following acute (Study 1) and repeated rugby exposure (Study 2). Specifically, these studies will (a) attempt to investigate the time course of change in inflammatory markers pre and post rugby play, (b) whether or not there is a possible down regulation in host immunity, and (c) if changes in stress state are exacerbated with repeated exercise over a rugby tournament.

Following initial documentation of player opinions and attitudes to contemporary game demands in Wales (Study 3), the second section of the thesis will focus on chronic stress responses and potential changes in immunoendocrine markers over a rugby year. In particular, analysis of changes in weekly illness incidence and markers of mucosal immunity will be investigated in Study 4. Further studies will attempt to document changes in seasonal training load with changes in hormonal state (Study 5) and lastly, psychological state (Study 6).

In addition to these main studies, supplementary case studies are included to endorse methods and findings documented in the thesis. The first case study (CS-1) will be to determine an appropriate method of collection for analysis of salivary lysozyme, a key marker of mucosal immunity investigated in study 4. In addition to this, attempts will be made to validate an appropriate method for quantification of training loads in elite rugby union players. This is outlined in case study 2 (CS-2) and was deemed necessary given the longitudinal nature of
studies 4-6. Adjunctive to thesis goals and in particular, to study 4, it was deemed necessary that a valid method of weekly illness assessment was determined. This is outlined in case study three (CS-3) where two methods of illness assessment, namely medical reporting and web-based technology will be compared. Finally, an attempt was made to evaluate contemporary physiological demands of game play at the elite level using objective global positioning technology. This is outlined in the last of the cases studies (CS-4). It is hoped that this investigation will provide fitness coaches and the games regulatory body (IRB) with a greater understanding of potential stressors in contemporary rugby. This may aid in the future development of appropriate seasonal structure and training periodisation.
Chapter 2

Review of the literature

2.1 RUGBY UNION: THE GAME

Rugby union is one of the world’s most popular team sports played in more than 100 countries across 5 continents (IRB, 2007) since it first came into existence in 1871 (Quarrie and Hopkins, 2007). The recent 2007 Rugby World Cup in France was the world’s third largest sporting event after the 2004 Athens Olympic Games and the 2006 FIFA World Cup, attracting over two million spectators and viewed by a worldwide audience of over three billion people (Mellalieu et al., 2008). Despite a general decline in sports interest within the UK (1996-2003), participation levels in rugby union have not suffered to the similar degree as other sports. Recent research has suggested that rugby union is now Britain’s second most popular sport (Ipsos MORI, 2003). This arrest in declining playing number seems to be related to increasing exposure of rugby union as a game and success brought about by national teams. In the case of England, a 16% growth in participation levels was observed across all age groups 12 months after the world cup success in 2003 (RFU, 2007). In Wales, rugby union is considered the national sport and is played by an estimated 59,900 players (http://www.irb.com, 2003). Of these, ~150 players participate on a full-time professional basis. The majority of elite players born in Wales are employed within one of four recognised professional regional club teams, namely the ‘Llanelli Scarlets’, ‘Neath-Swansea Ospreys’, ‘Newport-Gwent Dragons’ and ‘Cardiff Blues’. In turn, each region has feeder clubs which make up 14 teams (~384 players) in a semi-professional club setting (WRU, personal communication). A small percentage of Welsh qualified players are employed outside of Wales, principally within the English professional club system (Guinness premiership).

2.1.1 Player characteristics and physiological demands

Rugby is considered one of the most intense and physically demanding field games in the world (Mashiko et al., 2004). Indeed, the incidence of injuries in professional rugby union is among the highest in professional team sports (Brooks et al 2005b, 2005c; Targett, 1998). It is a game played by 15 outfield players of which there are two main position based player groups, forwards and backs. Forward players, numbered 1 to 8, are often recognised as the ‘ball winners’, while the backs, numbered 9 to 15, are described as the ‘ball carriers’. In recent
years, a game consisting of 7 players per side has also been derived. Within the 15-man game, two 40 minute playing periods are separated by a break of 10 minutes. During this time (80-min game) the ball is in play for approximately 30-35 minutes (Quarrie and Hopkins, 2007; McLean, 1992). Today’s game usually involves players performing repeated bouts of high intensity exercise interspersed by low intensity periods of walking/jogging. During this time, players are also involved in a high number of collisions through the games many contact situations. Typically, backs display an exercise pattern focused on running and speed, in addition to some tackling, while the forwards, in addition to running and tackling, take part in scrums involving physical impact and muscular performance (Mashiko et al., 2004). It is well accepted that body mass and height for forwards is greater than backs (Quarrie et al., 1996; Duthie et al., 2003). Positional demands also require forwards to have considerable strength and power in order to gain and retain possession of the ball, while speed and agility are prerequisite for backs in open play.

Since rugby union involves a host of game related activities, detailed and accurate assessment of the games physiological requirements has been difficult. Conventionally, the demands of rugby union have been assessed using movement analysis (Docherty et al., 1988; McLean, 1992; Deutsch et al., 1998, 2007; Roberts et al., 2008), assessment of heart rate (Deutsch et al., 1998) and lactate profiling (McLean, 1992). Data from movement analysis studies have found that 95% of activities last less than 30 seconds, and rest periods are generally greater than preceding work efforts (Duthie et al., 2003). Therefore, elite rugby union can be considered an intermittent sport with a considerable anaerobic component interspersed with activities of lower level intensity, primarily aerobic in nature. Backs generally cover a greater distance during a game, but the high degree of physical contact undertaken by the forwards usually results in greater total work by this player group (Duthie et al., 2003). Data on professional players from the southern hemisphere has recently shown that forwards spend significantly more time (12-13%) in high-intensity work than backs (~4-5%) (Deutsch et al., 2007). This is most likely because of their greater involvement in rucking, mauling, and scrummaging. In comparison, backs were shown to spend approximately two or three times more time in high-intensity running modes than forwards (Deutsch et al., 2007). All of the latter studies used video analysis in categorising game demands. Few detailed studies have analysed player movement patterns using objective and reliable software. In a recent study using time-motion analysis software (Roberts et al., 2008) within elite rugby union in England, backs were shown to travel a greater total distance than forwards (6127m vs. 5581m). Of this distance, greater distances were observed in walking (2351m vs. 1928m) and high-intensity running (448m vs. 9
298 m) categories. In turn, forwards were shown to spend greater time performing high-intensity activity than backs. This was attributable to more time spent in activities involving static exertion (Roberts et al., 2008).

Surprisingly, few published studies have documented levels of aerobic fitness using laboratory data in elite rugby union players. Recorded values for aerobic fitness (estimated field and laboratory \( \text{VO}_{2\text{max}} \) data) have been shown to be in the region of 45-55 ml.kg\(^{-1}\).min\(^{-1}\) for elite players (see Nicholas, 1997 and Duthie et al., 2003 for reviews). When expressed relative to body mass, values are traditionally higher in backs (Nicholas and Baker, 1995; Scott et al., 2003) although absolute values are greater in forwards (Jardine et al, 1988). Forwards appear to be able to produce higher absolute peak and mean anaerobic power outputs across a range (7-40 sec) compared with backs, although results are similar when expressed relative to bodyweight (see Duthie et al., 2003 for review).

While it is generally perceived that forwards undergo a greater amount of game related stress than their back counterparts, few acute (invasive) studies have been carried out in rugby union to substantiate this. No differences in blood parameters, other than blood urea nitrogen, were recorded between forwards and backs following a game in university student rugby players (Mashiko et al., 2004). Authors concluded that this increase in protein catabolism within forwards was most probably due to this player group experiencing more contact play. No match statistics were recorded. In a subsequent study, increases in creatine kinase (CK) levels and decreases in host immunity (neutrophil function) were observed after a university game in Japan (Suzuki et al., 2004). No differences between player groups were observed.

### 2.1.2 Evolutionary trends within the game

Since its emergence as a professional sport, rugby union appears to have become a faster, ruck dominated game that contains more phases of play (Eaves and Hughes, 2003). Game phases involving player-player contact have become an increasingly important aspect of play across time (Quarrie and Hopkins, 2007; Eaves and Hughes, 2003). This trend seems to have been influenced by the onset of professionalism. A recent investigation into changes in player characteristics and match activities within southern hemisphere rugby (Quarrie and Hopkins, 2007) showed a considerable increase in tackles, rucks and ball-in-play time across the years 1994-2004. Number of rucks per match was found to have increased almost four-fold since the onset of professional status while a large increase in the number of tackles (1994: 160 ± 32;
2004: 270 ± 25) was also noted. Authors concluded that both events were a consequence of the ‘use it or lose it law’ introduced in 1994 and has ultimately led to an increase in contact area within the game. These findings substantiate those shown previously where authors have demonstrated a large increase in incidence of ruck events over time (years 1988-92: 62 events per game; 1993-99: 111.6; 2000-02: 134.4) (Eaves and Hughes, 2003). This trend is noteworthy since a number of studies have shown that the majority of injuries at senior and elite levels result from contact phases of play (Fuller et al., 2007). Furthermore, injury incidence has increased since the onset of professionalism (Garraway et al., 2000). Recent detailed analysis of contributory factors to injury risk in the English premiership have shown tackles to be the game event responsible for the highest number of injuries (Quarrie and Hopkins, 2008) and the greatest loss of time out of rugby union (Fuller et al, 2007). In the latter study, this was attributable to the frequency of tackling as a contact event although collisions were the contact event with the highest propensity to cause injury. Of the 25 games analysed, an average of 456.8 contact events were recorded per game (Fuller et al., 2007), reflecting the physical nature and high level of attrition experienced by contemporary players.

Additional to the increasing physical nature of rugby is an apparent paralleled increase in player size. Like game intensity, this increase seems to be, in the main, a result of professionalism. In a recent study by Quarrie and Hopkins (2007), clear trends exist for increased player body mass between the years 1994-2004 in both forwards (102.3 ± 1.2 vs 111.1 ± 2.9 kg) and backs (83.4 ± 2.1 vs 95.7 ± 2.3 kg) and is in line with previous observations (Olds, 2001; RFU, 2007). In rugby union, a larger body size correlates significantly with scrummaging force (Quarrie and Wilson, 2000) and competitive success (Olds, 2001). Unfortunately changes in game speed and player speed were not evaluated in these studies. However, an increase in game pace has been inferred from studies showing an increased amount of game phases and decrease in phase-participation time per player (Quarrie and Hopkins, 2007). Such findings have also been mirrored in other professional sports across time (Norton et al., 2001).

2.2 EXERCISE STRESS DEFINED

Many definitions have been used to describe the role of stress on the human biological system. Stress is defined as a physiologic response to events perceived as potentially or actually threatening the integrity of the body (Paccotti et al., 2005). From a physiological perspective, this may represent a stimulus capable of activating the hypothalamic pituitary adrenal axis (HPA-axis) or the sympathetic nervous system (SNS) to help an organism adapt
physiologically to deal with a threat (Glaser and Kiecolt-Glaser, 2005). Alternatively stress has been described as an unspecific reaction orientated syndrome that is characterised by deviation from the biological homeostatic state of the organism (Janke and Wolffgramm, 1995; Pacak and Palkovits, 2001). These stress reactions, from whatever context they arise, may be accompanied by corresponding changes in mood state, altered activation of the central and autonomous nervous system as well as humoral and hormonal responses (Kellman and Gunther, 2000). Immune cell functions are also modified by stress. Chronic stress is associated with the activation of the hypothalamic-pituitary-adrenal (HPA) axis, as well depression of immune function (Soo-Quee Koh and Choon-Huat Koh, 2007). When homeostasis is disturbed or threatened by internal or external challenges, activation of both SNS and HPA axis result in increasing peripheral levels of catecholamines and glucocorticoids (Elenkov et al., 2000). Following release, these substances begin to affect cell traffic, circulation, and the functional activity of immune cells (see Elenkov et al., 2000 for review). Strenuous exercise is a prototype of physical stressor and as such, represents an intriguing tool for monitoring the effects of stress on immune, psychological and hormonal state in athletes.

2.2.1 Stress and Rugby Union

Stress in the athletic domain has been investigated using a variety of techniques depending on the research domain i.e. psychological, physiological, or biochemical. From a systems point of view, athletic stress may be viewed as a destabilization or deviation from the norm in a biological/psychological system (psychophysical balance) (Kellmann and Kallus, 2001). Deviations from this psychophysical balance are therefore characteristic of demands which are either too high, too low, or those which prove excessive in terms of an individual athlete’s recovery limit or coping ability. Like most professional sports, participation in rugby union affords a level of ‘stress’ upon its playing population. This may be intensified within the contemporary playing population, due in principle, to current game trends, professionalism and demands of the game itself. Such stress afforded instances may take many forms. These may vary from those directly related to physical participation in the sport itself i.e. games/training, to stresses on players indirectly affected and supported by the playing environment (e.g. contractual issues, injury concerns, selection issues etc). Furthermore, seasonal variations in game fixtures may place disproportionate exercise loads on players in relatively short time periods. This may increase potential risks such as immune suppression and injury development. Increased frequency of competition, changes in game intensity from domestic to European/International competition, and daily conditioning are other areas of interest. Supplementary to this stress model may be a failure to incorporate adequate recovery
throughout training cycles. This will ultimately affect stress-recovery balance as described by Kellmann and Kallus (2001). With the above in mind, the object of the present investigation will be to highlight the potential role exercise stress has on player well being and more specifically immunoendocrine function. Detailed analyses of potential areas of stress in professional rugby are outlined in figure 2.1.

2.3 EXERCISE-INDUCED IMMUNOENDOCRINE DISTURBANCES

An acute bout of physical activity is accompanied by responses remarkably similar in many respects to those of infection (Gleeson, 2002). As well as elevations in circulating leukocyte level, there are also increases in various substances known to influence leukocyte function. These include inflammatory cytokines, activated complement fragments and acute phase proteins like C-reactive protein (Mackinnon, 1998). Hormonal changes also occur in response to exercise with rises in several plasma hormones such as adrenaline, cortisol, prolactin and growth hormone, all of which are know to have immunomodulatory effects (Khansari et al., 1990). These processes serve to attract scavenging cells, which can engulf and destroy microbes at the site of infection and inflammation. One important function of the swelling and oedema is to divert organisms and antigenic material associated with infection to the lymphoid circulation so that they can be carried harmlessly to the lymphoid organs to stimulate the full armoury of an immune response and, hence, recovery (Clow and Hucklebridge, 2001). Almost any form of local tissue damage results in similar processes. These immune processes (defence against infection and recovery following injury) are subject to modulation by the autonomic nervous system (ANS) and the neuroendocrine hypothalamic pituitary adrenal (HPA) axis. Both of these regulatory systems, and how they impact upon the immune system, are affected by both physical and psychological stress (Clow and Hucklebridge, 2001). This makes investigation of professional rugby players intriguing given their exposure to physical and psychological stress by the very nature of their occupation. Before reviewing the effect of exercise stress on modulation of the immune and endocrine systems, it is first necessary determine the distinct roles these systems have in biological functioning.

2.3.1 Circulating cells and functional role

Leukocytes

Leukocytes or white blood cells consist of granulocytes (60-70% of circulating leukocytes), monocyte (10-15%) and lymphocytes (20-25%) (Gleeson and Bishop, 2005). Depending on
their form, they have different functions, including ingesting bacteria, protozoa, or infected or dead body cells; producing antibodies and regulating the action of other leukocytes.

**Neutrophils**

Neutrophils represent 50-60% of total circulating leukocyte pool and are the most predominant source of leukocyte granular proteins secreted into blood plasma during physical exercise (Morozov et al., 2003). They are the first barrier against infection due to their ability to migrate rapidly into loci of infection where they phagocytise and kill pathogens. The sequence of response that occurs in neutrophil response to microbial invasion includes adherence, chemotaxis, phagocytosis, oxidative burst, degranulation, and microbial killing (Wolach et al., 1982). Neutrophils also play a key role in the early stages of the inflammatory response as chemotactic factors promote their migration from peripheral blood to extravascular tissue (Faurschou and Borregaard, 2003). Neutrophils are involved in release of immunomodulatory cytokines that influence other components of the immune system such as B and T lymphocyte activities (Lloyd and Oppenheim, 1992). Once reaching an inflammatory site, neutrophils are able to eliminate pathogens by phagocytosis and by the release of specific animicrobials into their immediate environment during the process of degranulation (Janeway et al., 2001).

**Monocytes**

Like neutrophiles, macrophages (mature monocyte) invade muscle after injury or modified use (Tidball, 2005). Since macrophages are rich sources of growth factors, cytokines and free radicals as well as antigen presenting cells, they can play important roles in regulating cellular immune response to injured tissue (Tidball, 2005).
Figure 2.1 Overview of contemporary playing demands and stress contributors within rugby union: implications for player welfare. IRB; international rugby board, URI’s; upper respiratory infections.
Lymphocytes
Various subsets of cells make up what is collectively known as lymphocytes. These include B-cells, T-cells and natural killer (NK) cells. T helper cells (CD3⁺CD4⁺CD8⁻) are necessary for B-cell and monocyte activation, while T cytotoxic cells (CD3⁺CD4⁻CD8⁺) can kill virus infected cells and tumour cells (Abbas, 2000). T-helper cells (Th) may be classed into either of two categories depending on their function. Th-1 cells function in cell-mediated immune activities while Th-2 cells, which are involved in antibody production (Marshall et al., 1998). Th-1 and Th-2 lymphocytes can be distinguished by the type of cytokines they produce (Gleeson, 2005).

NK cells
Approximately 10-15% of peripheral blood lymphocytes are neither T nor B cells (Gleeson, 2007). These large cells are known as NK (natural killer) cells. The primary role of NK cells is lysis of virus infected cells and tumour cells (Abbas, 2000). This occurs through release of their granule contents, ultimately causing break up of the virally infected cell membrane. NK cells are defined by the expression of CD56 and/or CD16 and the absence of CD3. Activation of NK cells does not require recognition of an antigen-MHC II combination (Gleeson and Bishop, 2005) and thus serve as a front line of defence before a specific response can be mounted by T and B cells. The potential role of NK cell activity in upper respiratory illness (URI) symptomology has been previously shown in soldiers (Gomez-Merino et al., 2005), sedentary subjects (Niemans et al, 1990) and the elderly (Ogata et al., 2001).

2.3.2 Effects of exercise on circulating cell number
During intense exercise, an immediate leukocytosis (increase in circulating number of leukocytes) is typically observed. This consists mainly of elevations in neutrophils which begin to recover leaving a developing neutrophilia, peaking between 2-3 h post-exercise (McCarthy and Dale, 1988; Robson et al, 1999). Blood neutrophils show a biphasic response to exercise. A transient increase is usually observed within the first 30 min after exercise through adrenaline induced demargination from the endothelium and marginated pools. A second delayed augmentation several hours later is thought to be resultant from glucocorticoid induced release of neutrophils from the bone marrow and spleen (Peake, 2002; Tzai li and Gleeson, 2005). In turn, cortisol mediated redistribution of other leukocyte subsets from the circulation into the bone marrow, lymphoid, skin and injured tissue may also occur (Toft et al., 1994; Wira et al., 1990).
Acute exercise also elicits a biphasic change in the number of circulating lymphocytes. Numbers have been shown to increase during and immediately after exercise before falling below pre-exercise values during the early stages of recovery (lymphocytopenia). Decreased numbers usually return to pre-exercise resting values steadily throughout recovery (Gleeson and Bishop 2005). Not surprisingly, circulating T-cell numbers (CD3$^+$) of lymphocytes also exhibit a biphasic response to acute exercise, with increases in T-cell number during and immediately after exercise before falling during recovery (Gleeson and Bishop, 2005; Malm et al., 2004). This response appears to be largely mediated by exercise intensity. During exercise, most investigators have found an increased number of T-cells in circulation, although this number can decrease below resting values for several hours after exercise (Malm et al., 2004). In studies on lymphocyte subsets, a decreased ratio of T-helper to T-cytotoxic (CD4:CD8) cells have been observed in athletes after exhaustive exercise (Berk et al., 1986; Lewiki et al., 1988; Haq et al., 1993). This is reflected by a greater increase in CD8$^+$ lymphocytes than CD4$^+$ lymphocytes (Pedersen et al., 2000). Exercise typically induces recruitment to the blood cells expressing characteristic NK cell markers (Pedersen et al., 2000). NK cell numbers have been shown to be markedly reduced for up to 16 hours after heavy exercise (marathon) (Castell et al., 1997).

Few studies have investigated changes in blood leukocytes and lymphocytes over prolonged periods. No significant changes in the resting leukocyte profiles were observed during a 6 week progressive overload training period in rugby league (Coutts et al., 2007), 2 weeks of intensified training in cyclists (Halson et al., 2003), in professional soccer players over the course of a season (Filaire et al., 2003) or with 18.5 weeks of basic infantry training (Brenner et al., 2000). Little or no effects of overtraining has been observed on circulating immune cell counts (Gabriel et al., 1998; Rowbottom et al., 1995; Mackinnon et al., 1997) although in the latter study, declining leukocyte counts after 4 weeks of training was observed. Lower lymphocyte counts in overreached athletes have been observed when compared to well-trained athletes after 2 weeks of intensified training (Mackinnon et al., 1997). Low leukocyte counts have been found in athletes engaged in heavy training programs (Mackinnon, 1998) while decreases in circulating leukocyte and lymphocyte counts were shown to coincide with increases in illness during army training (Whitham et al., 2006). In summary, changes in circulating cell numbers of leukocytes do not seem to change greatly with training/exercise load although further investigation is warranted.
Table 2.1. Effect of strenuous exercise on the immune system

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<th>During Exercise</th>
<th>After Exercise</th>
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<td>Neutrophil count</td>
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<td>Monocyte count</td>
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<tr>
<td>Lymphocyte count</td>
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<td>CD4+ T cell count</td>
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<td>CD8+ T cell count</td>
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<tr>
<td>CD19+ B cell count</td>
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<td>CD16+56+ NK cell count</td>
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<td>Lymphocyte apoptosis</td>
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<td>Lymphocyte proliferative response to mitogens</td>
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<td>Antibody response in vitro</td>
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<td>*Saliva IgA</td>
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<td>Delayed type hypersensitivity response test (skin test)</td>
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<td>NK cell activity</td>
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<td>C-reactive protein</td>
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<td>Plasma concentration of TNF-α</td>
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<td>Plasma concentration of IL-1</td>
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<td>Plasma concentration of IL-1ra</td>
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<td>Plasma concentration of IL-10</td>
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</table>

↑, increase; ↓, decrease; ↑↑, marked increase; *, increases & decreases reported in literature.

TNF-α, tumour necrosis factor alpha; IL, interleukin. Data taken from Pedersen et al., (2000).

2.3.3 Effects of exercise on cellular function

Adverse effects of exercise have been reported on many aspects of immune function. Decreases in lymphocyte responsiveness to mitogen stimulation have been observed post-exercise (Gmunder et al., 1988). Significant decreases in lymphocyte proliferative ability have also been observed after a marathon, with function not restored until 16 hours after the race (Castell, 1996). Acute exercise typically displays a biphasic response on NK cell cytolytic activity, with an initial enhancement followed by a delayed suppression (Pedersen 1991; Nieman et al., 1993; Kappel et al., 1991). This suppression is more evident with intense exercise of at least 1 h duration, with values reported to decrease 2-4 h after exercise (Pederson et al., 2000). Moderate exercise has been shown to boost neutrophil function, including chemotaxis, phagocytosis, and oxidative burst activity (Pedersen et al., 2000). Intense exercise, however, has been shown to diminish responsiveness of neutrophils to stimulation (via bacterial liposaccharide; LPS) and reduced oxidative burst activity (killing capacity) (Robson et al., 1999). This reduced ability can last for several hours. Furthermore, changes in
the ability of neutrophils to incorporate bacteria (phagocytic ability) on a per cell basis have been shown to be decrease (34%) after an ultramarathon (Gabriel et al., 1995). Transient decreases in neutrophil chemotaxis have also been seen following brief (30 min) aerobic (70% \( \text{VO}_{2\max} \)) exercise (Wolach et al., 2005) while the production of immunoglobulins by B lymphocytes is also inhibited following prolonged strenuous exercise (Gleeson, 2002). A summary table of exercise effects on the immune system is shown in table 2.1.

### 2.3.4 Hormonal response to exercise

Stressors such as exercise are characteristically met by a series of co-ordinated hormonal responses controlled by the central nervous system (Jonsdottir, 2000). These hormonal responses are thought to play a significant role in tissue growth as well as the regulation of energy substrate metabolism (Kraemer, 2000). Stress-induced activation of the hypothalamic-pituitary-adrenal axis (HPA axis) influences the immune system by the release of neuroendocrine hormones from the pituitary gland. Mediated by receptors for neuroendocrine hormones and neuropeptides, immune cells are able to respond to signals from the HPA axis and their activities are either activated or down-regulated. In addition, these cells have receptors for catecholamines, adrenaline and nor-adrenaline that enable them to respond to the sympathetic-adrenal-medullary (SAM) axis (Yang and Glaser, 2002). During exercise, adrenaline is released from the adrenal medulla and noradrenaline is released from sympathetic nerve terminals. Plasma concentrations of both of these catecholamines have been shown to increase almost linearly with duration of dynamic exercise and exponentially with intensity (Kjaer, 1989). The effects of catecholamines on target cells are mediated via adrenoreceptors, of which are known to be present within a variety of immune cells (Benschop et al., 1996). Adrenaline in particular has been shown to be important in recruiting lymphocytes and neutrophils into the circulation during intensive exercise 90 min (Nieman, 1997). Increases in plasma cortisol and testosterone are also known to occur with acute exercise (Häkkinen and Pakarinen 1993). Increases in plasma cortisol concentrations are associated with exercise intensity and duration greater than 1 hour. Cortisol is a key neuroendocrine modulator of the immune system playing a major role in metabolism and immune function as a potent agent for gluconeogenesis and immunosuppression (Pedersen et al., 1997). Furthermore, it’s effects that tend to be anti-inflammatory in nature (Miles, 2005) with recorded values 8 h post eccentric exercise associated with the amount of tissue swelling (Miles et al., 2008).

Testosterone is a steroid hormone secreted from the Leydig cells of the testes and has both anabolic and anticatabolic effects on muscle tissue (Volek et al., 1997). Like cortisol, acute
exercise increases testosterone linearly once a specific intensity threshold is reached, with peak concentrations usually occurring at the end of exercise (Wilkerson et al., 1980). High intensity resistance exercise is known to increase testosterone levels (Volek et al., 1993) as does prolonged endurance exercise (Daly et al., 2005). More detailed discussion on cortisol and testosterone is included in section 2.13.

2.4 IMMUNE SUPPRESSION: POSSIBLE CAUSES?

There are several possible causes for diminution of immune function with heavy training. Analysis of these concepts are discussed in greater detail in the following sections.

- The cumulative effect of repetitive bouts of intense exercise and concomitant elevations in stress hormones, particularly glucocorticoids, may be one reason for observed decreases in immune function with heavy exercise (Khansari et al., 1990). It is known from in vitro studies that stress hormones such as cortisol have an inhibitory effect on immune cell function (see Gleeson et al., 2007; Yang and Glaser, 2002). It is possible that when exercise is repeated frequently, there may not be sufficient time for the immune system to recover (Gleeson, 2002).

- Growing evidence suggests that very strenuous exercise can cause substantial subclinical injury, initiating and excessive inflammatory reaction and immunosuppression (Northoff et al., 1995). This, in many respects, mimics the immune reactions observed in clinical sepsis (Shephard and Shek, 1998). An excessive inflammatory response along with release of stress hormones and in particular cytokines, are thought to account for most of the signs and symptoms previously associated with overtraining syndrome (Smith, 2004). Recently excessive exercise stress has been proposed to induce a shift in T-helper cells (Th) from Th-1 cells, which function in cell-mediated immune activities, to Th-2 cells, which are involved in antibody production (Marshall et al., 1998). This suppressed cell mediated response has been proposed to increase risk of susceptibility to infections (Smith et al., 2003).

- Exercise results in initiation of an innate immune response, of which neutrophils play a key role. The bone marrow contains a 3 day store of mature neutrophils for release (Pyne, 1994). While it is questionable whether a single exercise bout impacts significantly on this store (Bishop et al., 2003), repeated exercise exposure, tissue injury and
inflammation may affect neutrophil defence capacity. It may be possible that repeated prolonged, intense bouts of exercise could deplete bone marrow stores of mature neutrophils. These less mature cells have a lower phagocytic capacity and content of granular digestive enzymes compared with more mature neutrophils (McCarthy and Dale, 1988, Yang and Hill, 1991). Repeated stimulation could theoretically increase an athlete’s predisposition of infection.

- Reduction in local host immune responses as a result of daily exercise may provide an important mechanism for infection risk in athletes. Decreases in immunoglobulin-A (Gleeson et al., 1999) and other antimicrobial peptides (West et al., 2006) within mucosal secretions such as saliva may serve to increase illness risk in athletes.

- Falls in plasma glutamine levels known to occur after exercise may become chronically depressed after repeated bouts of prolonged strenuous training (Parry-Billings et al., 1992; Walsh et al., 1998). The clinical effect of these changes remain inconclusive however.

- Other mechanisms may also occur. Increases in lymphocyte apoptosis (programmed cell death) following exercise has been previously shown (Mooren et al., 2002; 2004). The long term effects of repeated exercise on lymphocyte apoptosis has not been explored.

2.4.1 Exercise and blood neutrophil function

Unlike most lymphocytes, neutrophils die within a few days of leaving the bloodstream (Castell and Newsholme, 1997). An increased release of immature neutrophils from bone marrow has also been suggested to occur in response to strenuous exercise (Hansen et al., 1991). Furthermore it’s speculated that high volume training may leave a significant proportion of neutrophils in a chronic refractory state, unable to respond effectively to pathogenic stimuli (Pyne, 1994). This, along with the above would theoretically increase an athlete’s susceptibility to opportunistic infections. Decreases in neutrophil function have been observed during intensified training (Robson-Ansley et al., 2007). In this study it was thought that depletion of mature neutrophils occurring during repeated exercise resulted in an influx of immature neutrophils to the circulation. These less mature cells have a lower phagocytic capacity and content of granular digestive enzymes compared with more mature neutrophils (McCarthy and Dale, 1988; Yang and Hill, 1991). Also, following exposure to a bacterial stimulant, neutrophils respond to a lesser degree to secondary stimulation, with recovery
occurring gradually over time (Prasad et al., 1991). Changes in neutrophil function have also been reported with increasing training load. A 5-week period of endurance training in previously sedentary subjects caused a 30% reduction in the LPS stimulated neutrophil degranulation (elastase release) response (Blannin et al., 1997). A 2-week period of intensified training (IT) in already well trained triathletes was also associated with a 20% fall in the Lipopolysaccharide (LPS) stimulated neutrophil degranulation response (Robson, 1999b). Changes in neutrophil function with exercise are dependent on both exercise intensity and duration (Robson et al., 1999). In the latter study, increased serum cortisol concentrations were implicated in altered neutrophil responses (Robson et al., 1999). In summary, repeated stimulation and infiltration of immature blood neutrophils may downregulate function of circulating neutrophils. In theory, this could lead to a decrease global host protection although the impact of reduced neutrophil function on susceptibility to infection has not been investigated in detail. Extremely low levels of elastase (released during neutrophil degranulation) have been found in patients with Chediak-Higashi syndrome of whom were associated with an increased susceptibility to infection (Ganz et al., 1988; Vassalli et al., 1978).

2.4.2 Exercise and Plasma Glutamine/Glutamate

**Plasma Glutamine (Gln)**

Plasma glutamine is a neutral amino acid (AA) found in high levels in a number of human tissues (Rowbottom et al., 1996). It is the most abundant AA found in muscle tissue and plasma (Newsholme et al, 1990). Normal resting plasma glutamine concentration is 500-700 µM (Rennie et al., 1981) although values may vary between athletes of different sports (Hiscock and Mackinnon, 1998). Glutamine is synthesized from ammonia and glutamate by glutamine synthetase (Hiscock and Pedersen, 2002). Glutaminase catalyzes the reverse reaction to form ammonia and glutamate from glutamine (Rowbottom et al., 1996). Glutamine has been suggested to be a versatile AA with differing roles. These include transfer of nitrogen between organs and detoxification of ammonia, maintenance of acid-base balance during acidosis, as a nitrogen precursor for the synthesis of nucleotides, a fuel for gut mucosal cells and as a possible regulator of protein synthesis and degradation (Rowbottom et al., 1996). One of the more essential roles of this AA is in immune cell metabolism (Castell, 2003) where it provides an important fuel for immune cells, in particular macrophages and lymphocytes (Hiscock and Pedersen, 2002, Parry-Billings et al., 1990). In humans, glutamine has been shown to influence the in vitro proliferation of lymphocytes (Rohde et al, 1996; 1995). Traditionally lowered plasma levels following heavy exercise have been linked with
compromised immune function (Castell et al., 1996; Keast et al., 1995; Parry-Billings et al., 1992). Low levels of this marker have also been implicated as a marker of overreaching (Halson et al., 2003), overtraining (Rowbottom et al., 1995; 1996) and in elite athletes showing signs of chronic fatigue and infection (Kingsbury et al., 1998). Lower levels in athletes diagnosed with overtraining have been shown when compared to nonathlete controls (Mackinnon and Hooper, 1996; Parry-Billings et al., 1992; Rowbottom et al., 1995).

However, studies involving administration of glutamine prior to exercise have failed to show consistent beneficial effects on observed immune responses (Rohde et al., 1998). Glutamine concentration has been shown to decrease by a similar degree in both glutamine and placebo-supplemented groups (Castell et al., 1997). Mackinnon and Hooper (1996) failed to show significant difference in glutamine levels between subjects who did or did not develop a URTI in a study examining the effect of intensified training (IT) on swimmers. Furthermore, no differences in a host of immune responses were reported in a glutamine supplemented group compared to a non supplemented (Rohde et al., 1998). Decreases in plasma concentration with exercise and associated changes in glutamine metabolism have not been shown to alter lymphocyte proliferation in exhaustive trained rats (Santos et al., 2007). Conversely it has been recently shown that supplementation of hydrolyzed whey plus glutamine resulted in partial prevention of lymphocyte apoptosis induced by exhaustive exercise (Cury-Boaventura et al., 2008). Although decreases in glutamine may occur with heavy exercise, evidence is lacking to suggest that low levels of plasma glutamine is associated with impaired immune function and increased susceptibility to infection (Bassit et al., 2000; Castell et al., 1997; Castell and Newsholme, 2001; Mackinnon and Hooper, 1996).

Large discrepancies in the literature exist relating to regular effects of exercise on this marker. Elevated levels (~30%) above resting plasma glutamine have been observed following 6 weeks of progressive endurance training (Kargotich et al., 2007). Similar increases have been observed in top level triathletes sampled across a training season (Rowbottom et al., 1996) while increases in glutamine levels were also seen in elite swimmers during four weeks (Mackinnon and Hooper, 1996) and six weeks (Kargotich et al., 2005) of intensified training. Interestingly, in the latter study, stable plasma glutamine levels were recorded in ‘overtrained’ swimmers undertaking the same protocol. These findings are in contrast to studies where decreased glutamine concentrations have been recorded and associated with overtraining/underperformance (Keast et al., 1995; Parry-Billings et al., 1992; Rowbottom et
al., 1995). More recent work suggests that decreases in glutamine concentration may not be evident until two weeks into an intensified training program (Halson et al., 2003).

Several reasons for observed declines with exercise have been proposed. Output of glutamine from skeletal muscle is increased by high concentrations of glucocorticoids such as cortisol (Rennie et al., 1981). Observed declines in plasma glutamine concentration with heavy training have been suggested to occur from increased demand by tissues that require glutamine as a fuel and/or decreased production or altered transport kinetics of this AA (Walsh et al., 1998). A recent hypothesis suggests that repeated bouts of high intensity exercise over days and weeks may induce an adaptive increase in renal uptake of glutamine in buffering acidosis (Walsh et al., 1998). Hence net overutilization may explain the fall in post-exercise plasma glutamine. It’s possible that this concept may hold true for overtrained athletes, although interference in rate of glutamine release from muscle has been suggested for lower resting plasma glutamine concentration in this athlete group (Newsholme, 1994). Finally, occurrence of a mitochondrial lesion within skeletal muscle has also been suggested as a possible reason for decreased glutamine concentrations during heavy exercise (Rowbottom et al., 1995).

In summary, available evidence within the literature would suggest that measuring the dynamics of glutamine may be more indicative in tracking training tolerance and impending overreaching than alterations in immune function per se. Differences in study methodology, timing of sampling, training duration and study population may account for observed discrepancies.

**Plasma Glutamate (Glu)**

Glutamate, along with glutamine, has a role in acid-base balance and de novo synthesis of nucleotides (Rowbottom et al., 1996) and TCA cycle intermediates (Mourtzakis et al., 2008). It is also an important regulator of protein synthesis and degradation (Rowbottom et al., 1996). With the above in mind, it has been suggested that glutamate plays an important role in the repair/regenerative responses to daily muscle loading (Coutts et al., 2007). Elevated levels of plasma glutamate have been reported following intensified training in soccer (Filaire et al., 2003), cycling (Halson et al., 2003), rugby league (Coutts et al., 2007a; 2007b) and in trained athletes (Mackinnon and Hooper, 1996). In addition to lower plasma glutamine levels in overtrained athletes, Parry-Billings et al (1992) have reported significantly higher plasma glutamate concentrations compared with controls. In another study, recovery of glutamate to previous resting levels following intensified training was shown to occur with a 7 day taper.
(Coutts et al., 2007a, 2007b). Purported underlying mechanism(s) for these elevations remains unclear. High glutamate concentrations have been observed in catabolic conditions such as sepsis, cancer and HIV (Hack et al., 1996), possibly as a result of decreased muscle uptake. Furthermore high plasma glutamate levels along with insufficient baseline glutamine levels has been suggested to result from catabolism or loss of body cell mass (cachexia) in healthy subjects after very high intensity exercise (Kinscherf et al., 1996). Increased generation of glutamate during high intensity exercise may be a consequence of known bodily requirements to increase ammonium ion production so that acid-base balance can be maintained (Goldstein et al., 1980). Since glutamine is required to support renal ammonia genesis (Damian and Pitts, 1970), resulting increases in glutamate (via hydrolysis of glutamine to glutamate and ammonia) may occur. It has also been speculated that with repeated high intensity exercise, observed increases in glutamate concentration may occur as a result of cell damage in skeletal muscle or the lungs, thereby curtailing the removal of glutamate by glutamine synthetase (Smith and Norris, 2000). The same authors concluded that glutamate concentration increases significantly when the recovery from high intensity training is impaired through either repeated high intensity training or lack of recovery time.

**Plasma Glutamine/Glutamate ratio**

Reduced plasma glutamine (Gln) and elevated plasma glutamate (Glu) levels have been previously been observed following periods of high intensity training (IT) or overreaching (OR) in professional soccer (Filaire et al., 2003), cyclists (Halson et al., 2003) and swimmers (Mackinnon and Hooper, 1996). Together these markers [glutamine/glutamate ratio] have being suggested as been useful in tracking training tolerance and in identifying training stress (Smith and Norris, 2000). Elevated plasma glutamate and a reduced Gln/Glu ratio were observed in athletes who were classified as ‘overtrained’ (Smith and Norris, 2000). Other studies have reported lowered glutamine and increased glutamate levels in overtrained athletes (Parry-Billings et al., 1992). More recently changes in plasma glutamine and glutamate have been shown to be a sensitive marker of overreaching (OR) in rugby league (Coutts et al., 2007a; 2007b). In these studies, increases in plasma glutamate were observed in two groups of players following 6 weeks of normal and IT. However significant decreases in resting Gln:Glu ratio were observed in the player group undertaking additional intensified training only, observations which were coupled with decreases in key performance tests. Authors concluded that this maker may be useful in monitoring IT within team sport athletes. These results confirm those of a previous studies, where a decrease in Gln:Glu ratio below the value of 3.58 has been suggested to indicate a state of overreaching (Smith and Norris, 2000, Halson et al.,
This decreased ratio has been shown to return to baseline with two weeks of recovery (Halson et al., 2003). Authors did not speculate as to why Gln:Glu ratio decreased with increasing training load in the rugby league study. Incidentally both studies by Coutts et al. (2007) and Smith and Norris (2000) used the same analysis technique. This is important since variation in analysis technique has been suggested to influence findings (Castell, 2003). Finally, athletes who have developed a load tolerance to training, principally through aerobic work, tend to have higher Gln:Glu ratios (Smith and Norris, 2000).

2.4.3 Tissue trauma and associated immune response

Tissue injury normally induces a rapid but complex sequence of immune reactions (Shephard and Shek, 1998). In particular, changes in innate immunity have been typically observed with exercise related muscle damage. In these studies, increases in blood neutrophils and monocytes have been observed over the first 12 h post-exercise (Smith et al., 1998; MacIntyre et al., 1995; Pizza et al., 1995; Paulsen et al., 2005). Findings have been substantiated from immunohistochemical analysis of muscle biopsies where infiltration of neutrophils and monocytes into injured muscle tissue after eccentric muscle damage have been shown (Paulsen et al., 2005). Rapid invasion of neutrophils has been suggested to occur within 1 h of increased muscle use, after which concentrations may remain elevated for as long as 5 days (Fielding et al., 1993). During tissue injury, it is thought that the main functional role for neutrophils is their ability to release proteases to degrade cellular debris caused by muscle damage (Tidball, 2005). While playing a key role in muscle repair and regeneration, other findings show a role for neutrophils in promoting muscle damage soon after muscle injury or modified use. During proteolysis and removal of cellular debris, neutrophils release high concentrations of cytolytic and cytotoxic molecules that can damage muscle or healthy bystander tissues (Tiidus, 1998). After acute injury, macrophages also move into injured tissue and have been shown to be capable of promoting muscle damage through the release of free radicals. Other findings indicate that this cell group also play a role in repair and regeneration through release of growth factors and cytokine-mediated signalling (Tidball, 2005, Shephard and Shek, 1998).

In the early inflammatory stage, cellular debris is removed by infiltrating neutrophils and is followed by a regenerative response during which satellite cells proliferate to replace previously damaged and phagocytosed muscle (Toumi and Best, 2003). Increases during exercise are thought to be resultant from complement activation, hormonal and cytokine stimulation (Johnson et al., 1998) and are typically biphasic in response. After stimulation, activated neutrophils function through a series of actions including adherence to endothelial
cells (attachment), migration to the inflammatory site (chemotaxis), and phagocytosis and killing of many microbial, bacterial and viral pathogens (Parslow et al., 2001; Robson-Ansley et al., 2007). They destroy invading pathogens and tissue fragments via both oxygen dependent (release of reactive oxygen species) and oxygen independent (release of proteases) mechanisms (Tzai Li and Gleeson, 2005). Once activated the secretary function of neutrophils results in their degranulation and the efflux of granule content from the cell or into the phagosomes (Morozov et al., 2003) and extracellular surrounding area. Elastase, a proteolytic enzyme, is subsequently released by neutrophils during this process (Pyne, 1994). With respect to exercise, these proteinases are used to digest foreign agents and any tissue debris resultant from tissue damage. The role of neutrophils during rugby exercise has been previously shown (Suzuki et al., 2004). In this study, significant decreases in neutrophil phagocytic capacity and oxidative burst were observed in collegiate rugby players immediately post match. Changes in neutrophil degranulation have not been explored with rugby exercise.

### 2.4.4 Cytokine release and overtraining

Tissue trauma from exercise has been recently implicated as an underlying cause of overtraining syndrome (Smith, 2004). It has been suggested that if acute inflammation is not resolved, then acute inflammation may be transformed into an ongoing, undesirable condition of chronic inflammation (Buckley et al., 2001). In an athletic context, continuation of a training regime without sufficient recovery is thought to compound this initial local inflammation. Resultant increases in release of inflammatory mediators and pro-inflammatory cytokines from activated monocytes are thought to lead to systemic inflammation (Smith, 2000). It has been suggested that release of these pro-inflammatory cytokines may account for some of the symptoms observed in overtrained athletes (Halson et al., 2003). Cytokines are key molecules secreted under trauma (e.g. injury or illness) and are involved in communication between many cells and organs (Smith, 2000). It is thought that if high volume/intensity training persists without sufficient rest/recovery, elevated levels of circulating cytokines (IL-1-β, TNF-α and/or IL-6) interact with various systems of the body, accounting for most of the signs and symptoms previously associated with overtraining syndrome (Smith, 2004).

Under the model proposed in figure 2.2, its has been suggested that systemic inflammation induces sickness behaviour (fatigue, appetite suppression, depression), activation of the sympathetic nervous system and the HPA axis, suppression of the hypothalamic-pituitary-gonadal axis, up regulation of liver function and possibly immunosuppression (Smith, 2000). Few studies in exercise science have verified this theory however. Furthermore, mild tissue
trauma, followed by recovery, is an integral part of the training process (Armstrong and VanHeest, 2002; Smith, 2000). This is often referred to as adaptive microtrauma (Smith, 2000). No changes in resting IL-6 and TNF-α were observed after a 2 week period of intensified training in cyclists despite changes in performance, fatigue, and mood state (Halson et al., 2003). Conversely chronic elevations in IL-6 have been recently observed in endurance athletes following a period (4 weeks) of heavy training (Robson-Ansley et al., 2007). These elevations were associated with suppression of the innate immune system and an increase in fatigue and generalised malaise. Future studies examining the role of other pro-inflammatory cytokines like IL-6 and muscle damage on cognitive state and performance may help in substantiating this link. Further discussion of IL-6 during inflammation is discussed in section 2.5.1.

Figure 2.2: Cytokine theory of overtraining: proposed events leading to, and sustaining the overtraining syndrome From: SMITH: Med Sci Sports Exerc, Volume 32(2).February 2000.
Chapter 2

LITERATURE REVIEW

2.5 TISSUE INFLAMMATION AND ACUTE PHASE RESPONSE

The term inflammation is used to describe the environment produced by activated macrophages, including a milieu of chemokines, cytokines and other proteins that are a central aspect of the innate immune response (Woods et al., 2006). Major events include (1) tissue injury, (2) release of vasoactive substances by the injured tissue, (3) vasodilation, (4) leukocyte adhesion, (5) leukocyte migration to the injured site and eventually (6) tissue repair (Malm, 2001). The inflammatory response may be acute, as in transitory physical injury or infection, or low grade and chronic, as in long term infections and auto-immune diseases (Janeway et al., 2001). The association between tissue damage through exercise and associated inflammation is clear. Modified muscle use or injury typically initiates a rapid and sequential invasion of muscle by inflammatory cell populations that can persist for days to weeks, while muscle repair, regeneration and growth occur (Tidball, 2005). Chronic exercise is traditionally associated with a decrease in systemic inflammation whereas acute exercise results in varying degrees of microtrauma to the muscle, connective tissue, bones or joints. These micro-traumas lead to an inflammatory response to repair the damaged tissue (Woods et al., 2006). As acute exercise induces an inflammatory response, its magnitude and subsequent resolution can provide information on the degree of stress and subsequent recovery of the athlete. In the absence of adequate rest, the damaged tissue does not have time to repair, and a chronic inflammatory condition connected with overtraining may occur (Smith, 2000).

2.5.1 Interleukin 6 (IL-6)

Acute inflammation is accompanied by changes in the concentrations of acute phase proteins (APPs) (Tilg et al., 1997) and is termed the acute phase response (APR). Of the many mediators in the APR, IL-6 plays a key role. It is a pleiotropic cytokine and its secretion is stimulated by infection, trauma or immunological challenge (Tilg et al., 1997). One of the physiological roles of IL-6 is to induce release of cortisol to the circulation, and exercise induced changes in cortisol have been linked to IL-6 (Pedersen et al., 2001). Levels of this cytokine are thought to be released by virtually all multinucleated cells including skeletal myocytes where it is produced in response to contraction (Pedersen, 2007). Considerable debate exists as to whether this cytokine is classified as inflammatory or anti-inflammatory in nature. Many previous studies have shown increases in plasma IL-6 with acute exercise (Pedersen, 2007) with levels usually returning to pre-exercise values within a few hours of rest (Pedersen et al., 2001). Bruunsgaard et al (1997) have found close correlations between elevations in IL-6 and muscle damage with exercise while others have not (Miles et al., 2008).
Therefore, the notion that post-exercise cytokine production (inflammation) is related to skeletal muscle damage remains unclear.

Elevated resting IL-6 levels have been previously implicated as a key indicator of unexplained underperformance syndrome (Robson, 2003). Chronic elevations in IL-6 have been recently observed in endurance athletes following a period (4 weeks) of heavy training (Ronson-Ansley et al., 2007). Elevations were associated with suppression of the innate immune system and an increase in fatigue and generalised malaise suggesting additional roles of IL-6 within the athletic domain. Exogenous administration of IL-6 increases the sensation of fatigue at rest (Spath-Schwalbe et al. 1998) and during exercise (Robson-Ansley et al., 2004). IL-6 has also been suggested to act on the CNS to elicit the release of adrenocorticotropic hormone (ACTH), and ACTH in turn increases the synthesis of glucocorticoids in the adrenal gland (Akira et al., 1993). Higher resting plasma levels of IL-6 have been shown in professional rugby players when compared to controls (Pool et al., 2002). In the same study IL-6 concentration following endotoxin stimulation was significantly lower in the rugby players when compared to controls. This led authors to conclude that mononuclear cells appear to be chronically activated and less responsive in this athlete group. Steensberg et al (2003) have suggested that elevated plasma levels of IL-6 in such individuals represents low grade inflammation rather then its cause.

The same authors showed that infusion of recombinant human (rh) IL-6 induces an anti-inflammatory environment in humans through up-regulation of known anti-inflammatory cytokines IL-10, IL-1 receptor antagonist (IL-1ra) and C-reactive protein. Furthermore, IL-6 infusion failed to result in up-regulation of pro-inflammatory cytokine TNF-α, while an increase in cortisol was observed in the same study. It has been suggested that IL-6 may contribute to the resolution of acute and chronic inflammatory processes by the direct suppression of IL-1 and TNF, the induction of glucocorticoid release and induction of natural antagonists of IL-1 and TNF (Tilg et al., 1997).

### 2.5.2 C-reactive Protein (CRP)

Along with IL-6, C-reactive protein (CRP) represents a key protein involved in the acute phase response following tissue damage and infection. Primarily produced in the liver following induction by IL-6 (Petersen and Pedersen, 2005), CRP is known to reflect the impact of trauma on the body (Brewster et al., 1994) and is associated with tissue damage (Gebhard et al., 2000). Recent evidence has revealed that CRP is also expressed and secreted by peripheral blood mononuclear cells (Haider et al., 2006). Low grade chronic inflammation is reflected by
increased CRP concentrations and increased systemic levels of some cytokines (Ross, 1999). Its basic features are the control of inflammation, the stimulation of clearance of damaged cell and tissue components, and the initiation of repair functions (Pepys, 1981). It is thought that CRP may have a role in the induction of anti-inflammatory cytokines in circulating monocytes and in the suppression of the synthesis of pro-inflammatory cytokines in tissue macrophages (Tilg et al., 1997). It has also been implicated in preventing excess complement activation (Bíró et al., 2007). Therefore it provides a pivotal role in limiting inflammation by inducing and orchestrating regulatory processes. While acute exercise induces increased concentrations of CRP it has been suggested that tissue injury associated with heavy training may lead to decreases in resting levels of serum CRP (Shephard, 1997). CRP concentrations have been shown to be increased in subjects after an intensive training program in elderly subjects (Schuit et al., 1997). Serum concentration of CRP has been shown to peak 2 days after high force eccentric exercise (Paulsen et al., 2005) and 1 day after long duration exercise (Pedersen and Hoffman-Goetz, 2000, Mooren et al., 2005). In the latter study a significant correlation was found between reductions in force generating capacity and appearance of CRP and CK.

Increased levels of CRP have been found 16 h after a marathon but not immediately after the event (Castell et al., 1997). Neidhart et al (2000) have found elevated CRP levels at 24 h and 48 h after a marathon run. Regular exercise appears to significantly lower circulating CRP and other inflammatory cytokine concentrations (Okita et al., 2004; Stewart et al., 2007; Toft et al., 2002) possibly as a result of a training effect (King et al., 2003). Higher circulating levels of CRP (and IL-6) are independently associated with lower levels of aerobic fitness ($\mathrm{VO_{2\text{max}}}$) in asymptomatic men (Kullo et al., 2007). Mattusch et al (2000) have found reductions in CRP in athletes after 9 months of endurance running. Furthermore, regular soccer training led to lower CRP levels several months later (Fallon et al., 2001). Low resting levels of CRP have been reported professional cyclists (Semple et al., 2006) while elevated levels of CRP have been reported in response to overtraining (Uusitalo, 2001; Fry and Kraemer, 1997)

2.5.3 Exercise-induced muscle damage: Creatine Kinase (CK)

Muscle damage after exercise results in a substantial increase in myocellular protein levels within blood (Armstrong et al., 1983). Proteins levels have been found to be more pronounced during eccentric exercise when compared to other forms of exercise (Saxton et al., 1994; Armstrong et al., 1983). Traditionally changes in plasma/serum creatine kinase (CK) or myoglobin (Mb) levels have been used as markers of tissue damage (Clarkson and Hubal, 2002). Their release has been associated with disruption to the muscle cell membrane (Friden
et al., 1983; McNeil and Khakee, 1992). Extent of muscle damage is related to both intensity and duration of exercise, with intensity playing a greater role (Tiidus and Ianuzzo 1983). With respect to serum CK, levels can be raised as a consequence of both metabolic and mechanical causes (Brancaccio et al., 2007). Severe muscle damage has been shown to result in muscle pain and stiffness, higher than normal perceived exertion during exercise and decrements in muscle strength and maximal power output which can last for 5-10 days (Clarkson et al., 1992; Jones et al., 1986; Gleeson et al., 1995). Exercise induced muscle damage also impairs restoration of muscle glycogen (O’Reilly et al., 1987).

At least five isoforms of CK exist: three isoenzymes in cytoplasm (CK-MM, CK-MB and CK-BB) and two isoenzymes (non-sarcomeric and sarcomeric) in mitochondria. In normal serum, total CK is provided mainly by the skeletal muscle and is almost exclusively of the MM fraction (Brancaccio et al., 2007). In contact sports like rugby union, muscle damage may occur during game instances outside of areas associated with traditional eccentric damage. High frequency direct body-body contact as occurs during tackling and collisions, may result in additional tissue damage as shown in a recent study (Smart et al., 2008). This ‘trauma’ induced muscle damage has been previously observed in other contact sports such as boxing (Zuliani et al., 1985). In this study significantly larger increases in serum CK and Mb were observed over a comparable non-contact activity (shadow boxing). In rugby union, significant positive correlations have been observed between the number of tackles and peakMb (r = 0.85) and CK (r = 0.92) respectively (Takarada, 2003). Authors concluded that direct muscle damage as a result of tackle play and damage normally attributable to eccentric muscle contractions were responsible for the large elevations in both markers. The effect of tissue trauma on immunological indices was not investigated. The direct relevance of CK in evaluation of muscle damage has been subject of recent debate (Martinez-Amat et al., 2005). Nevertheless this marker has been used in more recent exercise related studies. During intensified training in rugby league, Coutts et al (2007) found that CK levels within the IT player group were double those of the normal training group. Levels were shown to be reduced after a 7 day taper, similar to findings following 2 weeks of IT in cycling (Halson et al., 2003). Research in other team sports such as American football (Hoffman et al., 2005) have shown increased CK levels following heavy training. Few longitudinal investigations have looked at changes in CK levels over a season. Assessment of plasma CK activity has been suggested to be potentially useful, not as a marker of impending overtraining, but as a means in identifying a state of recent muscle damage or temporary overreaching (Gleeson, 2002).
2.6 BRAIN-IMMUNE SYSTEM INTERACTION: PSYCHOLOGICAL STRESS

The ability of the CNS to modulate the immune system has been well established (Ader et al., 1991). Previous studies have shown that psychological stress can down-regulate or dysregulate immune responses by causing dysregulation of the signals within the complex CNS network (Besedovsky and Del Ray, 1991). Two pathways by which the immune system is modulated by psychological stress include the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal medullary (SAM) axis (Yang and Glaser, 2002).

In addition, primary and secondary lymphoid tissues are innervated by the autonomic nervous system (Besedovsky and Del Ray, 1991). It is thought that stress induced activation of the HPA axis influence the immune system by release of neuroendocrine hormones from the pituitary gland. These hormones subsequently effect regulation of immune cells via presence of neuroendocrine receptors (Yang and Glaser, 2002). In addition, these cells have receptors for catecholamines, enabling them to respond to signals from the SAM axis. The presence of stress hormones can subsequently dysregulate immune responses and have potentially harmful effects on health (Yang and Glaser, 2002). Limited research exits examining the direct role of psychological stress on immune function in high performing athletes. Most evidence comes from occupational medicine. Psychological academic stress has been shown to be involved in modulation of cellular immune responses (Glaser et al., 1997) where investigators have shown stress induced reactivation of latent Epstein barr virus (EBV). Psychological stress has also been implicated in prolonging wound healing time (Kieholt-Glaser et al., 1995), decreases in

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**Figure 2.3:** Schematic representation of brain-immune system connections. Loop A and loop B represent the outflow of “information” from the central nervous system (CNS) to the periphery. Loop C represents the manner by which cytokines convey “information” from the periphery to the CNS (inflow) CRH, corticotropin releasing hormone; ACTH, adrenocortico-tropic hormone; CORT, corticosterone; NE, norepinephrine; E, epinephrine; Enk, enkephalin; SP, substance P; NPY, neuropeptide Y; GH, growth hormone; Mo, macrophages; IL1, interleukin-1; TNF, tumor necrosis factor; IL6, interleukin-6. From: SMITH: Med Sci Sports Exerc, Volume 32(2).February 2000.317
NK-cell activity and leukocyte response to mitogens as well as decreases in virus specific T-cell response to vaccination (see Yang and Glaser 2002 for review). Chronic psychological stress has been shown to down regulate cell immune responses as evidence by decreased T-cell proliferation response to infection and T-cell responses to antigenic stimulation (Kiecolt-Glaser et al., 1987; Glaser and Kiecolt-Glaser, 1997). In these studies, subjects (caregivers) also had a higher incidence of respiratory infections confirmed by physicians. The role of psychological stress on mucosal immunity and respiratory illness is discussed further in section 2.12.

2.7 EXERCISE STRESS: CLINICAL OUTCOMES

2.7.1 Overreaching, Overtraining and Burnout

Excessive physical training, along with incomplete recovery and high general stress may manifest in short-term performance reduction and altered mood states, now referred to as overreaching (OR) (Kreider et al., 1998; Kuipers and Keizer, 1988). In high level sport, OR is sometimes deliberately induced in athletes prior to a period of recovery to induce performance improvements or super compensation (Kuipers and Keizer, 1988). This short-term OR has been labelled functional overreaching (Meeusen et al., 2006) and involves initial temporary deterioration in performance, usually lasting a few days (Fry and Kraemer, 1997). If adequate recovery is not provided during periods of intensified training, non-functional overreaching (NFOR) or overtraining syndrome (OT) may occur (Budgett, 1990; Kuipers and Keizer, 1988; Meeusen et al., 2006; Coutts et al., 2007b); figure 2.4. Overreaching has been previously shown to occur in semi-professional level rugby league players (Coutts et al., 2007b). In the latter study, significant changes in plasma glutamine:glutamate ratio were observed between players undertaking intensified training than those who did not. Corresponding declines in endurance and power related performance tests led authors to conclude that OR can occur in rugby league players with only a relatively small increase in training load above what is considered ‘normal’ (Coutts et al., 2007).

It is important to distinguish OT from OR, the latter reflecting the time period between the application of an exact stimulus, and subsequent recovery and adaptation (Smith, 2000). This may last less than 2 weeks (Fry et al., 2006). To date, no reliable clinical tests for OT or NFOR have been discovered although, like OR, performance decrements and altered mood states are usually evident throughout the recovery period. OT may also cause immuno-suppression, with higher incidence of infection and slower wound healing observed in the
over-trained athlete (Hiscock and Mackinnon, 1998) and last longer than a few weeks or even months (Coutts et al., 2007). Diagnosis of OT has been scientifically difficult because OT can result from a multitude of factors including anabolic/catabolic imbalance, hormonal dysfunction of hypothalamic-pituitary axis, amino acid imbalance, and autonomic dysfunction together with non-training stressors (Smith and Norris, 2000).

![Overreaching vs Overtraining](image)

![Training Continuum](image)

**Figure 2.4:** Training continuum showing difference between overreaching (OR) and overtraining (OT) occurring as a result of applied training stimulus/exercise stress or intensified training program (ITP).

It has been reported that < 0.1% of the general population (Shephard, 2001) show symptoms of OT or NFOR. Documentation of the prevalence of this condition is also limited in team sports (Filiare et al., 2001; Naessens et al., 2000) with most reports stemming from anecdotal evidence (Kentta et al., 2001; Koutedakis and Sharp, 1998; Naessens et al., 2000). Symptoms of OT have been reported to occur in 30-50% of high level soccer players during a competitive season (Naessens et al., 2000; Lehmann et al., 1992). Others have reported that between 7-30% of all elite athletes may show signs of OT at any given time (Kentta et al., 2001) with rates of up to 21% previously noted in swimmers (Hooper et al., 1995). In two separate studies in elite US distance runners; Morgan et al (1987b; 1988) it was found that 60% of women and 64% of men reported being overtrained at some point in their career. Care should be taken when interpreting prevalence of OT given the varied diagnosis criteria between studies. Causative factors pertaining to this condition are thought to arise from excessive training fatigue, inappropriate training structures, sharp increases in training load, monotonous training, travel and other social factors (Meeusen et al., 2006; Lehmann et al., 1997; Foster, 1998).
Despite the use of the term athlete ‘burnout’ in sport, it is somewhat anomalous to find that no common definition exists (Fender, 1989). One thing agreed is that burnout represents a reaction to chronic stress (Smith, 1986, 1989, Cresswell and Eklund, 2005). According to Fender (1989), burnout in sport should be considered as a ‘reaction to the stresses of athletic competition that can be characterized by feelings of emotional exhaustion, an impersonal attitude toward those the athlete associates with, and decreased athletic performance.’ Other researchers have termed burnout as an ‘experiential syndrome that is characterised by a sense of mental and physical exhaustion’ (Cresswell and Eklund, 2006). One of the consequences associated with burnout experiences in athletes is the shift from a self-determined and passionate engagement within their sporting activity to a less motivated and lethargic state, often leading to complete withdrawal (Gould et al., 1996). In a recent study in rugby union, changes in burnout were assessed using the Athlete Burnout Questionnaire (ABQ; Raedeke and Smith, 2001). Authors showed that ‘Burnout’ experiences amongst professional players in New Zealand were associated with injury, non-selection, rugby experience and team membership (Cresswell and Eklund, 2006). Furthermore, changes in burnout were associated with playing position; with greater scores for exhaustion reported during in season within the forward playing group. Previous burnout research in professional rugby revealed that players participating in England exhibited two to three times the level of exhaustion compared to their New Zealand counterparts (Cresswell, 2005). This was substantiated in a following study leading into the autumn internationals (2006) where half of the England squad showed signs of mental and physical exhaustion (Ipsos MORI, 2006). Few studies have assessed burnout from physiological endpoints. Evidence suggests that burnout or emotional exhaustion results in reduced HPA activity with hyposecretion of cortisol under basal conditions (Pruessner et al., 1999), similar to findings seen in people suffering from chronic fatigue syndrome (Demitrack, 1994).

2.7.2 Changes in athlete mood

It has been suggested that the best gauge of overreaching and overtraining is how the athlete feels. The athlete may be transformed from an outgoing, enthusiastic, sociable, highly competitive individual, to being constantly tired, depressed, and uninterested in training and competing (Armstrong and Vanheest, 2002; Foster and Lehman 1997; McKenzie, 1999; Morgan et al., 1987; Stone et al., 1991, Urhausen and Kinderman, 2002). It is known that athletes tend to develop dose-related mood disturbances with increases in training load and stress (Raglin, 1993; Coutts et al., 2006). Low scores for vigour and rising scores for negative
moods such as depression, tension, anger, fatigue and confusion have been previously found with increasing training load in swimmers (Morgan et al., 1987, 1988). Changes in mood state are also evident before drops in performance (Armstrong and VanHeest, 2002). Conversely training volume reductions usually result in improved psychological state (Morgan et al., 1987, 1988, Berger et al., 1999; Martin et al., 2000). The exact reasons for changes in mood and psychological well-being with heavy training are unknown but may represent an athlete’s need for rest and recuperation.

It is thought that mood changes reflect underlying biochemical or immunological changes that are communicated to the brain via hormones and cytokines (Gleeson, 2002, Smith, 2004). A recent theory linking changes in mood state with preceding training and tissue trauma has been proposed (Smith, 2004). The author speculates that parallels between heavy training and lethargy can be made with studies of sick or injured animals. In animals, changes in mood and lethargy whilst sick have been suggested to represent a protective survival response and enhance healing. The author states that similar changes in mood are seen in overtrained athletes and that these factors encourage rest and protection from future stressors. Studies directly investigating this in humans remain elusive but it is thought that elevated levels of proinflammatory cytokines (IL-β, IL-6, and TNF-α) are able to communicate with the brain hypothalamus and induce such behavioural changes (Dantzer et al., 1998; Maier and Watkins, 1998). IL-6 in particular has been suggested to act as a biological mediator in linking changes mood state with stress and elevations in this cytokine are known to occur with physical exercise (Pedersen et al., 2001) and psychological stress (Cohen et al., 1999). Furthermore, IL-6 has been associated with stress and depression in humans (Maes, 1995) and has been linked with changes in positive emotional style and illness expression during infection (Doyle et al., 2006). In a previous study (Cohen et al., 1999) the illness symptom/sign response and nasal IL-6 level was reported for 55 adults experimentally infected with influenza. Results were monitored and compared between groups defined by pre-exposure perceived stress. Authors showed that greater stress predicted greater IL-6 production, symptoms and nasal secretions while analysis supported IL-6 production as a potential pathway linking perceived stress to illness (Cohen et al., 1999). In a recent study investigating innate immune responses to intensified training in athletes, chronic elevation in IL-6 was observed along with increased feelings of fatigue and generalised malaise (Robson-Ansley et al., 2007). Consequently the role of this cytokine in documented mood disturbances through the HPA axis seems justified.
2.7.3 Increased predisposition to Illness

Aside from burnout and overtraining, athletes engaged in heavy training programs, appear to be more susceptible to infection (Gleeson, 2006). Sore throats and flu like symptoms have been reported to be more common in athletes than in the general population, and once athletes are infected, colds may last longer (Heath et al, 1991; Nieman et al., 1990, Peters and Bateman, 1983). Epidemiological and experimental studies show that, during the incubation period of an infection, depending on the pathogen, exercise may worsen the disease outcome (Friman and Ilback, 1992). It is thought that this increased predisposition to illness in athletes undertaking heavy training is resultant from changes in host protective immunity. While people get sick for a variety of reasons, preliminary studies have indicated that the incidences of banal infections are related to chronic or acute heavy exertion (Foster and Lehmann, 1997; Lehmann et al., 1993., Peters and Bateman, 1983). Athletes engaged in heavy training programs appear to be more susceptible to infection (Gleeson, 2006) and the ability to compete is reduced during sickness (Friman and Ilback, 1998). Most of these infections are upper respiratory in nature. Indeed, upper respiratory illness (URI) is the most common medical condition affecting elite athletes (Nieman, 1994; Fricker, 1997; Neville et al., 2006, Mackinnon, 2000). It is also the most common medical condition affecting athletes at both the summer and winter Olympic Games (Hanley, 1976; Reeser et al., 2003), the most frequently reported cause of disability among athletes and have been suggested to cause more lost training days than all the other diseases combined (Weidner, 2001; Beck, 2000).

2.8 EXERCISE AND ILLNESS

Illness is experienced by almost 90% of athletes during training for competitions at some point during a given calendar year (Fricker et al., 2000). Increased incidence of banal infections has also been indicated as an early marker of overtraining in athletes (Foster and Lehmann, 1997; Lehmann et al., 1993., Budgett, 1998). This is thought to be related to excursions above individually identifiable thresholds of training load and strain (Foster et al., 1998). Thus exercise and illness have been linked indirectly.

2.8.1 How much is too much?

Among elite athletes and their coaches, a common perception is that heavy exertion lowers resistance and is a predisposing factor to URTI’s (Hanley, 1976; Jokl, 1974). Much evidence linking heavy exercise with increased infection incidence comes from acute studies. It was
reported that runners who completed an ultramarathon (56 km) reported a 2-fold greater incidence of URTI (33% vs 15%) within 2 weeks of race completion compared to controls (Peters and Bateman, 1983). In a similar study, Nieman et al (2006) showed that nearly 1 in 4 runners reported a URTI episode during the 2-week period following a 160-km race. These results were not confirmed in a similar study (Ekblom et al., 2006). Authors failed to observe a difference in infection incidence 3 weeks after a marathon race compared to the 3 weeks leading up to the race. However, among the group of runners who experienced a URTI episode in the 3 weeks before the race, 33% of these also experienced an URTI episode after the race (vs 16% in runners with no URTI preceding the race). This suggests that exercise stress may have allowed for reactivation of the virus responsible for the pre-race infection or an extended duration of the infection when the latter occurred within the few days before the marathon. Data from animal studies have supported the finding that one or two periods of exhaustive exercise after inoculation leads to more frequent appearance of infection and higher illness rate (Davis et al., 1997). In the latter study mice were exposed to rest, 30 min of moderate exercise, or 2.5-3-h of exhaustive exercise after intranasal infection with herpes simplex virus (HSV-1). Investigators showed that when mice were exercised to fatigue, overall mortality during a 21 day period was greater in exercised mice than controls or moderately exercised mice.

![Figure 2.5: J-shaped Curve as devised by Nieman, (1994).](image)

Limited data exists investigating the effects of chronic exercise on infection incidence. In a previous survey on Danish elite orienteers, an average of 2.5 URI's per year were reported compared with 1.7 for age, sex and occupation matched controls (Linde, 1987). Although non-significant, the average duration of URI was 7.9 days in the study group and 6.4 days in the
control group. The amount of physical activity reported by the control group was not reported. No association has been found between training load and illness incidence in collegiate women’s basketball across a season (Anderson et al., 2003). Clear classification of illness criteria was not reported while markers of immune status were not measured. Increased URTI incidence has been reported in male cadets over 3 weeks of physical conditioning (Gomez-Merino et al., 2005). Interestingly a significant correlation was observed between illness incidence and NK cell number. In a recent study by Spence et al (2007), elite athletes were more likely to experience a URTI or unidentified URI’s when compared to recreationally competitive athletes over a 5 month training and competition season. In turn, sedentary controls displayed higher illness rates then recreationally competitive athletes. It is thought that this increased predisposition to illness in athletes undertaking heavy training may be resultant from exercise induced changes in host protective immunity (Nieman et al., 1990; Davis et al., 1997).

It is not known whether or not observed increases in illness incidence are resultant from changes in exercise intensity, preceding volume or overall training history. In the study by Heath et al. (1991), it was shown that running mileage for a year appeared to be the most important determinant in URTI development. Individuals who ran more than 3.8 miles per day, on average had a 2-fold greater incidence of URTI than those running 1.3 miles or less per day. In another study on marathon runners (Nieman et al., 1990), training more than 97 km-per-week doubled the odds of self-reported URI’s in the 2 months prior the race, compared with runners training less than 32 km-per-week. In summary, these reports suggest that athletes engaged in heavy training do display a higher incidence of common colds or URTI’s than corresponding controls. This increased illness potential seems to occur in or around time periods where athletes are involved in heavy training and/competition. Conversely regular moderate exercise is associated with a reduced vulnerability to URTI’s (Nieman et al., 1990; Matthews et al., 2002; Spence et al., 2007). This apparent exercise-illness relationship has been described in the form of the J-shaped curve (Nieman, 1994; figure 2.5).

This model predicts that individuals who exercise moderately are at less of a risk of infection. In comparison, those who exercise heavily are at an enhanced risk when compared to sedentary and recreational counterparts. Substantial evidence showing a protective effect of moderate exercise on illness potential is lacking. In one randomized controlled study of 36 women, exercise subjects walked briskly for 45 minutes, 5 days a week over a 15-week period (Nieman et al., 1990). It was shown that those who exercised experienced one-half the number of days
with URTI symptoms than that of the sedentary control group. In another study, incidence of common cold in elderly women during a 12-week period was found to be significantly lower in subjects who exercised moderately for ~1.5 hours per day than those in the sedentary control group (Nieman et al., 1993). Furthermore, an increased incidence of URTI’s have been reported in subjects with either low or extremely high total daily energy expenditures, with lowest URTI incidence in subjects with moderate energy expenditures (Novas et al., 2002). A recent comprehensive surveillance study has provided the most compelling evidence for this exercise-illness relationship to date (Spence et al., 2007). In this study, a J shaped curve was observed within elite, recreational and sedentary subjects via pathogenic identification of illness (Spence et al., 2007).

### 2.8.2 Effects of upper respiratory illnesses (URIs) on performance

Upper respiratory infections have the potential to significantly disrupt the athletes training schedule (Midgley et al., 2003). During the symptomatic stages of infection, not only is quality of training time lost, but attaining pre-infection performance may take several more weeks after the symptoms of the acute infectious stage have subsided (Midgley et al., 2003). In some cases, URTI's may persist for up to 10-20 days (Shepard, 2000) while in others, a viral infection may lead to a debilitating state know as ‘postviral fatigue syndrome’ (Maffulli et al., 1993; Parker et al., 1996). Athletes with this condition have been suggested to suffer from lethargy, easy fatigability, and myalgia which can last for months (Nieman, 2000).

Several case histories have been published demonstrating sudden and unexplained deterioration in athletic performance which can be traced to a recent URTI or subclinical viral infection running a protracted course (Parker et al., 1996; Friman and Ilback, 1998). Pyne et al. (2000) reported that swimmers who had not reported illness during competition tended to have a higher level of performance than those people who had reported illness. In a subsequent study (Pyne et al., 2005), mild illness was shown to have a trivial effect on the competitive performance within elite female swimmers; and a substantial though small harmful effect on male swimmers. No severe episodes of systemic illness were noted in this study and so the future effects of this on performance could not be established. Previous data has shown decreases in various measures of physical performance such as isometric strength, isokinetic strength, and submaximal exercise during an infectious illness (Daniels et al., 1985). A 30% decrease in isotonic muscle strength has been observed following viral inoculation, (Friman et al., 1985). Short-term exercise and pulmonary function does not seem to be affected despite viral (rhinovirus) inoculation (Weider et al., 1997). Conversely, decreases (17%) in oxygen
uptake at the onset of blood lactate accumulation have been reported following viral illness before a slow recovery (Jakeman, 1993). In this study, pronounced tachycardia and differences in energy metabolism were also reported.

2.8.3 Symptomology and clinical course

The incidence of colds has been reported to vary between 2-4 per individual in a given year (Turner, 1998; Gwaltney et al., 1966; Myint and Taylor-Robinson, 1996; Larsson, 1996). Rhinoviruses and coronaviruses are thought to account for between 50% and 75% of upper respiratory tract infections (Johnston and Holgate, 1996). Viral URI’s, commonly known as the ‘common cold’ symptomology is normally characterised by rhinorrhoea (runny nose), nasal obstruction, pharyngitis and cough. While the hallmark of a URTI or cold is nasal discharge and obstruction, this can vary considerably. Some colds may qualify as ‘dry’ in nature (Larsson, 1996). Although fever, myalgia and systemic illness are infrequent, symptoms relating to such nonetheless occur. The incubation period for rhinovirus infection (most common virus) proceeds very rapidly (8-12 h). It is generally considered that most symptoms should recede within 7-8 days of initial infection, with peak occurrence between the second and third days (Johnston and Holgate, 1996; Gwaltney, 2002; Spence et al., 2007). However, 25% of cases may last longer (Gwaltney, 2002). In a recent study, those subjects who displayed symptoms with an identified pathogen had significantly worse functional impairment scores on days 3–4 than those without an identified pathogenic cause (Spence et al., 2007). On occasion, symptoms may also involve the lower respiratory tract. The viruses most likely to produce these effects in adults are thought to be influenza, para-influenza, respiratory syncytial (RS) virus and especially adenoviruses (Larsson, 1996).

It has been proposed that a number of non-infectious agents (Spence et al., 2007) or upper airway inflammation (Müns, 1993; Bermon, 2007) may contribute to upper respiratory illnesses in athletes. In a recent comprehensive study, Spence et al (2007) showed that the presence of infectious agents were responsible for only 30% of observed URTI cases in elite, recreational and sedentary controls over a 5 month period. In another study, the existence of viral etiology was found in 69% of illness cases reported by 200 young adults over a 10-month period (Makela et al., 1998). Susceptibility is not related to cooling of the body (Johnston and Holgate, 1996) as is traditionally viewed. However, lower rates of respiratory illness occur in summer, with rhinoviruses still the most frequently isolated virus at this time (Monto, 2002).
2.9 MUCOSAL IMMUNITY: ROLE IN HOST DEFENCE

Mucosal immunity in association with innate non-specific defence factors, form the first line of defence against pathogens, allergens, and antigens presented at mucosal surfaces (Kimura et al., 2007). There is a higher risk of infection at epithelial surfaces of the body, such as the respiratory tract, gastrointestinal or urogenital tract, and the skin, that interfere with and separate the host from, the external environment (West et al, 2006). More than 90% of all infections involve the mucosa with regard to microbial colonization or entry into the body (Brandtzaeg, 2003). Such surfaces are protected from invading micro-organisms by an array of immune related mechanisms, many of which primarily belong to the innate mucosal defence system. Although the mucosal immune system does not function independently from the systemic immune system, it is nevertheless regarded as a distinct entity given its many localized defence factors (Toy and Mayer, 1996).

2.9.1 Exercise, viral entry and mucosal protection

It has been reported that each day, an average individual warms, filters and humidifies ~ 14,000 L of air (Cole, 1982). This air contains suspended particulate matter that is contaminated with viruses, bacteria and fungi (Eccles, 2002). Particulate matter > 10 µm in diameter is first deposited in the nose, trapped in the thin mucus blanket that covers the nasal respiratory epithelium and then slowly moved by rapidly beating cilia to the nasopharynx. Here it is swallowed and sterilized in the acid of the stomach (Larsson, 1996). The mucociliary transport system clears the airway from dust, pollen, and microbes while mucus, secretory antibodies (mainly IgA) are present as the first line of immunological defence (Larsson, 1996). Anything which acts to compromise this defence may lead to an increased chance of respiratory infection. Impaired nasal mucociliary clearance has been previously shown in long distance runners for several days after a marathon (Müns et al., 1994). These findings are important in terms of pathogenic protection since ventilation rate and depth may change considerably with exercise. Increases in ventilation volume from 5 l.min⁻¹ at rest to 160-180 l.min⁻¹ during heavy exercise in healthy males are typically observed (McArdle et al., 1996). Inhalation of largely unfiltered air may pose additional challenges to local immune responses in the fight against environmental pathogens. Furthermore, with incremental exercise athletes start to breathe through their mouth rather than through the nose, thus bypassing the nasal filter mechanism (Niinimaa et al., 1980). Supplementary to this may be a drying of oral mucosa and subsequent decrease in saliva flow rate. The importance of saliva flow has been previously established in oral health (Atkinson and Wu, 1994; Rantonen and Meurman, 2000).
2.9.2 Constituents of human saliva: role in host protection

Saliva contains a large number of organic and inorganic compounds of which the predominant constituent is water (97-99.5%), which enters saliva from plasma across the acinar cells (Young and van Lennep, 1979). A wide array of antimicrobial proteins and peptides protect the oral cavity against microbial infection (Rudney et al., 1991; Bosch et al., 2002). Two mechanisms forming part of mucosal immune defences in saliva involve a group of humoral agents called antimicrobial peptides (AMP’s) and antibody mediated mucosal defence, of which immunoglobulin A (s-IgA) is predominant. AMP’s are constituent and inducible factors of secretions at mucosal surfaces that display activity against a broad range of pathogens (West et al, 2006). One of these AMP’s, lysozyme may be important in mucosal defence to pathogens given its ability to destruct bacterial cell walls (West et al., 2006). Salivary IgA is also thought to be important in defence against infections via its ability to immobilize microorganisms or prevent their attachment to mucosal surfaces (Gleeson et al., 2005). Prevention of infection is assisted by immune exclusion, a process mediated by these antibodies in cooperation with innate non-specific defence mechanisms (Gleeson et al., 2005).

2.9.3 Saliva release and flow

Whole unstimulated saliva is a mixture of secretions which enter the mouth in the absence of exogenous stimuli such as chewing. Saliva is secreted from submandibular glands (65%), parotid glands (23%), and sublingual glands (4%) collectively known as the major salivary glands. Secretions from minor glands (8%) situated on the tongue, palate, and buccal and labial mucosa is also known to exist (Crawford et al. 1975, Ferguson, 1999). Salivary glands are innervated by both the sympathetic and parasympathetic nerves (Garrett, 1987; Chicharro et al., 1998). Generally, an increase in sympathetic stimulation (via noradrenaline) leads to higher salivary protein concentrations, whereas increased rates of fluid secretion are controlled principally by parasympathetic nerves (via acetycholine) (Proctor et al., 2003).

Continuous supply of both immune and non-immune factors in the mouth is another important functional role of saliva (Rantonen and Meurman, 2000). Therefore, changes in saliva flow may limit the availability of protective antibodies such as IgA at the oral surface, an effect that may be detrimental to host defence (Laing et al., 2005). Saliva secretions also protect the oral mucosa through a mechanical washing effect (Bishop et al., 2000) via flushing of microorganisms and their products into the gut. The secretary volume of saliva each day through salivary glands approaches 750 ml, equating to normal flow of approximately 0.3-0.5
ml.min\(^{-1}\) (Chicharro et al., 1998). Values below 0.1 ml.min\(^{-1}\) are considered as hyposalivation, and values between 0.1-0.25 ml.min\(^{-1}\) as low (Tenovuo and Lagerlöf, 1994). It has been suggested that saliva flow rate represents the single most important defence factor to affect oral health (Rantonen and Meurman, 2000).

Decreased flow rates have been observed after strenuous exercise (Blannin et al., 1998; Walsh et al., 2002), with values returning to normal after approximately 2-3 h after exercise (Li and Gleeson, 2004; 2005). To date, authors have cited a possible drying effect of breathing cold air (Tomasi et al., 1982), increases in SNS activation (Chicarro et al., 1998) and dehydration (Walsh et al., 2004) to explain the secretion of smaller amounts of more concentrated saliva after prolonged exercise (Laing et al., 2005). Its importance in oral protection can be seen in cases where disruption of normal mucosal processes occurs, as in patients suffering from autoimmune disorders like Cystic fibrosis and Primary Sjögren's syndrome (SS). Lin et al. (2001) have shown that increased incidence of oral candidial infections is related to lower flow rates in HIV-infected patients. Furthermore, increased incidence of oral infections has been shown to occur in individuals suffering from xerostomia (dry mouth syndrome; Fox et al., 1985). To summarise, mucosal protection is dependent on synergistic interactions between its various chemical agents and overall salivary output. In addition to evaluation of absolute concentrations, the role of saliva flow in the secretion of identified markers should be considered important when evaluating health status.

2.10 SALIVARY IGA

Secretory IgA consists of a J-chain-containing dimeric IgA (dIgA) antibody produced by IgA antibody forming cells (AFC’s) in the salivary glands (Kimura et al., 2007). It is the predominant form of antibody that mediates specific immunological defence at mucosal surfaces in response to an antigenic stimulus (Brandtzaeg, 1989, 1992; Proctor and Carpenter, 2001). Importantly, in contrast to IgG antibodies, IgA antibodies do not activate complement and are therefore considered non-inflammatory (Brandtzaeg, 2003). Within humans, two distinct subclasses of immunoglobulin (Ig) A have been identified based on their amino acid sequence and glycosylation (Mestecky and Russell, 1986). Subclass IgA1 predominates (approximately 90%) in serum, whereas IgA2 predominates in most mucosal secretions (Delacroix et al., 1982). Saliva however, contains approximately 60% IgA1 in normal adults (Delacroix et al., 1982; Tappuni and Challacombe, 1994) and has been found to be as high as 80% in elite swimmers (Gleeson et al., 1999).
Typically, IgA levels are expressed in terms of:

1. Absolute IgA concentration ($\mu g.ml^{-1}$).
2. IgA secretion rate ($\mu g.min^{-1}$) to account for the total amount of IgA appearing on the mucosal surface per unit time. Secretion rate is obtained by multiplying raw IgA concentration by the saliva flow (ml.min$^{-1}$).
3. IgA relative to total protein ($\mu g.mg protein^{-1}$) to account for changes in salivary protein content by drying of the oral surfaces with exercise (Koch et al., 2007). Exercise typically produces an increase in total protein in saliva; thus apparent decreases in IgA:Pro are likely to reflect changes in the total protein content of the saliva sample rather than fluctuations in IgA (Blannin et al., 1998).
4. IgA relative to saliva osmolality, IgA-Osm (mg.mOsm$^{-1}$) to correct for low salivary flow rates (Blannin et al., 1998; Sari-Sarraf et al., 2007).

2.10.1 Modulation

S-IgA is a J-chain-containing dimeric IgA (dIgA), of which 95% normally appearing in saliva is produced by local gland-associated immunocytes rather than being derived from the serum (Brandtzaeg, 1989; Kimura et al., 2007). After production in the submucosal glands, d-IgA then binds to a receptor (polymeric immunoglobulin receptor, pIgR) located on the mucosal epithelium. This complex is subsequently transported across the mucosal epithelium before being released into saliva as IgA via combining with the secretary component (SC) (Mostov, 1994; Brandtzaeg 1998; Kimura et al., 2007). Modulation of s-IgA secretion is regulated by its synthesis (days) (Toellner et al., 1998) or transcytosis (minutes) (Kugler, 1999) and so alterations with exercise are likely induced via modulation of the transepithelial secretory process rather than plasma (B-lymphocyte) activation and plasma cell differentiation. This theory is based on studies showing rapid changes in s-IgA concentration and secretion rate with selected stimuli (Hucklebridge et al., 2000) and the fact that salivary glands are densely innervated by the autonomic nervous system (Morse et al., 1983). Secretory IgA has a half life of 3-6 days and a synthesis rate of $\sim$66 mg/kg/day (Mestecky, 1993). Secretion of s-IgA has been shown to be increased by both parasympathetic and sympathetic stimulation in rodents (Carpenter et al., 2000). Adrenaline has been shown as a key mediator in s-IgA release via increased mobilization of the polymeric Ig receptor (Carpenter et al., 2004).
2.10.2 Role of s-IgA in oral protection

Specific protection is afforded by several recognized mechanisms: (i) interfering with microbial adherence to mucosal surfaces (Mestecky et al., 1999); (ii) inhibiting pathogen colonization via immune exclusion on the mucosal surface and within virus infected epithelial cells (Brandtzaeg, 2003); (iii) complexing with antigens across at the basolateral surface; and (iv) interrupting replication of intracellular pathogens during transcytosis (intracellular neutralization) (Mazanec et al., 1993b; Yoa et al., 1991), (v) binding antigens in the lamina propria and facilitating their excretion through the epithelium back into the lumen (immune excretion) (Mazanec et al., 1993; Lamm, 1998).

This anti-viral role at mucosal surfaces has been recently shown in an exercise setting (Gleeson et al., 2002). Changes in s-IgA were shown to mirror the pattern of Epstein Barr virus (EBV) reactivation in saliva, with low levels of s-IgA preceding the appearance of viral EBV-DNA and high levels appearing in response to EBV reactivation. While measurement of total salivary IgA antibody levels may not directly reflect resistance to specific respiratory infections, the role of sIgA in limiting respiratory infections has been recognized (Brandtzaeg, 1992). A previous meta-analysis has concluded that low levels of s-IgA could compromise immune resistance to respiratory infections (Jemmott and McClelland, 1989). Measurement of s-IgA is thought to be an indicator of the functional status of the entire mucosal immune system (Mestecky, 1993). Lowered concentrations of s-IgA or chronic s-IgA deficiencies are associated with an increased frequency of URTI episodes (Gleeson et al., 1999), recurrent URTI (Isaacs et al., 1984), or reduced protection against certain infections (Asahi et al., 2002) in mice.

2.10.3 Effects of exercise on s-IgA

Salivary IgA concentration has been shown to be reduced after intense exercise in various modes of exercise including swimming (Gleeson et al., 1995; 1999; Tharp and Barnes, 1990), triathletes (Steerenberg et al., 1997), kayaking (Mackinnon et al., 1993), running (Akimoto et al., 1998; Nieman et al., 2002) and short-term high intensity exercise (Fahlman et al., 2001). Analysis of changes immediately post-exercise have shown ambiguous results with some studies reporting increased (Dimitriou et al., 2002, Sari-Sarraf et al., 2007) or decreased s-IgA concentrations (Mackinnon et al., 1987; Tharp and Barnes, 1990, McDowell et al., 1991). Only one study has investigated acute responses in IgA levels in rugby union, with no changes in concentration reported after a game (Koch et al., 2007). Difficulties in accounting for
variations in fluid intake, sweat rate, game intensity (collegiate level) and relatively small sample size \((n = 16)\) make conclusions hard to interpret. Indeed, some players displayed decreased in IgA levels whereas others did not (Koch et al., 2007). In those studies reporting decreases in s-IgA, recovery of s-IgA levels to pre-exercise levels normally occurred within 1 hour. Exercise intensity of at least \(70\% \, \text{VO}_{2\text{max}}\) seems to be necessary for inducing decrease in IgA concentrations.

It is possible that differences in timing of sample, nutritional intake, exercise intensity/duration and/or degree of sympathetic/parasympathetic activation between studies may account for mixed findings. The latter point is worthy of consideration since salivary glands are innervated by both parasympathetic and sympathetic nerves (Chicharro et al., 1998). In a recent soccer specific study (Sari-Sarraf et al., 2007) involving two exercise trials, s-IgA concentration was shown to initially increase immediately post-exercise (90 min intermittent activity). S-IgA concentration further increased (200%) when two exercise bouts were completed on the same day while concentrations decreased (88%) below pre-exercise values (trial-1) when values were analysed ~2.5 h after exercise cessation on the second trial. Thus it is possible that the degree of sympathetic activation and subsequent timing of sample collection may account for differences in observed IgA results. Degree of sympathetic activity has been recently shown to influence IgA mobilisation in the high-intensity trials (Allgrove et al., 2008). In general, it has been suggested that any observable decreases occur between 2 and 24-h after prolonged strenuous exercise (Mackinnon et al., 1987; Gleeson et al., 2001; Sari-Sarraf et al., 2007).

Mixed findings have also been reported for s-IgA secretion rate (s-IgA concentration \(\times\) flow). Studies investigating changes with acute exercise have reported either no change (Blannin et al., 1998; Bishop et al., 1999) or a decrease in IgA secretion rate (Steerenberg et al. 1997; Nieman et al., 2002, Pacque et al., 2007). In the latter study, s-IgA secretion rate had not returned to normal the morning after an ultra-endurance race in male and female participants. Possible decreases in secretion rate could be resultant from changes in immunoglobulin concentration and/or saliva flow. In reviewing the literature it becomes apparent that changes in saliva flow are not always reported.

Few longitudinal studies have looked at s-IgA frequently enough to draw definitive conclusions regarding the effects of stress accumulation or exercise chronicity on this marker. Early studies have shown decreases in s-IgA levels with intense training in skiers (Tomasi et al., 1982) and chronic high intensity training over 3 months in swimmers (Tharp and Barnes,
In a recent study, Nakamura et al. (2006) failed to observe a significant relationship between decreased s-IgA levels and appearance of URTI in soccer players (2 month period). Putlur et al. (2004) observed lower (non-significant) levels of s-IgA in competitive female soccer students over the course of a 9 week competitive season in comparison to recreationally active controls. Quantitative training related data was not provided. Rama et al (2007) observed decreases in s-IgA after the second week in a 5 week football pre-season. Declines in s-IgA values were observed by week 2 and continued to decline (50%) until week 4. Significant decreases in both s-IgA and the secretion rate of s-IgA have been observed over a 12 month period in collegiate American footballers (Fahlmann and Engels, 2005). Concentrations of s-IgA have been shown to be significantly decreased at the end of an 18.5 week basic infantry training program (Brenner et al., 2000). No associations between s-IgA concentration and incidence of URTI were reported in the latter study although it’s worth noting that saliva was only taken twice over the monitoring period.

In studies on elite swimmers, a significant decline in salivary IgA concentration was shown over a 7 month season (Gleeson et al., 1995; 1999), possibly implicating the role in chronic exercise stress in s-IgA levels. The clinical significance of the reported decline however was questionable since not all swimmers undertaking the training program were prone to increased infection and saliva was only collected in the early and late phases of the program (4 months apart). Although exercise was reported as being intense, training loads in the time preceding saliva collection were not reported. Aside from its role in oral protection, regular monitoring of s-IgA has been suggested as a mechanism in monitoring overtraining (Mackinnon et al., 1994; Shephard and Shek, 1998) and excessive training in individual athletes prone to infection (Gleeson et al., 2000). Training at an intense level over many years has been suggested to result in a chronic suppression of salivary immunoglobulin levels (Gleeson and Pyne, 2000). It is possible that chronic exercise induced depression in s-IgA may be resultant from inadequate recovery between training and/or competition. Tiollier et al (2005a) demonstrated a significant decrease in s-IgA concentrations in army commandos after a 5-day combat course (preceded by 3 weeks of training). In this study, s-IgA levels were shown to recover to baseline levels within a week of pre-training values. In another study by the same investigators (Tiollier et al., 2005b), 2-weeks of active recovery (with reduced training stimulus) failed to allow for proper s-IgA recovery after progressive declines were reported over an 18-day living high-training low experiment within elite skiers.
2.10.4 Role of s-IgA in illness development

While it has been suggested that exercise induced suppression of s-IgA is associated with increased URTI risk (Gleeson et al., 1999), direct links between changes in s-IgA and increased illness potential remains equivocal. This is possible because of a time-lag between possible s-IgA decreases and development of URTI. Few studies have monitored s-IgA before and after development of a URTI on a regular basis, making it difficult to formulate definitive conclusions about s-IgA kinetics and URTI development. Although non-significant, notable decreases in saliva flow and s-IgA secretion rate were observed by Nakamura et al. (2006) 3 days before the appearance of a URTI in soccer players over a 2 month period. A previous study carried out in hockey and squash players (Mackinnon et al., 1993) showed that players who developed symptoms of URTI had reductions in salivary immunoglobulin A (s-IgA) of 22% and 23% within 2 days of symptom onset. In a recent study, significant reductions (28%) in s-IgA concentration were shown to occur 3 weeks prior to URI episodes within elite yachtsmen (Neville et al., 2008). In this study, recovery of s-IgA was shown to occur 2 weeks after appearance of a URI.

Nieman et al. (2006) have observed decreases in salivary IgA secretion rate in runners after completing an ultramarathon (160-km). Of those who reported a URTI episode (1 in 4 runners), the fall in IgA secretion was significantly greater than those who did not report a URTI. Rama et al (2007) observed decreases in s-IgA after the second week of a 5 week over a football pre-season. Declines in s-IgA were observed during the weeks with highest training loads while observed URTI’s reported by players occurred during weeks 3-4 when s-IgA was at its lowest. Reduced levels of s-IgA have been previously implicated in URTI development within elite Russian Olympic athletes (Levando et al., 1988). Coincidentally, these decreases in s-IgA and increase in URTI occurred during a period of maximum intensity training. Frequency of illness has been shown to be significantly greater in soccer players compared to a control group over the course of a 9 week competitive playing season (Putlur et al., 2004). Lower, although non-significant levels of s-IgA were also observed in the exercise (soccer) group. Previous studies in elite swimmers have revealed that low pre-season concentrations of total IgA (Gleeson et al., 1999a), and in particular IgA1 (Gleeson et al., 1999b) are associated with high numbers of infection episodes. A pre-season total IgA concentration below 35 mg/L was associated with high numbers of infections (Gleeson et al., 1999a) while regression analysis predicted an additional infection for each 10% drop in pretraining salivary IgA per month. A follow up study however found no correlation between changes in s-IgA levels and infection risk, despite significant decreases with training (Gleeson et al., 2000).
While supporting evidence does exist for this exercise-illness relationship, the majority of research investigations have been carried out on endurance trained or recreational athletes. Limited evidence exists on the aetiology of illness in other sporting populations, in particular those involved in team sports. A season of training in American football has been recently shown to result in a significant decrease in both s-IgA and the secretion rate of s-IgA as well as an increase in the incidence of URTI (Fahlman and Engels, 2005). In light of the indefinite findings, it can be concluded that any interpretation of changes in s-IgA should view low levels as a risk factor for infections only (Gleeson et al., 2004) and not a direct cause per se.

2.10.5 Reasons for observed change

Although the physiological mechanisms underlying observed declines in s-IgA are unclear, it is likely that a combination of both neural and endocrine factors are involved given their influence on immune responses to exercise (Fleshner, 2000; Pedersen and Steensberg, 2002). Regulation of secretion and synthesis of s-IgA is not only dependent on prior antigenic stimulation, but is also under strong neuroendocrine control. Hence anything which alters neuroendocrine functioning such as stress may affect s-IgA levels (Teeuw et al., 2004). It has also been suggested that mobilisation of s-IgA is inhibited by the effects of stress induced cortisol release in humans (Hucklebridge et al., 1998); thus implicating the role of exercise in reported IgA changes within athletes. Salivary composition during exercise is influenced by the autonomic nervous system and hypothalamic-pituitary-adrenal axis activity (Chicarro et al., 1998), of which salivary cortisol is considered a reliable index. Cortisol has been shown to inhibit transepithelial transport of s-IgA (Sabbadini and Berzi, 1995), inhibit in vivo B-lymphocyte antibody synthesis (Saxon et al., 1978) and has been implicated in the decreased B-lymphocyte antibody synthesis after exercise (Nehlsen-Cannarella et al., 1991).

Previous studies have failed to find a relationship between levels of s-IgA and cortisol in elite cross country skiers subjected to hypoxia and training (Tiollier et al., 2005), following high intensity swim training (Tharp and Barnes, 1990) or 10 weeks of running training (McDowell et al., 1992). Conversely, Hucklebridge et al. (1998) found a negative correlation between salivary cortisol and s-IgA within 30 min of awakening. A possible explanation for poor correlations between cortisol and declines in s-IgA may be because cortisol is the end product of the neuroendocrine stress response in humans. A cortisol mediated inhibitory effect on plasma cell antibody production is plausible since this process is known to require many hours to days (Hucklebridge et al., 1998; Viru and Viru, 2004). This may account for observed
exercise-induced falls in s-IgA concentration between 2 and 24 h after particularly long bouts of exercise (Gleeson et al., 2001; Mackinnon et al., 1987). This theory has been substantiated from studies involving dexamethasone (synthetic glucocorticoid) treatment where significant declines (24-48 h) in s-IgA were recorded following administration (Wira et al., 1990; Wira and Rossoll, 1991; Alverdy and Aoys, 1991). In the latter study, a 76% decrease in IgA concentration was observed. Interestingly, this decrease in IgA was associated with a corresponding increase in bacterial adherence (2.4 fold) and bacterial translocation to mesenteric lymph nodes. These studies were carried out on rats and therefore direct parallels to human IgA response cannot be made with certainty.

Other processes within exercise may account for observed changes in s-IgA. Significant decreases in pIgR mRNA expression were observed after intense exercise in mice, thus implicating the role of exercise on s-IgA transport (Kimura et al., 2007). In this study, a clear suppression in s-IgA was also observed. This is notable given the role of pIgR in the transepithelial process. Consequently, if suppression of pIgR occurs, the quantity of s-IgA secreted within saliva also falls (Shimada et al., 1999). These findings need to be replicated in humans. It has been suggested that chronic stress may decrease s-IgA by reducing recruitment of precursors of plasma cells in the salivary gland or by directly affecting the immunoglobulin producing activity of the plasma cell (Tsujita et al., 1999). Furthermore, strenuous exercise has been shown to result in an up regulation of humoral immunity and simultaneous suppression of cell-mediated immunity (McKune et al., 2006). This down regulation in cell mediated immunity is thought to result in reduced protection against the majority of protective agents (Smith, 2003).

2.11 SALIVARY LYSOZYME (S-LYS)

Lysozyme is a low-molecular-weight cationic protein and represents the main enzyme of the non-specific salivary immune defence (Meyer and Zechel, 2001). Therefore it is considered part of the innate immune system. It is synthesized and continually released by granulocytes, monocytes, and macrophages (Jolle’s and Jolle’s, 1984; Osserman et al., 1973; West et al., 2006) and is present in all mucosal secretions (Pruitt et al., 1999). Traditionally salivary lysozyme is expressed in terms of absolute concentration (mg.L⁻¹) or secretion rate (µg.min⁻¹) (Ng et al., 1999; Koh et al., 2002; Allgrove et al., 2008). However, previous studies have shown that salivary lysozyme is not affected by changes in saliva flow (Rudney, 1989, Perera et al., 1998).
2.11.1 Modulation

Lysozyme is found in saliva via secretion by the mucous membranes of the salivary glands, in particular the submandibular and sublingual glands (Nobel, 2000). Its appearance is also thought to arise from mononuclear cells entering the oral cavity (Perera et al., 1998) through gingival crevices, the production of which has been shown to be increased by inflammatory stimuli (Friedman et al., 1983). The regulatory factors governing salivary gland secretion of lysozyme are largely unknown at present, although systemic or constitutive factors are thought to be involved (Rudney et al., 1994).

2.11.2 Role of lysozyme in oral protection

The role of lysozyme in infectious disease resistance has been previously acknowledged (Jolle’s and Jolle’s, 1984). It exerts its immunological action via its enzymatic effects on the peptidoglycan layer of gram positive bacterial walls (Kmiliauskis et al., 2005). Here it hydrolyses the bonds (beta 1-4 glucosidic linkages) in exposed bacterial cell walls ultimately causing cell lysis and eventual death (Tenovuo, 1989). Other characterised functions of lysozyme include stimulation of neutrophils and macrophages and antimicrobial effects carried out in conjunction with immunoglobulins (Germaine and Tellefson, 1979). It has also been proposed to act as a lytic factor for bacteria which immunoglobulins have bound, mimicking in some respects the complement system in serum. Other antimicrobial properties such as inhibition of bacterial growth, metabolism and de-chaining have also been proposed (Schenkels et al., 1995; Soo-Quee Koh and Choon-Huat Koh, 2007).

2.11.3 Effects of exercise on salivary lysozyme

Very few studies have investigated the role of exercise on s-Lys. Koutedakis et al (1996) reported a significant reduction in s-Lys concentration at the end of both aerobic and anaerobic swimming sessions in elite males while a significant decrease in s-Lys secretion rate was noted after anaerobic sessions only. Furthermore, s-Lys concentrations showed a continual decline over the 2 sessions taken on consecutive weeks. No further studies were carried out on this subject group however. In a recent study, short-duration (~22 min), high-intensity exercise (75% VO2max) has been shown to increase the secretion rate of s-Lys despite no change in the saliva flow (Allgrove et al., 2008). The authors concluded that such changes were as a result of increased sympathetic activity occurring with acute exercise. To the author’s knowledge, no
longitudinal studies have investigated changes in s-Lys with exercise or its association with illness potential.

2.11.4 Role of saliva lysozyme in illness development

Few studies have attempted to correlate changes in salivary lysozyme with appearance of URTI's or other reported illnesses. In those reporting correlations in illness and sickness, findings have been mixed. Rudney et al. (1991) found no significant associations between antimicrobial proteins (including s-Lys) in whole saliva and measures of health status amongst 216 student subjects while Moder and Twetman (1979) showed an inverse relationship between s-Lys and gingivitis in children. Development of oropharyngeal candidiasis in HIV-infected patients has been thought to be a consequence of inefficient lysozyme and lactoferrin concentrations (Laibe et al., 2003). Kmiliauskis et al. (2005) failed to find statistically significant differences between lysozyme concentrations in saliva of immunodeficient subjects and those of healthy controls. s-Lys has been suggested to play an important role in determining resistance or susceptibility to acute bronchitis caused by *Haemophilus influenza* (Clancy et al., 1995) while low levels have been correlated with disease susceptibility (Taylor et al., 1995) and in patients with autoimmune disorders (Koh et al., 2004). Moreover, recent studies have indicated s-Lys as a promising marker into the effects of stress and humour on natural immunity (Perera et al., 1997, 1998).

2.11.5 Reasons for observed change

Lower levels of lysozyme concentration and secretion rates have been reported in a cross sectional study of emergency department nurses who reported higher self-perceived stress (Yang et al., 2002). A negative correlation has been found between perceived stress scales and salivary lysozyme in 39 students (Perera et al., 1997). In the same study, increased levels were found after an examination. Together these findings point to the possible role of neuroendocrine control or degree of sympathetic/parasympathetic activation on subsequent release. As speculated by Perera et al. (1998), macrophages like lymphocytes, contain adrenergic receptors and as such, increases in the levels of catecholamines with stress may be responsible for decreased levels of s-Lys. Additionally, stress whether it be physical or psychological, is known to increase glucocorticoid levels through increased adrenal medulla and sympathetic nervous activity. Increased glucocorticoid levels inhibit the functions of macrophages and monocytes. It has been hypothesized that since macrophages are one of the principle sources of salivary lysozyme (Perera et al., 1997), stress can result in decreased lysozyme secretion (Koh et al., 2002).
2.12 PSYCHOLOGICAL STRESS: MUCOSAL IMMUNITY AND ILLNESS

Psychological stress is associated with susceptibility to the common cold in a dose dependent manner (Cohen et al., 1991). Chronic stress, less diverse social networks, and low levels of positive emotional style have all been shown to predict an increased probability of developing a common cold in persons experimentally infected with an upper respiratory virus infection (Cohen et al., 1991, 1998, 1999, 2003). Changes in illness rates with psychological stress may also be linked to observed changes in mucosal immunity.

Similar to exercise, chronic psychological stress is thought to have down-regulatory effects on mucosal immunity (Bosch et al., 2002) whereas acute psychological stress has been shown to induce increased mobilisation of s-IgA (Ring et al., 2005). Kugler et al. (1996) have reported elevations in both salivary IgA concentration (independent of saliva flow) and cortisol in soccer coaches during competition stress. These findings have been substantiated from previous studies showing increases in s-IgA concentration and/or secretion rate during mentally stressful situations (Bosch et al., 2001; Zeier et al., 1996) or a psychological challenge (Evans et al., 1997; Bristow et al., 1997). Increased secretion rate of s-IgA has been associated with positive mood (Hucklebridge et al., 2000). In turn, chronic psychological stress can lead to impairment of immune system function leaving the individual exposed to greater risk of infection and illness (Herbert and Cohen, 1993; Clow and Hucklebridge, 2001). Recent data have showed that stressful life events are associated with low secretion rates of s-IgA in middle aged and elderly (Phillips et al., 2006). In this study, authors speculated that decreases in IgA production by the local plasma cells or a reduction in the efficiency with which s-IgA is transported were resultant from chronic stress experiences. Lower levels of s-IgA have also been reported in a cross sectional study of 132 nurses who reported higher self-perceived stress (Yang et al., 2002). Incidentally, lower levels of lysozyme concentration and secretion rates were also reported in this study.

Psychological stress has been previously reported to impact negatively on other adaptive immune parameters. In a previous study, reactivation of three latent herpesviruses, Epstein-Barr virus (EBV), herpes simplex virus type-1 (HSV-1) and human herpes-virus 6 (HHV-6) was shown in army cadets (Glaser et al., 1999). Examination and training stress were thought to be contributors.
2.13 HORMONES IN SALIVA

In addition to the many antimicrobial proteins found, human saliva also contains steroidal hormones. Within plasma, these hormones circulate either as free hormones or as hormone bound proteins. Such binding may be non-specific (predominantly bound to albumin) or in the case of some hormones, bound to specific carrier proteins such as sex hormone binding globulin and cortisol binding protein (CBG). With reference to saliva hormone sampling, concentrations reflect the free fraction only in plasma (Hofman, 2001; Lac, 2001) and so offer a better alternative to plasma derivatives.

2.13.1 Saliva Cortisol

Cortisol, the major circulating human glucocorticoid, is a key mediator of systemic and psychological stress responses. Its use as an index of global athletic stress marker is obvious since its release is highly responsive to a variety of stress states. These include physiological stress, nutrition and exercise status (Brandenberger and Follenius, 1975; Brandenberger et al., 1982), and sleep (Pietrowsky et al., 1994). Cortisol (C), is also a powerful natural immuno-suppressant (Petrovsky et al., 1998) making it a hormone of interest in studies evaluating immune function. Salivary C has been shown to positively correlate with anxiety and psychological stress scores (Aardal-Ericksson et al., 1999; Yang et al., 2001). Levels have been shown to increase with acute stress and shown to correlate with pre-examination stress scores in university students (Ng et al., 2003). Parallel increases in serum and saliva C concentrations have been shown to with acute moderate-intense exercise (O’Connor and Corrigan, 1987). Peak plasma values have been shown to occur 30 min after exercise (Hansen et al., 1991). In elite swimmers, the higher the cumulative distance swum, the higher saliva C concentrations were observed over a 37 week period (Chatard et al., 2002).

The use of salivary C represents a non-invasive and reliable estimate of circulating cortisol (Port, 1991; McCracken and Poland, 1989). Its function in the oral cavity is not known and is thought to enter saliva by passive diffusion or by other means of active transport (Kirschbaum and Hellhammer, 1994). Authors speculated that because cortisol appears to enter saliva intracellularly by diffusing through the cells of the salivary glands, the diffusion rate is high enough to maintain concentration equilibrium between the saliva fraction and the plasma unbound fraction. Thus, salvary C is independent of changes in saliva flow (Vining and McGinley, 1987; Kirschbaum and Hellhammer, 1994). Values in saliva may be as much as 20% lower than plasma free C values (Read, 1989, Raff et al., 2002). Good correlations have
been previously reported between salivary C and the serum/plasma ‘free’ concentrations; correlations usually ranging between $r = 0.80$ to $r = 0.90$ (Read, 1989; Poll et al., 2007). In contrast, correlations between 0.81 to 0.85 have been reported in studies comparing salivary values to ‘total’ serum cortisol (Gallagher et al., 2006; Poll et al., 2007; Gozansky et al., 2005) which also contains the physiologically inactive protein-bound C fraction (Kirschbaum and Hellhammer, 1994). Salivary C has been suggested to represent a better measure of adrenal cortical function than serum C (Mandel, 1990; Soo-Quee Koh and Choon-Huat Koh, 2007).

2.13.2 Saliva Testosterone

Testosterone is considered a key anabolic hormone with multiple physiological functions in the human body. In males, testosterone is mainly produced and secreted by the Leydig cells of the testes (Brownlee et al., 2005). With respect to exercise, testosterone (T) is especially important in the growth and maintenance of skeletal muscle, bone, and red blood cells (Zitzmann and Nieschlag, 2001). Like C, acute exercise increases T linearly once a specific intensity threshold is reached, with peak concentrations usually occurring at the end of exercise (Wilkerson et al., 1980). High intensity resistance exercise is known to increase T levels (Volek et al., 1993) as does prolonged endurance exercise (Daly et al., 2005). In the latter study, free and total T levels decreased below pre-exercise values 90 minutes into recovery following volitional exhaustion. A negative relationship between C and total T was also observed.

The effects of chronic heavy exercise, in particular endurance exercise are thought to have a suppressive effect on the hypothalamic-pituitary-gonadal (HPG) axis in men (Kraemer et al., 2008). Lower basal circulating levels of testosterone have been reported in men who have performed chronic endurance exercise for many years (Hackney, 1996). Furthermore, T levels have been shown to decrease in athletes participating in long duration stressful events such as a marathon (Kussi et al., 1984; Fournier et al., 1997) or a wrestling tournament (Kraemer et al., 2001). Decreases in T up to 48 h may occur after acute exercise, especially in events exceeding 3 h in duration (Tanaka et al., 1986). In the latter study it has been suggested that catecholamines may be responsible for the prolonged inhibitory effect of stress on T biosynthesis. However, decreases may be attributable to increased utilisation of T during exercise to preserve protein tissue or via a decreased rate of production because of inhibitory mechanisms (Kraemer et al., 2008). It has also been postulated that reduced levels of T with heavy training result from training induced adaptations in the hypothalamic-pituitary axis and/or peripherally by alternations in the negative feedback loop that regulates production via
pituitary luteinising hormone (Hackney, 1996; Barron et al., 1985; MacConnie et al., 1986). Other potential mediators explaining observed decreases in T with exercise involves the role of stress hormones such as cortisol. It is thought that elevations in C with exercise may interfere with T production, either acutely during exercise or chronically as a result of training (Kraemer et al., 2008). It has been previously shown that hypercortisolism of endogenous or exogenous sources suppress T secretion by a direct action on the testis (Cumming et al., 1983). Good correlations have been previously reported for T levels between saliva and total serum testosterone (r = 0.71) and free T (r = 0.67) (Shirtcliff et al., 2002).

### 2.13.3 Testosterone:Cortisol ratio

Changes in hormone testosterone/cortisol (T/C) ratio have been suggested as possible indicator of physiological strain (Urhausen et al., 1995; Fry and Kraemer, 1997) and overtraining (Banfi et al., 1993; Adlercreutz et al., 1986; Alen et al., 1988). Testosterone administration increases muscle protein synthesis and promotes muscle mass growth in humans (Ferrando et al., 1998). On the other hand, cortisol administration has a catabolic effect on myofibrillar proteins and suppresses protein synthesis (Kayali et al., 1987). Therefore alterations in T and C are thought to represent a disturbance in overall anabolic/catabolic balance of the athlete (Banfi et al., 1993; Hoogeveen and Zonderland, 1996). Changes in T/C are known to affect the recovery state and duration of recovery after exercise (Fry et al., 1991; Kuipers and Keizer, 1988). A change in this balance is dependent on intensity and duration of exercise (Hoogeveen and Zonderland, 1996).

Repeated heavy training without sufficient recovery period may cause a persistent disturbance in this balance, in which resting levels of T may be decreased (Alen et al., 1988; Fry et al., 1991; Hakkinen et al., 1989) and C levels are increased (Cumming et al., 1983; Houmard et al., 1990; MacConnie et al., 1986). Alterations in T:C have been suggested to result from changes in the response of the testes or adrenals, uptake by target organs and hormone catabolism (Hoogeveen and Zonderland, 1996). Strong negative relationships have been observed between C and T in some studies (Doerr et al., 1976; Cumming et al., 1983; Daly et al., 2005) while others have not (Kraemer et al., 2008). Additionally, increased levels of C and CRH have been suggested to be responsible for inhibition of testicular secretion of T (Cumming et al., 1983; Macconnie et al., 1986). It’s possible that individual variations among athletes, time course/magnitude of the stress response as well as pulsatile nature of release may have accounted for variations in observed findings.
In exercise studies, a fall in T/C ratio is regarded as an indication of tiredness (Adlercreutz et al., 1986) and possible indicator of overtraining in rugby players (Maso et al., 2004). A significant decrease in T/C ratio and T concentration has been found previously in rugby league players undergoing 6 weeks of progressive overload training (Coutts et al., 2007). This reduced anabolic state was also associated with a reduction in player body mass and did not return to baseline despite a 7 day taper in players who undertook preceding intensified training. Other studies have shown that that T/C ratio measures are useful in monitoring training adaptation and recovery to training as well as game play stress in team sport (Filaire et al., 2001; Kraemer et al., 2004). A decrease in free T/C ratio of 30% or more in plasma has been shown to indicate temporary incomplete recovery from intensive training in elite speed skaters (Banfi et al., 1993). However a decreased T/C ratio with heavy training was not shown to correlate with performance change in professional cyclists (Hoogeveen and Zonderland, 1996) or swimmers (Hooper et al., 1999). Conversely, Mujika et al (1996) found that increases in T/C ratio were positively correlated with performance improvements in swimmers. Also, Bonifazi et al (1995) observed decreases in C concomitant with performance improvements while T did not change. Other studies (Kirwin et al., 1988; Hakkinen et al., 1989) failed to show performance changes with increased C concentrations.

2.13.4 Transportation of analytes into saliva

According to Read (1989), the presence of a molecule in saliva can occur through four mechanisms

1. Passage through tight junctions of the cells of the salivary glands. This passage is dependent on the molecular weight of the molecule and has been deemed unimportant for compounds of 200 Da or more. This excludes almost all hormones (Vining and McGinley, 1987).

2. Passage through the lipid-rich cell membranes of the acinar cells of the salivary glands. Concentration reflects the free fraction in plasma and is available to lipophilic molecules such as cortisol.

3. Active exocrine gland secretion. Such secretion is energy dependent and possible metabolism in the salivary gland may have an effect on the concentration. An active transport mechanism operates for many proteins such as s-IgA (Young and Van Lennep, 1979).

4. Contamination. It is possible that minor abrasions and leakage from the gingivae could facilitate transportation of hormones into saliva.
2.13.5 Other Methodological issues

In addition to the above, pH and metabolism of the analyte in the salivary gland may effect overall concentration in saliva. With reference to contact sports like rugby, the possibility of contamination via gum/mouth abrasions is a clear possibility when hormones are to be analysed. It is also possible that subjects with poor oral hygiene and/or those wearing oral protection devices during exercise e.g. mouthguards, may have artificially elevated concentrations in selected analytes. Blood contamination has been previously shown to significantly affect the quantitative estimate of salivary concentrations (Granger, 2000). Brushing of teeth prior to sampling should therefore be avoided. Consequently, care should be taken with analysis and interpretation of values. Mixed saliva usually contains suspended matter, including bacteria, leukocytes, oral squames and mucoid. Mucins contained in saliva may make pipetting difficult by virtue of their viscosity. It has been suggested that freezing and thawing the sample will denature these mucins (Vining and McGinley, 1987). This is important since denatured mucins tend to absorb much of the debris from the sample. Furthermore, freezing eliminates much of the froth from fresh saliva samples. Consequently, most workers have advocated freezing and thawing, then centrifuging samples before analysis (Vining and McGinley, 1987).

2.14 CIRCADIAN VARIATION IN SELECTED IMMUNOEENDOCRINE MARKERS

Many biological indices of the human body display a known natural circadian or biological rhythm. Knowledge of this within-day variation will increase the possibility to detect ‘true’ change, resultant from the experiment or trial.

Cortisol: follows a diurnal rhythm with a large peak in concentrations after awakening in the morning (first 45 minutes) before declining throughout the day resulting in an evening nadir (Newsholme and Leech, 1983; Edwards et al., 2001; Hucklebridge et al., 1998). Data collected in our lab support these findings.

Testosterone: On average, testosterone levels follow a diurnal rhythm, with the levels highest in the morning, declining dramatically before noon, and then declining more slowly in the afternoon and evening hours (Granger et al., 2003). Diurnal variation has been suggested to be more stable in males. Data collected in our lab support these findings.
Salivary flow: Individual variations in saliva flow can be as high as 50% over a 24-h period (Ship et al., 1991) with unstimulated flow rates usually at their maximum in the mid afternoon (Dawes, 1972).

s-IgA concentration: It has been shown that s-IgA concentration peaks in the early morning (Gleeson et al., 2001; Dimitriou et al., 2002; Li and Gleeson, 2004) in rested subjects. After 4 h of awakening, observed decreases begin to plateau for the rest of the day (Hucklebridge et al., 1998). This morning peak may be partly due to decreased flow in the morning/and or storage of IgA in salivary glands so that concentration is higher upon first sampling (Rantonen, 2003).

Lysozyme: has been shown to relatively stable until midday, after which small gradual increases in concentration occur thereafter (Rantonen and Meurman, 2000).

IL-6: plasma IL-6 has been found to be higher in the morning (7 am) before decreasing throughout the afternoon (12 am & 4 pm) in rested healthy controls (Miles et al., 2008).

CRP: does not vary by time of day (Meier-Ewert et al., 2001; Miles et al., 2008)

Glutamine: Limited data exists on variations in plasma glutamine across the day. Concentrations are greatly influenced by meal intake with plasma values showing increases just 30 min after food intake. Generally, within a fasted state, little or no change has been observed (Castell et al., 1995).

Circulating leukocytes and leukocyte subsets: The absolute numbers of neutrophils and monocytes do not exhibit significant circadian fluctuations (Kronfol et al., 1997). Previous research has suggested that the number of lymphocytes does show some rhythmicity with lower values (lowest at 10 am) during the day before rising steadily until midnight (Kronfol et al., 1997). This circadian variation in circulating lymphocytes appeared to reflect largely a change in the number of T cells rather than B cells (Kronfol et al., 1997; Miyawaki et al., 1984). In contrast, circulating NK cell counts have been shown to peak in the early morning with values low at night (Bourin et al., 1993).
2.15 PSYCHOMETRIC ASSESSMENT: RESTQ-SPORT

Research studies focusing on emotional state in the sporting domain have predominantly used the profile of mood states (POMS) (McNair et al., 1992), its abbreviated form (Morgan et al., 1987) or the Daily analysis of Life Demands in Athletes (DALDA; Rushall, 1990). Until recently, questionnaires have failed to address the issue of sport specific stress and the importance of recovery on global mood state. This may be important since an increase in performance is only achievable when athletes optimally balance training stress with recovery (Rowbottom et al., 1998). It has been suggested that restricting analysis to the stress dimension alone is insufficient, leading authors to develop the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport; Kellman and Kallus, 2001).

The RESTQ-52 Sport is a psychometric questionnaire that can be used to assess the extent to which athletes are physically and/or mentally stressed. It has also purported to help classify if athletes become overreached or over-trained (Kellmann and Kallus 2001) and is designed to incorporate different paradigms that affect an athlete’s recovery-stress state. These include twelve basic scales and seven additional sport specific scales. The questionnaire uses a self-report approach in order to evaluate physical, subjective, behavioural and social aspects of stress and recovery. Without essential changes in the internal consistency of the scales, the period of reference can be extended up to four weeks, depending on the research question (Kallus, 1995; Kallus and Kellman, 2000). The RESTQ-52 Sport has been previously shown to display consistently high short-term stability with test-retest reliability reported at $r = 0.51-0.81$ (Kellmann and Kallus, 2001) and has been compared to other psychological questionnaire used in sports performance. Considerable correlations have been shown between RESTQ-sport and the POMS (Kellman et al 2001; Kallus and Kellman, 2000), despite the fact that both questionnaires differ in terms of scales.

Physical aspects are addressed through questions relating to bodily symptoms associated with stress and recovery while subjective and behavioural aspects are assessed by emotional mood and performance related items. In turn, social aspects are addressed by stress-related and recovery-orientated social behaviour (Kellmann and Kallus, 1999). Each item is rated by the subject according to its frequency on a seven point Likert-like rating scale ranging from 0 (never) to 6 (always), indicating how often the subject participated in various activities during the previous three days/ nights (72 h) (Appendix G). In total, the RESTQ-52 Sport contains 24 non-specific and 28 sport-specific items. The first seven scales tackle different subjective aspects of strain such as ‘General stress’, ‘Emotional stress’, and ‘Social stress’. The scales
‘Conflicts/Pressure’; ‘Fatigue’ and ‘Lack of Energy’ are concerned with performance aspects whereas ‘Somatic Complaints’ is thought to address the physiological aspects of stress and strain (Kellmann and Kallus, 1999). ‘Social Relaxation’, ‘Somatic Relaxation’, and ‘General Well-Being’ are the basic scales of the recovery domain which also incorporates an additional sub-scale for ‘Sleep Quality’. In addition, the questionnaire differentiates non-specific and sport-specific areas of stress and recovery through the use of additional sport specific paradigms such as ‘Success’ and ‘Physical Recovery’, ‘Being in Shape’, and ‘Injury’. These latter two scales address the physical fitness of the athlete while burnout scales such as ‘Emotional Exhaustion’ and ‘Personal Accomplishment’ are developed to assess psychological symptoms of burnout (Kellmann and Kallus, 1999).

This questionnaire has been used previously in assessing physical and psychological stress over training phases in a variety of sports (Kellman and Gunther, 2000, Coutts et al, 2006, Kellman et al, 2001, Steinacker et al. 1998). In the study by Kellman and Gunther (2000), a dose response relationship was observed between daily endurance training (minutes) and subjective assessment of stress and recovery in elite rowers prior the 1996 Olympic Games. Additionally, similar relationships between training load and the RESTQ-Sport have been found in soccer players (Ferger, 1998; Hogg, 2000), triathletes (Coutts et al., 2006), cyclists (Bouget et al., 2006) and rowers (Purge et al., 2005). Changes in RESTQ-index were shown to parallel with changes in resting cortisol levels during world cup competitions in rowing (Purge et al., 2005) and with cortisol and DHEA-S/C ratio in cyclists undertaking heavy training (Bouget et al., 2006). Despite no change in biochemical markers with increasing high intensity training, impaired recovery-stress states using the RESTQ-76 sport questionnaire were noted by Coutts et al (2007c) in triathletes. Authors concluded that monitoring mood changes using this questionnaire represented a practical tool in monitoring training status and early stages of overreaching in athletes (Coutts et al., 2007c).
2.16 SUMMARY

In this chapter an attempt was made to provide an overview of immuno-endocrine and psychological stress responses within the athletic domain. Specific emphasis was placed on characterising current knowledge of immune suppression including potential causes and clinical significance. In particular, literature concerning the effect of heavy exercise on hormonal state as well as mucosal immunity was highlighted in the competing athlete. Key emphasis on muscle damage and inflammation was discussed with respect to rugby union and how both interact with host immune responses and recovery from exercise. The available evidence suggests that repeated daily exercise in the absence of sufficient recovery will place an athlete at increased risk of developing illness. Potential progression of overtraining and burnout are also correlates of this stress-recovery imbalance. Limited information on stress within team sports like rugby union exists at the elite level. Therefore the effects of acute and chronic exercise stress will be investigated in the current thesis. Development of illness related symptoms and changes in mood state will also be discussed.
Chapter 3

General methodology

[Acute studies]

A general methods section is included below detailing preliminary measurements and laboratory analysis of investigated markers used in studies 1 and 2.

3.1 STUDY ENTRY AND ETHICAL APPROVAL

Approval of these studies was issued by the institutional ethical committee of the University of Glamorgan. The nature and purpose of each study was explained both verbally and in writing (Appendix B) to all players. In the majority of instances, the study goals and procedures were also provided via visual presentation (Microsoft PowerPoint) so that all players had the opportunity to ask any questions to investigators. All players completed informed consent forms (Appendix A) and were made fully aware that they could withdraw from the studies at any time. Players were also required to complete a medical history questionnaire (Appendix C) to determine suitability for the related studies. If the player answered ‘yes’ to any of the questions on the medical questionnaire, they were referred to the medical staff to determine future entry/exclusion from study participation.

3.2 PRELIMINARY MEASUREMENTS

3.2.1 Determination of Peak Oxygen Uptake ($\dot{V}O_{2\text{peak}}$)

Peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) was determined using a standardized incremental maximal running test protocol. Players were required to exercise on a treadmill (Woodway®) until volitional exhaustion after firstly warming up for 2 min at 10 km.h$^{-1}$. Players then dismounted the treadmill for 5 min to carry out a gentle stretch. Upon recommencement of the running protocol, treadmill speed was further increased to 12 km.h$^{-1}$ ($0^\circ$ incline). Speed and treadmill inclination were then increased alternatively every 3 min by 2 km.h$^{-1}$ or 2$^\circ$ increments until exhaustion. Players were instructed to indicate with hand signals when they felt they were within one minute of volitional fatigue. During exercise, sample collection was taken with the player wearing a nose-clip whilst breathing through a rubber mouthpiece attached to a two-way non-re-breathing valve. This in turn was connected to the Douglas bag via lightweight Falconia.
tubing (Cranlea, Ltd.). Mouthpiece and nose clip were presented to players 30 s prior to gas collection to negate any disruptions in breathing and evacuate ‘dead space’ on expiration. Heart rate (Polar Electro, Finland) and rating of perceived exertion (RPE; Borg, 1985) were monitored during the final 30s of each stage. In the event of the player not being able to finish the final stage, a hand signal was used to notify the experimenters of immediate gas collection.

Peak $\dot{V}O_2$ was defined as the highest continuous full minute $\dot{V}O_2$ during the test and included at least one of the following criteria:

1. A respiratory exchange ratio (RER) greater than 1.15.
2. Failure of the player to continue/complete exercise stage.

Expired air samples were collected during the final minute of each stage into 250 L Douglas bags at the inspiratory stage of each ventilatory cycle. Samples were analysed for $O_2$ and $CO_2$ content using a gas analysis system (Servomex 1440, Crowbridge, UK). The analyser was calibrated against chemically checked $O_2$ and $CO_2$ gas mixtures (4.09% $CO_2$, 16.1% $O_2$) and also zeroed against and nitrogen. Calibration was performed on the morning of testing day and frequently between the $\dot{V}O_2peak$ tests themselves. A dry gas meter (Harvard Ltd. Kent, UK) and a thermometer were used to measure the volume and temperature of each air sample, respectively.

3.3 BLOOD SAMPLING AND ANALYSIS

3.3.1 Blood collection and treatment
Venous blood samples were taken from the antecubital vein of the arm by venepuncture with the player in a supine position. Samples were collected into three Vacutainer tubes (Becton Dickinson, Oxford, UK). Blood collected in $K_3$EDTA vacutainers (4 ml) were kept at room temperature and used for standard haematological analysis and assessment of changes in plasma volume. Analysis was carried out within 4 h of collection. The remainder of the samples were separated immediately by centrifugation (1800 g x 15 min) for collection of plasma. Aspirated plasma was aliquoted into pre-coded eppendorf tubes and stored at -80°C for future analysis. In addition, whole blood (7 ml) was collected into sterile lithium-heparin vacutainer tubes (Becton Dickinson, Oxford, UK), kept at room temperature and analysed within 4-6 h following collection for analysis of neutrophil function (described below). Upon collection, 1 ml of heparinised blood was added to an eppendorf microcentrifuge tube containing 50 $\mu$l of 10 mg.ml$^{-1}$ bacterial lipopolysaccharide (LPS) solution (Stimulant, Sigma-
Aldrich, 840-15, Gillingham, UK). The blood and stimulant were mixed by gentle inversion, and then incubated immediately for 60 min in a water bath (37°C). After 30 min of incubation, the contents of the tube were gently mixed again via inversion (Robson et al., 1999). When the incubation period had elapsed, eppendorfs were centrifuged (10000 g x 2 min). The supernatant (plasma) was aspirated off into appropriate aliquots and stored at -80°C until analysis (detailed below). Remaining plasma from the lithium-heparin vacutainer tubes was also aspirated off into appropriate aliquots by centrifugation (1500 g x 10 min) and stored at -80°C for future analysis. For serum measurements (high-sensitivity C-reactive-protein; hs-CRP and creatine kinase; CK), blood (7 ml) was collected into vacutainer tubes containing a clot activator (SST; Becton Dickinson, Oxford, UK). Following centrifugation (1500 g x10 min) serum was aspirated into appropriate aliquots and stored at -80°C for future analysis.

### 3.3.2 Determination of Total and differential leukocyte counts

Haematological analyses for determination of haemoglobin, haematocrit, total and differential leukocytes were performed using an automated cell counter (Coulter Electronics, USA). The intra-assay coefficient of variation was 0.4%, 4.3%, and 2.3% for haemoglobin, haematocrit and leukocyte counts respectively. Changes in blood and plasma volume were calculated from measurements of haemoglobin (Hb) and haematocrit (Hct) using the equation of Dill and Costill (1974). Where required, all immune cell concentrations were corrected for possible exercise-induced changes in blood volume relative to the previous day's pre-exercise value (Brenner et al., 2000). Measurements taken from plasma and serum were also corrected for possible changes in plasma volume using the same procedure.

### Determination of blood leukocytes phenotypes

Blood leukocytes phenotypes were analyzed by three or four colour flow cytometry (Beckman-Coulter FC500 MPL). Specific staining of cells were performed by incubating the test sample (whole blood) with a specific test reagent. The staining procedure involved adding combinations of anti-human monoclonal antibodies (mAbs), conjugated with fluorescien isothiocyanate (FITC), phycoerythrin (PE) or phycoerythrin-cyanin (PE-Cy). The red cells were then removed by erythrocyte lysis with OptiLyse C (Beckman Coulter, UK Ltd.) and the leukocytes (unaffected by this process) were analysed by flow cytometry. Non-specific staining of monoclonal antibodies with isotypic controls was also employed to determine existence of non-specific fixation/binding of specific conjugated monoclonal antibodies. The brightly fluorescent positively stained lymphocyte/monocyte population was measured in gates set to exclude the low level non-specific fluorescence.
**T Lymphocyte cell preparation**

10 µL of specific fluorochrome-conjugated monoclonal antibodies for CD3 (PerCP, Becton Dickinson BD), CD4 (FITC; BD) and CD8 (PE; BD) was added to 100 µL of well mixed whole blood (EDTA) into pre-labeled (12 x 75-mm) tubes. In addition to this, 10 µL of appropriate isotype control for CD3 (PC5; BD), CD4 (IgG1-FITC; BD) and CD8 (IgG1-PE; BD) was added to corresponding control tubes. Cells were mixed by a slight flush before being vortexed gently at low speed for 1 sec to reduce cell aggregation. Cells were then incubated for 30 min (dark; room temp: RT). After incubation, 500 µL of OptiLyse C (Beckman Coulter, UK Ltd.) was added to solutions for cell lysis before being vortexed again (1 sec). Samples were then left for a further 10 min in dark (RT) before being centrifuged and washed (x 2) with PBS and adjusted to 6 x 10^6 cells µl^-1. Cells were incubated for a further 5 min in dark (RT) before running them on the flow cytometer immediately.

**Natural Kill (NK) cells Preparation**

NK cell (CD3^-CD16^+CD56^+) number was assessed via the co-expression of cell surface markers CD16 and CD56 and lack of expression of CD3 (Cooper et al., 2001; Scharhag et al., 2006). Cells were labelled with PC-conjugated anti-human CD3 (10 µl; BD), phycoerythrin anti-human CD56 (20 µL; BD) and phycoerythrin conjugated CD16 (5 µl; PE-Cy7 BD) fluorochrome-conjugated monoclonal antibodies within 100 µL of well mixed whole blood (EDTA). Relevant antibodies for appropriate isotype control for CD16 and CD56 were also added into pre labelled (12 x 75-mm) tubes for analysis. Cells were mixed by slight flush before being vortexed gently at low speed for 1 sec to reduce cell aggregation and incubated for 30 min in the dark at room temperature (RT). After incubation, 500 µL of OptiLyse C (Beckman Coulter, UK Ltd.) was added to solutions for cell lysis. Samples were then vortexed (1 sec) and left for a further 10 min in dark (RT) before being washed with PBS and adjusted to 6 x 10^6 cells ul^-1. Cells were incubated for a further 5 min in dark (RT) before running them on the flow cytometer immediately.

**Determination of Lymphocyte Subsets**

The EDTA-treated whole blood was used to determine T cell and natural killer (NK) cell subsets. Lymphocyte subsets were classified as total T cells (CD3^+), T helper cells (CD3^+CD4^+), T cytotoxic cells (CD3^+CD8^+) and NK cells (CD3^-CD16^-CD56^-). Data was adjusted to minimize debris and ensure populations of interest were included. This was achieved through appropriate gating for lymphocyte population using forward-scatter versus side-scatter characteristics and 5000 events per lymphocyte gate were collected. Further analysis of lymphocyte subsets were
performed using WinMDI 2.8 software (Joseph Trotter, The Scripps Research Institute, CA, USA). Cell counts of each lymphocyte subset were calculated by multiplying the percentage of cells with appropriate fluorescence by the absolute number of lymphocytes.

3.3.3 Determination of LPS-stimulated neutrophil degranulation

Neutrophil degranulation was determined from release of elastase (measured in plasma) before and after LPS-stimulation. Plasma elastase was determined using a coated ELISA kit capture antibody specific for human PMN elastase (Merck Chemicals, UK). On the day of analysis thawed samples (stimulated and unstimulated samples) were initially diluted (x 20) by adding 30 µl plasma to 570 µl diluent in an eppendorf tube. After mixing (inversion), 30 µl of diluted stimulated samples were further diluted (300 µl diluent) in an additional labelled eppendorf tube. This completed the final sample dilution (x 220). In cases where samples were above detection, samples were re-analysed using a higher dilution factor (x 300). Upon reconstitution (via serial dilution), 100 µl of standards (0.156-10.0 ng/ml), samples and controls (high and low) were added to a pre-coated plate (polyclonal antibody to human PMN elastase) in duplicate. Unstimulated samples were added in singleton according to kit manufacturers instructions. Additionally, 100 µl of diluent was added as blank wells. Wells were incubated for 1 h on a rotator (600 rpm) before being washed (x 4). After washing, 150 µl of anti-α1-PI rabbit HRP-conjugate was added to all wells. The plate was further incubated for one hour at room temperature on a rotator (600 rpm). After incubation, the plate was washed again (x 4) before addition of 200 µl of TMB substrate solution. Following incubation (in dark, RT for 20 min), the enzyme reaction was stopped via addition of 50 µl of stop solution. The absorbance was measured at 450 nm (correction at 620 nm). The intra-assay coefficient of variation for this assay was 6.0%. All samples from the same sample lot were analysed together to avoid between assay variation. Since elastase is released under normal physiological conditions (non-stimulated), plasma elastase concentration was initially measured in all samples and corrected for changes in plasma volume (unstimulated baseline values). These values were then subtracted from stimulated elastase values, thus providing a value for total elastase release. As elastase concentrations were ascertained from neutrophils present in whole blood, total elastase release values were corrected accordingly via multiplication (1-hct value). This gave a true representation for neutrophil degranulation and was subsequently expressed as elastase release per neutrophil (per cell basis).
3.3.4 Determination of serum Cortisol and Testosterone

**Serum Cortisol (C)**
Analysis of C was done on thawed serum samples using a commercial pre-coated plate capture antibody ELISA (DRG Diagnostics, Germany; sensitivity 2.5 ng.ml\(^{-1}\)). All samples were analysed in duplicate. Appropriate volume (20 µl) of standards (0-800 ng.ml\(^{-1}\)), control and samples were dispensed into appropriate wells followed by addition of 200 µl of enzyme conjugate (anti-cortisol antiserum conjugated to horseradish peroxidise) as competition for binding site. After mixing and incubation (60 min), the unbound conjugate was washed off by rinsing wells (x 3) with wash solution. After washing, 100 µl of substrate solution (Tetramethylbezidine; TMB) was added to wells before incubation (15 min). The colorimetric enzymatic reaction was then stopped via addition of 100 µl of stop solution 0.5-M sulphuric acid. Wells were then read at 450 nm within 10 min of stopping the reaction. The intra assay CV was 3.2% for this assay.

**Serum Testosterone (T)**
Similar to C, thawed samples were analysed in duplicate using a commercial pre-coated plate capture antibody ELISA (DRG Diagnostics, Germany; sensitivity 0.083 ng.ml\(^{-1}\)). Appropriate volume (25 µl) of standards (0-16 ng.ml\(^{-1}\)), control and samples were dispensed into appropriate wells (in duplicate) followed by addition of 200 µl of enzyme conjugate (testosterone conjugated to horseradish peroxidise) as competition for binding site. After mixing and incubation (60 min), the unbound conjugate was washed off by rinsing wells (x 3) with provided wash solution. After washing, 200 µl of substrate solution (Tetramethylbezidine; TMB) was added to wells before incubation for 15 min. The colorimetric enzymatic reaction was then stopped (100 µl of stop solution; 0.5-M sulphuric acid). Wells were then read at 450 nm within 10 min of stopping the reaction. The intra assay CV for this assay was 3.4%.

3.3.5 Determination of high sensitive C - reactive protein (Hs-CRP)
Concentrations of high sensitive C-reactive protein (HsCRP) were determined in serum using an immunoturbidimetric assay (Randox Ltd, Antrim, UK; sensitivity 0.08 mg.l\(^{-1}\)). Thawed samples were measured via the formation of antibody-antigen complexes during reaction of the sample with provided assay buffer and anti-CRP latex. Sample turbidity (loss in light intensity of light beam as it passes through the solution) was measured at 570 nm using an automated clinical chemistry system (RX Daytona™). Concentrations were determined by comparison to a standard curve (in duplicate) established in the same set of measurements. Daily quality control was obtained by using a range of hs-CRP controls bought from same supplier (Randox...
Intra assay CV for this assay was less than 5%. All samples from the same sample lot were analysed together to avoid between assay variation.

### 3.3.6 Determination of interleukin 6 (IL-6)

Plasma IL-6 concentrations were analysed from K$_3$EDTA treated venous blood using commercially available high sensitivity ELISA (Diaclone Research, Besancon, France; sensitivity < 0.8 pg/ml). All samples were assayed in duplicate. 100 µl of sample, controls and re-constituted standards (0-50 pg.ml$^{-1}$) were added to a pre-coated IL-6 antibody specific plate. After addition of detection antibody (50 µl), the plate was allowed to incubate for 3 h. Following washing (x 3), the enzyme (streptavidin-peroxydase) was added before further incubation (30 min). The plate was subsequently washed again (x 3) to remove all unbound enzyme, before addition of substrate (TMB) to induce a colorimetric reaction (20 min incubation). The reaction was then stopped and all wells were read at 450 nm (reference wavelength 620 nm). The intra-assay coefficient of variation (CV) was 7.6%. All samples from the same sample lot were analysed together to avoid between assay variation.

### 3.3.7 Determination of Creatine Kinase (CK) activity

Total creatine kinase activity was quantitatively measured using VITROS CK slides (Ortho-Clinical Diagnostics, Buckinghamshire UK) in serum (range 20-1600 u/L). Thawed samples (11 µl) were evenly distributed on a multilayered slide according to manufacturer’s instructions. Slides were precoated with N-acetylcysteine (NAC) to serve as an activating agent for creatine kinase (CK). The test principle implies that upon deposition of sample, creatine kinase catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. In the presence of glycerol kinase (GK), glycerol is phosphorylated to L-α-glycerophosphate by ATP. Subsequent oxidation of L-α-glycerophosphate to dihydroxyacetone phosphate and hydrogen peroxide occurs in the presence of L-α-glycerophosphate oxidase (α-GPO). Finally, leuco dye is oxidised by hydrogen peroxide in the presence of peroxidase to form a dye. Samples were checked against known values (VITROS Chemistry calibrator kit 3, Ortho-Clinical Diagnostics, Buckinghamshire UK) bought from the same company. Samples outside of system range were diluted with 7% BSA and reanalyzed with multiplication of dilution factor for original sample’s CK activity. Reflection densities were subsequently measured (VITROS 950 Chemistry System) at 670 nm during incubation (5 min, 37°C), with rate of change in reflection density converted to change in enzymatic activity. Intra assay co-efficient of variation was < 3%. All samples were analysed in the same batch on the same day to avoid between assay variation.
Chapter 4

Immonoendocrine Responses to Acute and Repeated Exercise in Professional Rugby Union

Study One: Time-course of changes in immuneoendocrine markers following an international rugby game

SUMMARY

Intense exercise is known to cause temporary impairments in immune function. Few studies, however, have investigated the effects of intense competitive exercise on immunoendocrine variables in elite team sport athletes. The aim of this study was to evaluate the time-course of changes in selected immunoendocrine and inflammatory markers following an international rugby union game. Blood samples were taken from players (n = 10) on camp-entry, the morning of the game (pre), immediately after (post) and 14 h and 38 h into a passive recovery period. Players lost an average of 1.4 ± 0.2 kg in body mass over the course of the game (ambient conditions: 11°C, 45% RH). An acute-phase inflammatory response was observed as reflected through immediate increases in serum cortisol and IL-6 (post) followed by delayed increases in serum creatine kinase (CK; 14 h) activity and C-reactive protein (CRP; 38 h); P < 0.05. Decreases in the number of circulating T lymphocytes, NK cells and bacteria-stimulated neutrophil degranulation were also observed post-exercise (P < 0.05), indicative of decreased host immune protection. Following a large decrease in serum testosterone to cortisol (T/C) ratio immediately post and 14 h after exercise, T/C values then increased above those observed at camp-entry 38 h into recovery (P < 0.05). This rebound anabolic stimulus may represent a physiological requirement for recovery following intense tissue damage resultant from game collisions. Findings also suggest that a game of international rugby elicits disturbances in host immunity which last up 38 h into the recovery period.

4.1 INTRODUCTION

Rugby union is considered to be one of the most intense and physically demanding field games in the world (Mashiko et al., 2004). Since its emergence as a professional sport (1995), the game appears to have become a faster, ruck dominated game that contains more phases of play (Eaves and Hughes, 2003). Furthermore, players have become bigger and faster and are involved in more physical contact and collisions during game play (Duthie et al, 2003; Quarrie and Hopkins, 2007). Despite its growing popularity throughout the world, few scientific data exist to help us understand the competitive stresses inherent within the game.

Traditionally, the physical demands of rugby union have been assessed through analysis of game activities (Roberts et al., 2008; Deutsch et al., 2007; Duthie et al., 2005), injury analysis (Brooks et al., 2008; Fuller et al., 2008) or from questionnaire analysis for signs of player burnout (Cresswell and Eklund, 2006). While notational analysis type studies do provide
valuable insight into the various game activities undertaken by players, difficulties in the direct assessment of stresses arising from tackling and game contact instances inevitably occur.

Although competitive play is thought to elicit considerable physiological strain on players, few studies have investigated such through detailed biochemical assessment (Mashiko et al., 2004; Takarada, 2003). This is particularly evident at the elite level. In other combative sports like American football, structural tissue damage and associated biochemical disturbances have been associated with reductions in force production and athletic performance (Hoffman et al., 2002). In elite level rugby, it is possible that given the high-impact forces received during tackling, as well as eccentric damage from locomotion, a heightened inflammatory response and tissue trauma may be observed. Indeed, a significant link between the level of tissue trauma and the number of game related collisions has been established (Smart et al., 2008; Takarada, 2003) and that injury potential in rugby union is largely dependent on these contact phases of play (Fuller et al., 2007). However, it is not known if the degree of tissue disruption and biochemical disturbances is greater with higher levels of play or if these stress effects are capable of modifying host immune function.

Intense exercise is known to alter many aspects of the immune system, and in some instances depress host protection (see Gleeson, 2007 for review). Additionally, tissue trauma has been shown to influence immune cell recruitment into damaged muscle (Fielding et al., 1993; Paulsen et al., 2005). In particular, infiltration of blood neutrophils into injured tissue has been suggested as a key characteristic of the inflammatory process (Tidball, 2005). Rugby union is a game played over 80 minutes during which time players are required to run intermittently while participating in many game-related impacts such as tackling. It is possible that through investigation of biochemical and immunological status, a greater understanding of the game demands may be achieved. Knowledge of this at the highest level of play (international) may help in understanding recovery aspects of the game and potential adverse effects on host immune protection. To the author’s knowledge, no studies have previously attempted to investigate physiological responses before and after an elite rugby game using detailed biochemical analysis. With the above in mind, the purpose of this study was to investigate the effects of acute game stress on immunological and hormonal responses, with specific reference to recovery duration.
4.2 METHODS

Subjects
Ten international rugby union players [mean (± SEM): age 26.4 (0.7) yr, height 186.5 (2.5) cm, body mass 103.1 (3.9) kg, VO\textsubscript{2peak} 53.2 (1.1) ml.kg\textsuperscript{-1}.min\textsuperscript{-1}] agreed to participate in the current study. Players were informed of the purpose and risks of the study through visual presentation and player information sheets upon entry to the international training base. All players provided written informed consent (Appendix A) before volunteering for the study. Experimental procedures to be undertaken in the study were approved by the Research Ethics Committee of the University of Glamorgan and also by the medical committee of the Welsh Rugby Union. Any player who wished to withdraw from the study could do so at any time.

Sample collection
Peripheral venous blood samples were taken from players on entry to the camp (entry), the morning of the game (pre-game), within 10 min of the conclusion of the game (post-game) and again the following two mornings (14 h and 38 h post respectively). Starting time of the game was 17.00 hr. All blood samples were obtained from an antecubital forearm vein as described in section 2.1. Samples were collected into three Vacutainer tubes (Becton Dickinson, UK), one containing SST® Gel and Clot Activator (7 ml), the second containing K\textsubscript{3}EDTA (4 ml) and the third (7 ml), containing lithium-heparin. All blood samples were obtained from players while in a supine position and this was standardised throughout all sampling points. Blood samples were analysed for potential changes in markers of muscle damage (CK activity), inflammation (hs-CRP, IL-6), endocrine response (serum cortisol, testosterone), immune cell numbers (Blood leukocytes and leukocyte subsets), and immune function (neutrophil dregranulation response). To eliminate inter-assay variance, where possible, samples were analysed in the same assay-run. Detailed description of blood handling procedures as well as separation and analysis of markers can be seen in section 3.3.1.

Dietary control
Players were provided with dietary plans by the affiliated nutritionist in the lead up to international games and this was not altered for the current investigation. Meals generally included a variation of low fat yogurts, fruit, pastas/rice, cereal bars, vegetables, cooked meat and pancakes. Additionally, players were asked to consume their ‘typical’ diet on the day before, day of, and day(s) after both sampling points (games). All morning blood sample points were taken from players following an overnight fast. In the case of the post-game sample, players were 3-4 h from the last main meal. Players were asked to refrain from any
post-game fuel/fluid consumption until blood samples, in addition to body mass measurements, were taken for that time point. All players were asked to refrain from alcoholic beverages for the duration of sampling.

**Evaluation of fluid loss during exercise**

Players were weighed (in shorts only) 3 h before the start of the game and again, immediately after, using an electronic weighing scales (Seca Ltd, Birmingham, United Kingdom). Sweat loss was not estimated due to logistical difficulties. During the course of the game, all players were allowed to drink water ad-libitum whenever breaks in play permitted. Volume of fluids consumed was not noted. Whole body fluid loss was calculated from the reduction in body mass that occurred over the course of the game. Mean temperature and relative humidity values were recorded from four readings taken over the course of the game using an electronic wireless climate gauge (Thermo-Hygro). Post-game changes in plasma volume (PV) were computed using previously established methods (Dill and Costill, 1974).

**Game characteristics and analysis**

The game (international rugby union) involved players from teams ranked number six and two in the world at that time (IRB World Rankings November 2005). Game analysis and investigation of biochemical disturbances was performed on players from the home team only. Before the game, players participated in a standardised warm-up which included ball work, light tackling and pad work, stretching, calisthenics and position specific drills. This lasted approximately 30 min. In order to assess contribution of direct muscle trauma on markers of muscle damage/inflammation, game analysis was performed via use of video analysis software (Sports code, CoTm) and from video recordings following the event. The identified game statistics used in this study were contact events per player, player tackle number and game work:rest ratio. Player tackle number included the number of tackles received and made by each player while game work:rest ratios were defined as the period from the beginning of a play period to the interruption of that play period by the referee, and the period from the interruption of a play by the referee to the start of the next play (Takarada, 2003). The number of contact events included any element of play that included physical player-player contact (i.e. tackles, scrums, mauls, rucks). These specific game statistics were deemed important in determining the relationship between the contact element of rugby union and its possible effect on immunological and biochemical variables.
Statistical analyses

Normality of distribution was initially checked on variables using the Shapiro-Wilk test. Upon assumption of normality, data was analysed using a one way repeated measures analysis of variance with Bonferroni post-hoc correction. In the cases of CK, IL-6 and hs-CRP, analysis revealed skewed distributions and these data sets were subsequently log transformed prior to statistical procedures. Pearson product moment correlations were used to establish possible relationships between variables. Levels of significance were set at the P < 0.05 level (all variables). Data were evaluated using an SPSS for Windows version 13.0 software package (Chicago, USA) and presented as means ± SEM. For neutrophil degranulation, a sample size of 10 was deemed sufficient to detect a significant difference from analyses of power and effect size calculations used in recent published literature (Bishop et al., 2000, 2003)

4.3 RESULTS

Game data

Mean ambient temperature and relative humidity was 11.2°C and 45% RH respectively. Players were involved in 69 ± 9.0 minutes of game time and lost an average of 1.4 ± 0.2 kg in body mass (also 1.4% loss) over the course of the game.

Biochemical data

Serum cortisol and testosterone concentrations at camp-entry, before the game as well as throughout the recovery period (post, 14 h, and 38 h) are shown in figures 4.1a and 4.1b respectively. Main effects of time were observed for both cortisol and testosterone (P < 0.05). Compared to baseline levels (camp-entry), C decreased on the morning of the game (~18%; P < 0.05) before increasing considerably at the end of the game (post-game 40%; P < 0.05). Cortisol concentrations then decreased throughout the recovery period with values 14 h post similar to those observed at camp-entry. However, C decreased further during recovery period such that concentrations 38 h post were significantly lower (~33%) than those observed at camp-entry (P < 0.05). Unlike C, concentrations of T decreased immediately after exercise such that values were significantly lower (~ 42%) than those observed on the morning of the game (P < 0.05). Like C, serum concentrations of T 14 h post-exercise were similar to those observed at camp-entry. Notably, concentrations continued to increase into the recovery period with T values 38 h post higher than those observed at camp-entry (P > 0.05). T/C values were observed to decrease immediately post-exercise (P < 0.05) before rising steadily throughout the
recovery period. T/C values at 38 h post-exercise were significantly higher than those at camp-entry (Table 4.2; P < 0.05).

Serum creatine kinase activity is displayed in figure 4.2. Increases in CK activity were observed 14 h and 38 h after the game with values 14 h post significantly higher than those observed immediately post-exercise (P < 0.05). Unlike CK activity, the peak concentrations of hs-CRP were observed 38 h post (Figure 4.3). Pre-game CRP concentrations, as well as those observed immediately post, were significantly lower than those recorded at camp-entry. Concentrations then increased 14 h and 38 h following exercise such that values at 14 h post-exercise were significantly higher than at both pre- and post-game samples. Although the highest CRP values were observed 38 h POST, this increase did not reach statistical significance.

Highest concentrations of serum IL-6 were observed immediately post-exercise, after-which values decreased progressively during recovery. IL-6 concentrations immediately post were significantly higher than those at 14 h and 38 h post-game (Table 4.3; P < 0.05). Significant correlations were observed between the number of contact events (r = 0.78; P < 0.05) and tackles (r = 0.86; P < 0.05) completed by players with 38 h CK activity. Weaker but significant correlations between contact events (r = 0.65) and tackle number (r = 0.63) were observed with 14 h CK activity; P < 0.05. Significant correlations were also observed between the number of contact events (r = 0.82) and tackles (r = 0.64) with post-game neutrophil count; both P < 0.05.

![Figure 4.1a](changes_in_serum_cortisol_across_sample_points.png) *P < 0.05 from entry. Values mean ± SEM.

![Figure 4.1b](changes_in_serum_testosterone_across_sample_points.png) # P < 0.05 from pre-game values. Values mean ± SEM.
**Immune data**

Exercise induced changes in circulating number of blood leukocytes and leukocyte subsets are shown in table 4.1. Numbers of leukocytes and neutrophils significantly increased post-exercise, with concentrations decreasing gradually throughout the recovery period. Cell concentrations were still elevated 14 h into the recovery period (P < 0.05). In the case of blood neutrophils, cell numbers were elevated 38 h into recovery although data did not reach significance (P = 0.08). Similar findings were observed for blood monocytes. A significant effect of time (P < 0.05) was observed for numbers of total lymphocytes, CD3^+CD4^+ lymphocytes, CD3^+CD8^+ lymphocytes and NK [CD3^-CD16^+CD56^+] cells. Following an initial decrease in total lymphocytes immediately post-exercise (P = 0.08); a delayed increase was observed 14 h into the recovery period; concentrations significantly higher than 38 h post (P < 0.05); table 4.1

<table>
<thead>
<tr>
<th>Table 4.1: Immune cell concentrations at entry, before the game, immediately post, 14 h post and 38h post an international rugby union game. Values mean (SEM).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Entry</strong></td>
</tr>
<tr>
<td>Total Leukocytes (10^6.L^-1)</td>
</tr>
<tr>
<td>Neutrophils (10^9.L^-1)</td>
</tr>
<tr>
<td>Monocytes (10^9.L^-1)</td>
</tr>
<tr>
<td>Total Lymphocytes (10^9.L^-1)</td>
</tr>
<tr>
<td>CD4^+ cell number (10^6.L^-1)</td>
</tr>
<tr>
<td>CD8^+ cell number (10^6.L^-1)</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
</tr>
<tr>
<td>NK cell number (10^9.L^-1)</td>
</tr>
</tbody>
</table>

*P < 0.05 from all other sampling points. bP < 0.05 from camp-entry and pre-game values. aP < 0.05 from 14 h post-game values. bP < 0.05 from camp-entry.
Similar trends were also observed for lymphocyte subsets. A decrease ($P = 0.08$) in the number of circulating $CD4^+$ cells was observed immediately post-exercise, and was followed by a significant increase in concentrations 14 h post-exercise; values higher than all other sampling points ($P < 0.05$). A greater decrease in the number of $CD8^+$ cells was observed immediately post-exercise such that values were lower than all other sample points; $P < 0.05$. Concentrations of $CD8^+$ cells on the morning of the game (pre-game) were also lower than those observed at ENTRY (Figure 4.4; $P < 0.05$). Similar to $CD4^+$ and $CD8^+$ cells, concentrations of NK cells decreased immediately following the game; values significantly lower than all other sample points. Concentrations of NK cells then increased progressively.

Figure 4.2: Changes in CK across sample points. *$P < 0.05$ from pre-game values. #$P < 0.05$ from 14 h post. Values mean $\pm$ SEM.

Figure 4.3: Changes in high sensitivity C-reactive protein across sample points. *$P < 0.05$ from camp-entry. #$P < 0.05$ from pre-game & post-game values. Values mean $\pm$ SEM.

Figure 4.4: Changes in $CD4^+$ and $CD8^+$ lymphocyte concentrations across sample points. *$P < 0.05$ from all other sample points. #$P < 0.05$ from camp-entry. Values mean $\pm$ SEM.

Figure 4.5: Changes in NK cell concentrations across sample points. *$P < 0.05$ from all other sample points. Values mean $\pm$ SEM.
**Neutrophil function**

As mentioned above, a marked blood neutrophilia was observed. Following exercise, a non-significant increase (14%; P = 0.08) in plasma elastase concentration was observed after which values decreased below pre-game values 14 h (-47%) and 38 h (-29%) into recovery (P < 0.05; Table 4.2). Concentrations remained low throughout the remainder of the recovery period. Total LPS-stimulated elastase release followed a similar pattern with an initial increase in plasma elastase concentration observed immediately post-exercise. This increase was subsequently followed by decreases 14 h and 38 h post-exercise; P < 0.05.

Although total stimulated elastase concentrations remained lower than pre-game values, differences were non-significant. Adjusting data to take into account the number of circulating neutrophils revealed a 65% fall in LPS-stimulated elastase release per cell immediately post-exercise compared to pre-game values. Values began to increase gradually during recovery, with LPS-stimulated elastase release [per cell] 45% and 28% lower than pre-game values 14 h and 38 h post-exercise respectively (Figure 4.6).

![Figure 4.6: Change in lipopolysaccharide (LPS)-stimulated elastase release per neutrophil in response to an international game of rugby union. * P < 0.05 lower than all other sample points. Values mean ± SEM.](image)
Table 4.2: Blood and plasma concentrations of selected immunological variables before, immediately post, 14 h-post and 38 h-post an international rugby union game. Values mean (SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Game</th>
<th>Post-game</th>
<th>14 h post</th>
<th>38 h post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Neutrophil count</td>
<td>3.0 (0.35)</td>
<td>10.5 (1.1)</td>
<td>4.4 (0.30)</td>
<td>3.5 (0.36)</td>
</tr>
<tr>
<td>Plasma Elastase conc. (µg.L⁻¹)</td>
<td>119 (23)</td>
<td>136 (24)</td>
<td>#63 (8.2)</td>
<td>84 (19)</td>
</tr>
<tr>
<td>Stimulated Plasma Elastase conc. (µg.L⁻¹)</td>
<td>1623 (307)</td>
<td>2080 (222)</td>
<td>$1320 (168)</td>
<td>$1431 (178)</td>
</tr>
<tr>
<td>†Elastase release per neutrophil (fg.Cell⁻¹)</td>
<td>309 (78)</td>
<td>*110 (14)</td>
<td>167 (21)</td>
<td>221 (30)</td>
</tr>
</tbody>
</table>

†Plasma concentrations corrected (x 1-haematocrit); required before expression of elastase per neutrophil (neutrophils in whole blood).

# P < 0.05 different from pre-game. $ P < 0.05 different from post-game values. * P < 0.05 lower than all other sample points.

Table 4.3: Plasma/serum concentrations of selected biochemical variables at camp-entry, before, immediately post, 14 h post and 38 h post an international rugby union game. Values mean (SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entry</th>
<th>Pre-Game</th>
<th>Post-game</th>
<th>14 h post</th>
<th>38 h post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (nmol L⁻¹)</td>
<td>382 (12.9)</td>
<td>*313 (6.3)</td>
<td>*534 (47)</td>
<td>400 (21)</td>
<td>*261 (21)</td>
</tr>
<tr>
<td>Testosterone (nmol L⁻¹)</td>
<td>21.7 (1.6)</td>
<td>24.6 (0.6)</td>
<td>$13.8 (1.3)</td>
<td>20.2 (1.3)</td>
<td>24.3 (2.1)</td>
</tr>
<tr>
<td>T/C ratio</td>
<td>0.054 (.018)</td>
<td>0.075 (.012)</td>
<td>* $0.025 (.014)</td>
<td>0.049 (.014)</td>
<td>*0.093 (.015)</td>
</tr>
<tr>
<td>hs-CRP (mg.L⁻¹)</td>
<td>2.04 (0.36)</td>
<td>*0.97 (0.17)</td>
<td>*0.95 (0.19)</td>
<td>$2.13 (0.34)</td>
<td>2.91 (0.90)</td>
</tr>
<tr>
<td>IL-6 (pg.ml⁻¹)</td>
<td>0.85 (0.14)</td>
<td>1.15 (0.25)</td>
<td>3.69 (0.91)</td>
<td>#1.01 (0.29)</td>
<td>#1.08 (0.47)</td>
</tr>
<tr>
<td>CK (IU.L⁻¹)</td>
<td>497 (73)</td>
<td>333 (49)</td>
<td>519 (60)</td>
<td>$1182 (231)</td>
<td>$750 (99)</td>
</tr>
</tbody>
</table>

*P < 0.05 difference from values at entry. $ P < 0.05 difference from pre-game values. # P < 0.05 different from immediate post-game values.

hs-CRP: high sensitive C reactive protein; IL-6: interleukin 6; CK: creatine kinase
4.4 DISCUSSION

A few studies have examined the biochemical responses to game play in rugby union (Smart et al., 2008; Takarada, 2003; Mashiko et al., 2004; Elloumi et al., 2003). However a paucity of data exists on the physiological stresses imposed on players within the elite competitive environment. With the above in mind, the current study represents a unique body of data given that it is the first to examine detailed biochemical and immunological responses to competitive rugby at international level.

Decreased levels of testosterone and increased levels of cortisol are thought to represent disturbances in the overall anabolic-catabolic balance of the athlete (Banfi et al., 1993; Hoogeveen and Zonderland, 1996). In the current study, significant increases in C (~ 40%) and corresponding decreases in T levels (~ 43%) were observed immediately after the game. These changes resulted in a large decrease in T/C ratio, with values still not recovered after 14 h. Such findings corroborate those of a previous study in top level rugby union (Elloumi et al., 2003), although increases in C level were observed longer into the recovery period in the present study. It is possible that differences in playing standard (higher in present study) and playing environment contributed to this greater stress response. Interestingly, present findings also show that C decreased significantly below resting values 38 h into recovery. This was matched by corresponding increased levels of T, such that a significant increase in T/C ratio was observed for this time point when compared to camp-entry (taken post 48 h rest). These findings agree with those observed previously in rugby (Elloumi et al., 2003) and wrestling (Passelergue and Lac, 1999) and may represent a rebound anabolic stimulus during the recovery period. Future studies should attempt to investigate the performance related effects of this T/C rebound in athletes using physical performance tests and whether or not additional exercise during this period exacerbates the muscle repair processes required following heavy exercise.

Interestingly, significantly lower resting cortisol concentrations were observed on the morning of the game (pre-game) than corresponding C levels at camp-entry. This is in contrast to previous observations in American football where higher cortisol concentrations were observed in players closer to kick off time (Hoffman et al., 2002). However, it is possible that these higher C values were a consequence of heavy training and competition prior to players entering the international camp in the current study. Although samples were taken from players on the morning of camp-entry and players had refrained from previous exercise (min 48 h), the time point (camp-entry) occurred at the end of an intensified period in club competition (Heineken

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Furthermore, pre-game samples were taken 18 days into the international camp during which time conditioning type activity was considered ‘low’ relative to the previous phase of club competition. Consequently, these lower pre-game C levels may have been a product of decreased residual training stress when players were present at international squad headquarters. Higher resting levels of CRP and CK, along with lower T concentrations on camp-entry compared to pre-game values would seem to substantiate this point.

In accordance with previous studies (Ispirlidis et al., 2008), a delayed increase in CK levels was observed after exercise. Peak levels occurred 14 h following the game, although values were still elevated (2.3 fold) above pre-game values 38 h into the recovery period. This suggests that significantly more time may be required to obtain adequate recovery from preceding tissue damage. Increases in the in the serum activity of this enzyme are considered a result of increased permeability of skeletal muscle membrane, suggestive of skeletal tissue damage (Clarkson et al., 2002). As expected, resting CK levels were higher in athletes in the present study (rugby union) compared to those observed in other team sports like American football (Hoffman et al., 2002; 2005) and soccer (Ispirlidis et al., 2008). It is possible that differences in protein efflux, clearance rate or loss of membrane integrity (Baker et al., 2004; Sjodin et al., 1990) contributed to these findings. However, cumulative tissue damage arising from rugby related physical trauma represents the most plausible explanation for this finding. Indeed, despite a small subject number, significant correlations were observed between serum CK activity (both 14 h and 38 h) and player involvement in tackles and game contact events. Such correlations have been observed previously in other collision type sports such as boxing (Zuliani et al., 1985), rugby union (Smart et al., 2008; Takarada, 2003) and American football (Hoffman et al., 2002). Together, these findings are suggestive of a blunt trauma like effect on muscle damage which occurs during game contact events and resulting plasma CK leakage. The effect of collisions and tissue trauma on immunological indices was not investigated in the latter studies however.

In the current study, a significant leukocytosis was observed after the rugby game. This was dominated by a corresponding increase in blood neutrophils (>3.5-fold) and to a lesser extent, blood monocytes (2-fold increase). As observed previously, such findings are indicative of an exercise induced acute phase response needed for initiation of tissue repair processes following exercise (Malm et al., 2000; Fielding et al., 1993). Infusion of IL-6 has been shown to stimulate the release of neutrophils, hepatocyte-derived acute phase proteins such as hs-CRP, and cortisol production (Steensberg et al., 2003). In agreement with the latter study, increases
in serum cortisol and blood neutrophils were found in conjunction with corresponding increases in IL-6 post-exercise. Furthermore, a delayed increase in CRP level was also found (peak levels 38 h post-exercise). This CRP rise has been implicated in monocyte activation and adhesion molecule synthesis that recruit leukocytes (Torzewski et al., 2000). In the author’s opinion, it is possible that this prolonged anti-inflammatory response to tissue damage may be essential in further stimulation of necessary growth and repair processes following intense tissue damaging exercise like playing rugby. However future research into the effects of this inflammatory response and subsequent repair processes is required.

In contrast to increases in markers of inflammation, decreases in the circulating number of certain immune cell types were observed immediately post-exercise. In particular, a large drop in the number of circulating NK cells was found as previously observed in cycling (Scharhag et al., 2006). This, together with decreases in the number of total lymphocytes and T cell lymphocytes (CD4+ and CD8+ cells) may be indicative of reduced host protection, in particular to viruses (Faabri et al., 2003). With respect to CD8+ T cytotoxic cells, a greater decline (34%) from resting values was observed compared to CD4+ helper cells as shown previously (Gray et al., 1993). This indicates that CD8+ cells are preferentially recruited and removed from the circulation with rugby exercise. However a decrease in CD4/CD8 ratio was not observed. T lymphocytes play a fundamental role in the orchestration and regulation of the cell mediated immune response (Gleeson and Bishop, 2005) while defects in T cell function are linked to increased viral infections (Faabri et al., 2003). It is not known whether decreases in T cell number (this study) and function (Bruunsgaard et al., 1997; Ronsen et al., 2001) are due to exercise induced apoptosis (Mars et al., 1998) or redistribution of cells to other compartments (Gleeson and Bishop, 2005). It should be noted that lymphocyte functional capacity was not measured in the current study and requires further investigation.

A large neutrophilia was observed post-exercise and contributed to ~70% of the observed leukocytosis. Neutrophils are the most abundant blood leukocyte and form part of the body’s innate immune defence against pathogen entry into the body (Bishop et al., 2003). Despite an observed increase in cell number, when bacterially challenged (through LPS); a significant decrease (-65%) in the functional capacity of these cells (neutrophil degranulation) was observed. Although decreases in neutrophil degranulation has been previously shown following intense exercise (Laing et al., 2008; Bishop et al., 2003; Blannin et al., 1996), current findings suggest that this apparent suppression may last longer than previously thought. Indeed, findings revealed that neutrophil responsiveness was not fully resolved 38 h after the game. Such data point to the intense nature of rugby union. Decreased functional capacity of
this cell group has been suggested to result, in part, from an increased proportion of immature
neutrophils in the blood (Blannin et al., 1996; Bishop et al., 2003). Increased mobilization of
these immature neutrophils (from bone marrow) is in turn, thought to be glucocorticoid
induced (Peake, 2002; Blalock, 1989). Given the high cortisol levels observed post-exercise,
this represents the most plausible explanation in the present study. However, it should be noted
that other hormones (epinephrine, growth hormone) have been recently implicated in this
depressed degranulation response (Laing et al., 2008). Interestingly, although an increase in
plasma elastase content was evident after exercise, possibly as a result of increased number of
circulating neutrophils, a significant decrease in plasma elastase was also noted 14 h and 38 h
into recovery. This observation may be also suggestive that blood neutrophils enter a refractory
state following intense rugby play.

It has been previously shown that repeated rugby exercise, without adequate recovery causes
depression in other indices of neutrophil function (Takahashi et al., 2007). These studies,
together with current findings are suggestive that rugby exercise is capable of significantly
depressing the functional capacity of this cell group. Such findings are important in light of the
large amount of tissue trauma, resulting inflammation and subsequent neutrophilia that is
observed following game participation. It has been previously suggested that there is a limited
pool of circulating mature neutrophils at any one time (Pyne, 1994). Given observations to
date, it is feasible that with repeated tissue injury and inflammation, contact sports like rugby
may leave this cell group in a chronic refractory state and ultimately limit players defence
capacity to foreign agents and infection. Future studies should attempt to investigate the effects
of repeated game involvement and ultimately tissue damage, on neutrophil responsiveness.
Such data may help determine the possible effects of heavy competition periods on host
immunity and aid in training periodisation.

The limited amount of scientific data on acute physiological responses in team sports athletes
is perhaps not surprising given the often inaccessibility to players and logistical considerations
of data collection. In the current study, data was collected on an international group of players
in a game that was extremely competitive, with both teams within the top six nations of world
rugby at the time. Indeed, the game (which ended in a home team defeat) was played in front
of over 74,000 spectators and so it would be expected that environmental conditions would
have posed a high degree of psychophysical strain. Although every effort was made to
control for time of sampling, the samples taken post-game were representative of a different
point on the diurnal cycle (game kick-off: 17.00h) and could be considered a confounding
variable. This was in contrast to all other sample points (morning 8-9 am). However, since many of the biomarkers measured in the current study are purportedly linked to the effects of C, known to decrease throughout the day; changes in these biomarkers immediately after the game would therefore be suggestive of a real exercise induced effect. In conclusion, the current study demonstrates a marked inflammatory response to tissue damage resultant from playing rugby union. Such intense physical trauma is capable of resulting in transient but notable decreases in host immunity, factors all of which pose important considerations in terms of exercise recovery time and illness potential in the elite rugby player. Perturbations in measured biomarkers were suggestive that a recovery period of at least 38 h is necessary following elite rugby union game play. Future studies should attempt to investigate the repeated effect of muscle damaging exercise like rugby union on inflammation and associated immune responses.
Study Two: Evaluation of immunoendocrine responses throughout a three week international rugby series

SUMMARY
The aim of the present study was to evaluate the effect of repeated exercise exposure (competitive rugby union) on immunoendocrine responses over an international rugby series. Blood samples were taken from 8 professional players on camp-entry, as well as before and after (0h, 14h, 38h) two games spaced over a 21-day period. Samples were analysed for potential changes in serum C-reactive protein (CRP), cortisol (C), testosterone (T), blood leukocytes, IL-6 and creatine kinase (CK). Findings revealed that players entered the international camp with residual tissue damage and inflammation following previous club involvement. Analysis of game data revealed a large acute phase response immediately following and throughout the post-game period. Differences in the magnitude of this response appeared dependant on the number of collisions players experienced during game play. Complete resolution of these inflammatory processes was not evident after 38 h of recovery. In comparison to resting (camp-entry) levels, sharp increases in post-game C concentration (40%) were matched with corresponding decreases (37%) in T (both games); P < 0.05. During the recovery period, C levels decreased below pre-game values while progressive increases in pre-game T levels were also evident. This resulted in a gradual increase in serum T/C ratio throughout the tournament. Current findings highlight the impact international rugby union has on aspects of the immunoendocrine system and the time frame required achieving optimal recovery. Data also revealed that monitoring of previous club activity in players preceding participation in an international series is necessary in management of stress and recovery. Analysis of T/C ratio in the present study shows that improved hormonal profiles can be modified during a series despite ongoing training and intense international competition.

4.5 INTRODUCTION
Like most professional athletes, rugby players are required to train hard and frequently, with often more than one session planned on a given day. During the season, the quantity and intensity of weekly training may vary and is usually in line with team goals and weekend participation in club games. At the elite level, competition stress on players may be magnified from additional involvement in international fixtures. In a non-world cup year, these international windows (Northern hemisphere rugby) are normally confined to short intensified training-competition periods, namely the six nation’s series (run over February-March) and November international series. With respect to the latter competition, games are played during what is traditionally considered an ‘intense’ phase of the season. Players normally enter an international training camp on the back of intense European club games and may be required to play an international match inside 7 days. During this international window, up to four games may be played over a 28 day period, with recovery time and selection of players for games dependent on the individual home union. Therefore an obvious dilemma for athletes competing in these tournaments is time available for full physiological recovery between games, which is often limited. Recent studies have demonstrated that residual fatigue accumulated over successive matches can adversely affect team-sport performance (Ronglan et al., 2006; Spencer...
et al., 2005). Knowledge of the time course involved in stress-recovery balance throughout an international series may be of interest, particularly in terms of planning training and recovery time between successive games.

Rugby union is considered one of the most intense and physically demanding field games in the world (Mashiko et al., 2004). In addition to locomotor activity, players participate in a high degree of tackling and foraging for the ball; phases of play which are typically classed as game ‘contact events’. Studies have shown that contemporary rugby union players cover distances between 5.5-7.5 km (Roberts et al., 2008; Cunniffe et al., 2009) and that game phases involving player-player contact have become an increasingly important aspect of play across time (Quarrie and Hopkins, 2007; Eaves and Hughes, 2003). Indeed, recent findings have shown that an average of 456.8 contact events occur during a given game in English professional rugby (Fuller et al., 2007). All of the above data reflects the physical nature and high level of attrition experienced by modern players. Consequently, this makes recovery aspects of the game especially important, in particular during competitive tournaments.

To date, very little research on markers of stress and recovery has been documented in elite team sports over a competitive tournament (Andersson et al., 2008; Elloumi et al., 2003). It has been suggested that inappropriate recovery may predispose some players to overload injuries and reduced performance (Barnett, 2006) and that a period of one week may be necessary to restore anabolic-cATABolic balance in international rugby (Elloumi et al., 2003). To the author’s knowledge, few studies, if any have assessed changes in immunoendocrine markers and tissue damage with repeated rugby union activity. It is possible that given the high degree of attrition experienced by players, changes in these variables could provide a more objective view on the time course required for optimal recovery. Knowledge of such may help fitness and coaching staff in designing team specific recovery and training loads between subsequent games. With the above in mind, the goal of the present investigation was to monitor changes in immunoendocrine variables across an international rugby union series.

2.14 METHODS

Fifteen international rugby union players were sampled across the study series. However for reasons of clarity and with the repeated exercise focus in mind, data is reported on 8 players common to all sample points only. Players (3 backs, 5 forwards) were informed of the purpose and risks of the study by visual presentation and study information sheets in the week leading up to the series. All players provided written informed consent (Appendix X) before volunteering in the study. Anthropometric data for the study group is displayed in Table 4.4.
Experimental procedures to be undertaken in the study were approved by the Research Ethics Committee of the University of Glamorgan and also by the medical committee of the Wales Rugby Union. Any player who wished to withdraw from the study was free to do so at any time.

**Sample collection**

Testing was carried out on players from the home team over the course of an international rugby union series (November Internationals). In total, 9 blood samples were taken on each player over a period of 20 days, during which time there were three games. Sample testing points were distributed throughout the course of the period, with particular focus on games 1 and 3 (Figure 4.7). These two games involved opposition who were ranked number 1 and 2 respectively in the world at that time period (IRB World Rankings November 2005). The home team were ranked number 6. An additional game involving opposition ranked number 10 in the world (game 2) and peripheral home team squad players also took place in between both games 1 and 3. The series took place during the competitive phase of the rugby season and all players were involved in European cup game the week before camp-entry.

![Figure 4.7: Schedule for the whole sampling period throughout the international rugby union series.](image)

Peripheral venous blood samples were taken from players on entry to the camp (entry) after a minimum of 48 h rest from previous rugby activity. Other testing points included the morning of the games (pre), within 15 min of the conclusion of the games (post) and again on the following two mornings (14 h and 38 h respectively). All morning samples were taken between 8.00-8.30 am. Starting time of game 1 and game 3 was 16.00 h and 17.00 h respectively. Details of blood collection and analysis of biomarkers can be seen in sections 2.1 (Methods) and Study 2 respectively.
Evaluation of fluid loss during exercise

All players were weighed 3 h before both games and again immediately after, using an electronic weighing scales (Seca Ltd, Birmingham, United Kingdom). Players were partially clothed (shorts only) for both measurements. During the course of the games, players were allowed to drink water *ad-libitum* whenever breaks in play permitted. Due to logistical difficulties, volume of fluid consumed by players was not noted. Both games took place under similar environmental conditions and under a closed roof stadium (Millennium Stadium, Cardiff). Temperature and relative humidity values were recorded and are as described in Study 1. Post-game changes in plasma and blood volume were computed using previously established methods (Dill and Costill, 1974). All blood data were subsequently corrected accordingly.

<table>
<thead>
<tr>
<th>Table 4.4: Subject characteristic data (n = 8); values Mean (SEM).</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>27.1(0.8)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>102.5 (5.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>184.8 (3.2)</td>
</tr>
<tr>
<td>* VO_{peak} (ml.kg^{-1}.min^{-1})</td>
<td>52.4 (2.0)</td>
</tr>
<tr>
<td>*HRmax (beats.min^{-1})</td>
<td>186 (1.6)</td>
</tr>
</tbody>
</table>

* Determined in laboratory.

Game characteristics and Diet

For full description game analysis, key game related variables and dietary considerations please refer to Study 1.

Description of player training activity

Between games, players were typically involved in conditioning and resistance type exercise set out by affiliated fitness staff. Training activity was implemented with consideration of previous player involvement in high level European cup games upon camp-entry. In view of this, sessions involving long duration were kept to a minimum over the course of the series. Training intensity however was maintained throughout. In addition to conditioning type exercise, players undertook rugby specific training set out by team coaches and participated in tactical training in the lead up to both games. Specific forward and defence training sessions
were also carried out over the course of the series. As recovery between training sessions, players typically participated in light swimming/pool work or functional rehab.

**Statistical analyses**

Normality of distribution was initially checked on variables using the Shapiro-Wilk test. Upon assumption of normality, data was analysed using a repeated measures analysis of variance. Following a main effect (time), a Bonferroni post-hoc test was used to locate where the differences occurred. In the cases of CK, IL-6 and hs-CRP, analysis revealed skewed distributions and these data sets were subsequently log transformed prior to statistical procedures. Levels of significance were set at the $P < 0.05$ level. Data were evaluated using an SPSS for Windows version 14.0 software package (Chicago, USA) and are presented as means ± SEM.

### 4.7 RESULTS

**Game data**

Players were involved in $84.1 \pm 5.1$ min (Game 1) and $70 \pm 11.9$ min (Game 3) of game time, respectively. Five of the players were also involved in an additional game in between sampling points (Game 2; $64.4 \pm 9.1$ min). Mean ambient temperature and relative humidity over the two games was $11.3 \pm 0.18^\circ$C degrees and $41 \pm 1.3\%$ RH, respectively. Players lost on average, $0.85 \pm 0.3$ kg (Game 1) and $1.40 \pm 0.2$ kg (Game 3) body mass over the course of the games.

<table>
<thead>
<tr>
<th>Game variable</th>
<th>Game 1</th>
<th>Game 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ball out of play (min,sec)</td>
<td>59.52</td>
<td>62.23</td>
</tr>
<tr>
<td>Ball in play (min;sec)</td>
<td>33.3</td>
<td>32.02</td>
</tr>
<tr>
<td>Formed Rucks + Mauls</td>
<td>137</td>
<td>114</td>
</tr>
<tr>
<td>Lineout count</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>Scrum count</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>*Total Tackles</td>
<td>49</td>
<td>35</td>
</tr>
<tr>
<td>*Ruck/Maul involvement</td>
<td>128</td>
<td>81</td>
</tr>
<tr>
<td>*Total contact events</td>
<td>177</td>
<td>116</td>
</tr>
<tr>
<td>*Contacts per min (E/min)</td>
<td>1.89</td>
<td>1.23</td>
</tr>
<tr>
<td>*Total game instances</td>
<td>332</td>
<td>244</td>
</tr>
<tr>
<td>*Instances per min (I/min)</td>
<td>3.55</td>
<td>2.59</td>
</tr>
</tbody>
</table>

* Data from 8 players included in current investigation. All other variables are general game descriptors. Total tackles: number made and received; Total game instances: indication of game involvement including kicks, carries, offloads, recycles, passes, breaks, tackles and ruck/maul involvement.
Game data revealed that the ball was in play for a longer period in Game 3 compared to Game 1 (Table 4.5) although a higher number of contact events such as rucks/mauls (137 vs 114) and scrums (33 vs 28) were recorded in Game 1. Player involvement in number of game instances was also greater (322 vs 244 I/min) in Game 1 than Game 3. Analysis of game data revealed that players in the current study participated in more tackles (29%), rucks and mauls (37%) and subsequently more contacts events (34%) in Game 1 than Game 3.

**Biochemical data**

Exercise induced changes in the number of circulating blood leukocytes and leukocyte subsets are shown in Table 4.6. Numbers of leukocytes, neutrophils and monocytes significantly increased post-exercise, with concentrations decreasing gradually throughout the recovery period. The magnitude of the post-game neutrophilia was higher in game 1 (12.6 ± 0.90 10^9·L^{-1}) than game 3 (9.6 ± 1.24 10^9·L^{-1}) although differences were non-significant (P = 0.08); figure 4.11. No statistically significant changes in post-game blood lymphocyte numbers were observed.

Main effects of time were observed for both cortisol and testosterone (P < 0.05). Compared to pre-game values, significant increases in C were found immediately after both games (50% game 1; 76% game 2) and C concentrations were still elevated 14 h post-exercise. For both games, C decreased below pre-game values 38 h into recovery (-7% game 1; -15% game 3). A gradual decrease in resting C was also evident throughout the series with pre-game values lower than those observed at camp-entry, values significant for game 3 (P < 0.05; Table 4.7). Cortisol values 38 h post-game 3 were 30% lower than those observed in players on entry to the series (P < 0.05).
**Figure 4.9:** Changes in serum creatine kinase across sample points; *P < 0.05 from entry. 
^a^ P < 0.05 from pre-game 1. 
^b^ P < 0.05 from pre-game 3. 
^c^ P < 0.05 from post-game 1.

**Figure 4.8:** Changes in C reactive protein across sample points; *P < 0.05 from entry. 
^a^ P < 0.05 from pre-game 1. 
^b^ P < 0.05 from pre-game 3. 
^c^ P < 0.05 from post-game 1.

**Figure 4.10:** Changes in interleukin-6 across sample points; *P < 0.05 from entry. 
^a^ P < 0.05 from post-game 1. 
^b^ P < 0.05 from post-game 3.

**Figure 4.11:** Changes in blood neutrophils across sample points; *P < 0.05 from entry. 
^a^ P < 0.05 from post-game 1. 
^b^ P < 0.05 from post-game 3.
Significant decreases (~37%) in serum testosterone (T) were observed after both games and T values were fully recovered 38 h into recovery. A gradual increase in resting T was observed throughout the series with pre-game 3 values significantly higher than corresponding pre-game 1 values (P < 0.05). An increase in resting T/C ratio was observed throughout the series with pre-game 3 and 38 h post-game values significantly higher than those at camp-entry (P < 0.05; figure 4.12).

Significant elevations in IL-6 were observed immediately post-exercise (both games) with values returning to baseline levels inside 14 h (Figure 4.10). Although the magnitude of the post-game IL-6 response was higher for game 1 (6.3 ± 1.5 pg.ml⁻¹) than game 3 (3.9 ± 1.1 pg.ml⁻¹), between game differences were non-significant (P > 0.05). A delayed increase in blood CRP concentrations was observed after both games, with peak levels observed 14 h (game 1) and 38 h (game 3) into recovery. Following entry into the camp, CRP values decreased gradually with concentrations before and immediately after both games significantly lower than those observed at camp-entry (Figure 4.8). A delayed increase in serum CK activity was observed after both games with peak values 14 h after exercise. CK levels were still elevated 38 h into recovery (P < 0.05; for game 1). Like CRP and cortisol, a decrease in CK values was observed following camp-entry (day one, 513 ± 114 IU.L⁻¹) up to the morning of the first game (day five, 310 ± 92 IU.L⁻¹); P < 0.05. The magnitude of the CK response was higher following game 1 (1462 ± 221 IU.L⁻¹) than game 3 (999 ± 222 IU.L⁻¹); between game differences non-significant (P > 0.05).
Table 4.6 Immune cell concentrations across the international rugby series. Values are mean (SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entry</th>
<th>Pre</th>
<th>Post</th>
<th>14 h</th>
<th>38 h</th>
<th>Pre</th>
<th>Post</th>
<th>14 h</th>
<th>38 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Leukocytes (10^9.L^-1)</td>
<td>5.93 (0.22)</td>
<td>5.64 (0.30)</td>
<td>*15.65 (1.06)</td>
<td>*8.72 (0.58)</td>
<td>5.85 (0.35)</td>
<td>6.31 (0.54)</td>
<td>*14.51 (1.04)</td>
<td>*8.19 (0.49)</td>
<td>6.79 (0.42)</td>
</tr>
<tr>
<td>Neutrophils (10^9.L^-1)</td>
<td>2.52 (0.17)</td>
<td>2.40 (0.19)</td>
<td>*12.61 (0.90)</td>
<td>*4.63 (0.42)</td>
<td>2.74 (0.18)</td>
<td>3.14 (0.38)</td>
<td>*9.70 (1.24)</td>
<td>*4.37 (0.39)</td>
<td>3.55 (0.30)</td>
</tr>
<tr>
<td>Monocytes (10^9.L^-1)</td>
<td>0.62 (0.06)</td>
<td>0.54 (0.06)</td>
<td>*1.01 (0.05)</td>
<td>0.89 (0.10)</td>
<td>0.54 (0.06)</td>
<td>0.59 (0.06)</td>
<td>*1.08 (0.05)</td>
<td>0.78 (0.06)</td>
<td>0.69 (0.08)</td>
</tr>
<tr>
<td>Total Lymphocytes (10^9.L^-1)</td>
<td>2.56 (0.13)</td>
<td>2.43 (0.15)</td>
<td>1.85 (0.18)</td>
<td>2.99 (0.12)</td>
<td>2.32 (0.12)</td>
<td>2.31 (0.14)</td>
<td>2.55 (0.27)</td>
<td>2.78 (0.12)</td>
<td>2.31 (0.11)</td>
</tr>
</tbody>
</table>

*P < 0.05 from camp-entry and pre-game 1. a P < 0.05 from pre-game 1 and 38 h post-game 1. b P < 0.05 from post-game 1. c P < 0.05 from post-game 3.

Table 4.7: Plasma/serum concentrations of selected biochemical variables across the international rugby series. Values are mean (SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entry</th>
<th>Pre</th>
<th>Post</th>
<th>14 h</th>
<th>38 h</th>
<th>Pre</th>
<th>Post</th>
<th>14 h</th>
<th>38 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (nmol L^-1)</td>
<td>384 (11)</td>
<td>348 (22)</td>
<td>*523 (58)</td>
<td>432 (49)</td>
<td>321 (22)</td>
<td>*313 (10)</td>
<td>553 (42)</td>
<td>430 (17)</td>
<td>*266 (22)</td>
</tr>
<tr>
<td>Testosterone (nmol L^-1)</td>
<td>19.0 (2.0)</td>
<td>18.6 (2.1)</td>
<td>*11.4 (0.8)</td>
<td>16.2 (1.6)</td>
<td>17.9 (2.0)</td>
<td>21.3 (2.0)</td>
<td>*13.6 (2.0)</td>
<td>16.9 (2.0)</td>
<td>18.9 (2.0)</td>
</tr>
<tr>
<td>T/C ratio</td>
<td>0.051 (.006)</td>
<td>0.056 (.008)</td>
<td>*0.024 (.003)</td>
<td>0.040 (.005)</td>
<td>0.058 (.007)</td>
<td>*0.069 (.006)</td>
<td>0.026 (.004)</td>
<td>0.039 (.004)</td>
<td>*0.074 (.005)</td>
</tr>
<tr>
<td>hs-CRP (mg.L^-1)</td>
<td>1.99 (0.60)</td>
<td>*1.13 (0.32)</td>
<td>*0.89 (0.30)</td>
<td>2.89 (1.18)</td>
<td>2.80 (1.11)</td>
<td>0.97 (0.21)</td>
<td>*1.11 (0.2)</td>
<td>2.15 (0.27)</td>
<td>2.80 (0.41)</td>
</tr>
<tr>
<td>IL-6 (pg.ml^-1)</td>
<td>0.80 (0.19)</td>
<td>1.25 (0.33)</td>
<td>*6.28 (1.54)</td>
<td>1.17 (0.35)</td>
<td>0.85 (0.23)</td>
<td>1.28 (0.31)</td>
<td>*3.85 (1.12)</td>
<td>1.00 (0.40)</td>
<td>1.27 (0.61)</td>
</tr>
<tr>
<td>CK (IU.L^-1)</td>
<td>514 (114)</td>
<td>*310 (92)</td>
<td>532 (87)</td>
<td>*1462 (221)</td>
<td>842 (91)</td>
<td>322 (77)</td>
<td>493 (98)</td>
<td>*999 (222)</td>
<td>642 (154)</td>
</tr>
</tbody>
</table>

*P < 0.05 from entry. a P < 0.05 from post-game 1. b P < 0.05 from post-game 3. c P < 0.05 from 14 h post-game 3. d P < 0.05 from 14 h post-game 1. e P < 0.05 from pre-game 1. f P < 0.05 from pre-game 3. hs-CRP: high sensitive C reactive protein; IL-6: interleukin 6; CK: creatine kinase; T/C ratio: Testosterone/Cortisol ratio.
4.8 DISCUSSION

International rugby is a physically and mentally demanding game often played in competitions set out over short intense time periods. It is possible that when two or more games are played on consecutive weekends, incomplete recovery from the first game could result in an accumulative stress response. The goal of the present investigation was to monitor changes in immunoendocrine markers over an international rugby union series. It was hoped that greater clarity would be obtained on game related stress and whether or not subsequent training and competition augmented this stress response.

Current findings show that competitive international rugby union elicits profound increases in tissue damage as reflected by the 4.7 fold (game 1) and 3.0 fold (game 3) increases in serum CK. This data corroborates that in previous studies (Smart et al., 2008; Takarada, 2003), which have also shown that a large proportion of this tissue damage is attributable to the number of physical impacts received by players during play. Given the limited amount of players common to both games in the present study, correlation analysis between game impacts and observed variables was not performed. Nevertheless, exploration of differences in game data does help explain why a larger CK response was observed in game 1. Game data revealed that players were involved in a greater number of contact events (1.8 E/min game 1 vs 1.2 E/min game 3) such as tackles, rucks and mauls whilst other facets of game involvement (e.g. game instances) were also higher. Therefore, it is probable that differences in game physicality and intensity contributed to these observations. Interestingly, although findings were non-significant, the magnitude of the IL-6 response and blood neutrophilia appeared greater in game 1 when compared to game 3. Such data, although speculative, point to the role that game contact events in rugby have on the acute phase inflammatory response. Further research involving larger subject numbers is necessary to determine the impact of these game related statistics on other physical/cognitive markers of stress and fatigue. Given the level of tissue disruption and associated inflammation, it is likely that reductions in markers of physical ability were possible, as shown in soccer (Andersson et al., 2008). In the latter study, decreases in vertical jump scores, peak torque in knee extension/flexion, isokinetic knee extension and sprint performance were observed following an elite soccer game, with some markers still not recovered inside 69 hours. In rugby, this may have important implications in terms of recovery time and in particular, if players are required to participate in mid-week fixtures.

Previous research has found higher resting plasma IL-6 levels in rugby players when compared to triathletes and controls (Pool et al., 2002). However, in the same study it was also shown that following endotoxin stimulation, newly induced IL-6 concentrations were lower in rugby
players than recreationally trained individuals (controls) and triathletes. Authors speculated that mononuclear cells may be chronically activated to spontaneously release IL-6, possibly as a result of some counter-regulatory mechanism or immunosuppression. Interestingly, low resting (0.8 ± 0.19 pg.ml\(^{-1}\)) and moderate levels of exercise induced (6.3 ± 1.5 pg.ml\(^{-1}\) game 1; 3.9 ± 1.1 pg.ml\(^{-1}\) game 3) IL-6 were observed in the present study. This is perhaps surprising given the combative nature of international rugby union and, as one would expect, a vigorous inflammatory response. Other studies have shown a larger IL-6 response after intense prolonged exercise such as cycling (Scharhag et al., 2006) and running (Suzuki et al., 2003). Conversely similar increases in IL-6 levels to the current study have been observed after soccer (Ispirlidis et al., 2008). It is perhaps possible that given the intense and combative nature of rugby union, a blunted IL-6 response may occur. This could theoretically account for the low resting and exercise induced levels in the current subject group. IL-6 has been purported to display many functions including help in metabolic homeostasis (Pedersen et al., 2003; Febbraio et al., 2003), involvement in the acute phase response (Tilg et al., 1997) and induction of CRP release from hepatocytes (Pedersen et al., 2003; Petersen and Pedersen, 2005). IL-6 is also known to induce an increase in cortisol both directly and indirectly (Bethin et al., 2000). One would suspect that with repeated secretion of this cytokine through daily exercise, compensatory adjustments to effector organisms may occur such as increased sensitisation of tissues to lower IL-6 levels. Further investigation is necessary in exploring this concept and the effect of repeated exercise on cytokine secretion. Although regular physical activity is known to elicit an anti-inflammatory effect (Jankord and Jemiolo, 2004), knowledge of inflammatory and anti-inflammatory balance across a training season may be important in terms of season periodisation. For example, if a heightened post-exercise inflammatory profile were evident following periods of less activity (e.g. start of pre-season) or sudden increases in tissue damaging exercise (e.g. contact work, resistance training), recovery strategies may be developed accordingly.

Peak increases in CRP have been observed after 19 h of intense exercise (Scharhag et al., 2006). In the current study, peak CRP levels were observed between 14 h (Game 1) and 38 h (Game 3) after exercise. The time course of this CRP rise is in line with previous investigations (Steensberg et al., 2003; Scharhag et al., 2006) although no increases were observed immediately post-exercise as observed previously (Ispirlidis et al., 2008). Produced in the liver following induction by IL-6 (Petersen and Pedersen, 2005), CRP is known to reflect the impact of trauma on the body (Brewster et al., 1994) and is associated with tissue damage (Gebhardt et al., 2000). Its basic features are the control of inflammation, the stimulation of clearance of
damaged cell and tissue components, and the initiation of repair functions (Pepys, 1981). Analysis of current data suggests that a significant degree of tissue inflammation was still evident 38 h into recovery where CRP values were found to be ~1.7 fold (both games) higher than those on the morning of the games. This data indicates that a recovery period of 38 h is insufficient in allowing full resolution of rugby induced tissue inflammation.

Like the other acute phase proteins, significant increases in serum cortisol were observed after both games, approximately 40% of values compared to camp-entry. Cortisol is an anti-inflammatory stress hormone that has a glucregulatory function, interacts with IL-6 and acts as a natural immuno-suppressant (Petrovsky et al., 1998; Papanicolaou et al., 1996; Weicker and Werle, 1991). Inducible by many physical and psychological stressors, it provides an indication of global psychophysiological stress. In the present study, elevations in C were still apparent 14 h after exercise, providing an index of the intensity that playing rugby union elicits. In contrast to C, large decreases (~37%) in serum testosterone were observed after both games; findings which together with changes in C, negatively effected the T/C ratio. One of the main functions of testosterone is to stimulate cell growth and protein synthesis while conversely; C is known to have a catabolic effect on myofibrillar proteins and suppress protein synthesis (Kayali et al., 1987). Therefore, alterations in T and C are thought to represent a disturbance in overall anabolic/catabolic cellular balance and changes in both markers are known to affect the recovery state and duration of recovery after exercise (Fry et al., 1991; Kuipers and Keizer, 1988). As observed in figure 4.12, a large drop in T/C ratio was observed after both games, with values only achieving pre-game values 38 h into recovery.

Although alterations in T/C ratio may occur through changes in either marker alone, the large increases in C in conjunction with decreases in T reflect the significant effect that international rugby union has on both corticotrophic and gonadotropic axes. It is known that the corticotropic axis inhibits the gonadotropic axis at hypothalamic level by a direct effect (or via an increase of beta endorphin) of corticotrophin-releasing hormone (CRH) on gonadotrophin-releasing hormone (GnRH) secretion (Barbarino et al. 1989; Cumming et al. 1983). This, in conjunction with direct steroidogenesis inhibition at the Leydig cells, may explain these decreased levels in circulating T. Reciprocally, this is in agreement with (but does not explain) the rise of testosterone during the recovery phase when cortisol is low (Elloumi et al., 2003). Changes in both of these markers are evident from the coupling of mental and physical strain (Elloumi et al., 2003; Filaire et al., 2001). Given that both games were internationals and
played in front of over 74,000 spectators, the magnitude of this psychophysiological stress response may be the highest that will be observed in team sport.

Interestingly, a gradual increase in resting T/C ratio was evident from camp-entry up to and including pre-game samples, with further increases observed at both 38 h post-game time points. These changes were predominantly influenced by a gradual lowering in resting C throughout the series. Indeed, following camp-entry (Day 1), C decreased below camp-entry values on the morning of game 1 (Day 5), and continued to decrease on days 7, 19 and 21. Furthermore, significant increases in T levels were noted on the morning of game three (Day 19) when compared to corresponding values for game one (Day 5). These findings are indicative of decreased residual stress and together with T/C values, improved recovery. Such data is suggestive of a more favourable biochemical environment and theoretically would have been of benefit to the team in terms of training recovery and game readiness. Indeed, this T/C data would appear to substantiate fitness goals outlined by the team conditioning staff for that tournament. Given the time of the year (intense competition period) and previous involvement of players in European club fixtures, a strong emphasis was placed on player recovery. This was facilitated mainly through a decrease in training volume (while maintaining intensity) and appeared to have a beneficial effect on player anabolic status. Interestingly, the levels of cortisol 38 h into recovery decreased below pre-exercise levels in both games despite the fact that samples were taken at the same time of day. Although similar to findings observed previously in rugby (Elloumi et al., 2003) and wrestling (Passerellargue and Lac, 1999), it is unclear as to why this occurred. However, it may be speculated that enhanced uptake by target tissues, accelerated clearance or alternatively, other adaptive mechanisms to protect muscle and other cortisol sensitive tissues against increased post-exercise cortisol secretion may have occurred.

In addition to an improved T/C ratio throughout the series, another noteworthy observation in this study was that higher values of cortisol, CRP, CK and blood leukocytes were evident on camp-entry when compared to values observed five days later (pre-game 1). Taken in context, this data shows that players entered the international set up with residual stress as a result of preceding European club activity. In light of the fact that players had only six days to prepare before the first international game, against a team ranked number one in the world at that time, this was not ideal preparation. Incidentally, players reported the intensity, aggression and collisions associated with that game as being the highest over the series. This was reflected by the high number of game contact events and game instances (Table 4.5). Such findings re-
affirm the role of adequate recovery from competitive rugby union, irrespective of club or international involvement.

**Conclusion**

The main findings were that competitive rugby union elicits significant changes in immunoendocrine status. Logistical constraints did not allow us to analyse the residual effects of the game on markers of physiological and mental stress further into recovery. However, current data suggests that complete recovery from international games does not occur inside 38 hours. In the present study, an attenuated stress response and improved recovery was observed throughout the 20-day investigation and this was congruent with planned fitness goals. Residual inflammation and tissue damage in players upon entry to the international series indicate that close monitoring of previous club training and game involvement is essential when planning activity prior to and during preparation for international rugby matches.
Chapter 5

General Methodology
[Longitudinal studies]

This section outlines the longitudinal stress responses of professional rugby union players over the course of a playing season. The research consists of four further studies. Firstly, study 3 will consider player attitudes and opinions on current game demands and seasonal structure. This study will be followed by study 4 which will examine changes in illness incidence and mucosal immunity and study 5, which will analyse the effects of training load on hormonal and immunoenocrine markers over a competitive season. Study six will assess the associated changes in psychological stress and possible association with training load. A general methods section is included below given that the same playing squad was common to studies 4-6.

5.1 STUDY ENTRY AND ETHICAL APPROVAL
Ethical approval for these studies is similar to those discussed in the methods section for acute stress responses in rugby (section 3.1).

5.2 PRELIMINARY MEASURES

5.2.1 Determination of Peak Oxygen Uptake (\( \dot{V}O_{2\text{peak}} \))
Criteria for determination of \( \dot{V}O_{2\text{peak}} \) is described in section 3.2

5.2.2 Body mass and stature
Body mass was measured using a calibrated beam balance (Avery Ltd., UK). Prior to testing, players were weighed (in shorts only) to the nearest 0.1 kg. Stature was assessed using a fixed stadiometer (Holtain Ltd., UK). Stature was measured without footwear, with heels placed together resting against the stadiometer. Whilst the players breathed in deeply, the moveable indicator was placed in contact with the superior point of the head. Measurements were taken to the nearest 0.1cm.

5.2.3 Skinfold Assessment
Skinfold thickness was measured according to the ACSM guidelines for exercise testing and prescription (ACSM, 2000). All measurements were made on the right hand side of the body using calibrated Vernier callipers (mm) and performed by a trained Kineathropometrist.
Skinfolds (measured to nearest ± 1mm) were taken from the following nine sites: Waist, Chest, Bicep, Thigh, Abdominal, Tricep, Sub-scapular, Supra-Iliac, Calf. Skinfold site values were summed for comparative purposes across study duration.

5.3 SALIVA SAMPLING AND ANALYSIS

5.3.1 Collection of Saliva Samples

The standardized collection of saliva is most important to obtain reliable results (Tenovuo and Lagerlöf, 1994). All players were advised to maintain their normal lifestyle habits outside of club structure and avoid use of known immunostimulatory preparations such as probiotics. In order to limit known circadian rhythms in saliva flow, s-IgA and lysozyme, all saliva samples were taken following an overnight fast and between 8-9 am on the morning of sampling. Players were asked to refrain from physical activity and alcohol at least 48 h prior to collection and water consumption 10 min before sampling. To standardise conditions, collection took place on the Monday or Tuesday of designated testing weeks. In the event of a training session/game taking place on the preceding Saturday, sampling occurred on the Monday. Conversely, if intense exercise/game occurred on the preceding Sunday, sampling took place on the following Tuesday. Prior to collection, players were asked to sit quietly and swallow any residual saliva contents in the mouth before sampling. All players were assigned a reference code which was marked on each case/tube prior to collection. In the case of s-IgA and s-Lys analysis, the collection tubes/cases were pre-weighted for subsequent determination of saliva volume (below).

Collection of saliva for determination of saliva IgA (s-IgA)

Unstimulated saliva samples were taken using standard salivettes (Sarstedt, Leicester, UK) as used in previous investigations (Whitham et al., 2006; Walsh et al., 2004; Pacque et al., 2007; Nakamura et al., 2006; Brenner et al., 2000; Phillips et al., 2006; Klentrou et al., 2002; Ring et al., 2005; Akimoto et al., 2003). The saliva sample was collected by placing the cotton salivette swab (diameter 1 cm, length 4 cm) under the tongue. Players were asked to hold the swab as still as possible, keep eyes open, head tilted slightly forward with minimal orofacial movement for 2 min (timed using a portable electronic digital timer). After exactly 2 min, players then removed the swab before returning it to the salivette holder. Samples were placed on ice immediately after collection and transported to the laboratory where the swabs were centrifuged (1500 g x 15 min) to release saliva for analysis. After measurement of the saliva volume, saliva (centrifuged) was frozen at -80ºC until future analysis (detailed below).
**Collection of saliva for determination of salivary Lysozyme (s-Lys)**

Saliva was collected by expectoration into a pre-weighed vial (5 ml Bijou screw cap tubes, Sterilin, UK) for 2 min with eyes open, in a seated resting position. Player’s heads were tilted slightly forward and with minimal orofacial movement. Pre labelled tubes were immediately placed on ice during transport to the laboratory where they were subsequently stored at -80°C until future analysis.

**Collection of saliva for determination of salivary cortisol (C) and testosterone (T)**

Saliva was collected via passive drool into 5 ml plain Bijou tubes (Sterilin, UK). Samples were immediately placed on ice and frozen within 4 hours of collection at -80°C.

### 5.3.2 Determination of saliva flow

Saliva volume was estimated by weighing to the nearest mg and saliva density was assumed to be 1.00 g.ml⁻¹ (Chicharro et al., 1998). From this, saliva flow was determined by dividing the volume of saliva by the collection time (2 minutes) and expressed as (ml.min⁻¹). Saliva secretion rate (µg.min⁻¹) was subsequently calculated as the product of saliva flow (ml.min⁻¹) x s-IgA or s-Lys concentration (µg.ml⁻¹).

### 5.4 SALIVA ANALYSIS

#### 5.4.1 Determination of s-IgA

Salivary IgA was determined using a human IgA enzyme linked immunosorbant assay (ELISA). On the day of analysis, all saliva (separated) samples were allowed to thaw at room temperature. On analysis, a 96-well microtitre plate (Maxicorp, Nunc, Gibco, UK) was pre-coated with a primary antibody (100 µl of goat anti-human IgA; Bethyl Laboratories Inc., Montgomery, TX, USA) at a dilution of 1:100 in coating buffer (0.05 M Carbonate-Bicarbonate, pH 9.6) and kept over night (4 ºC). The plate was then incubated for 1 hour at room temperature (20ºC). After incubation, all wells were washed (x 3) with a suitable wash buffer (PBS, 0.05% Tween 20, pH 7.4) freshly prepared on the day of analysis. After washing, wells were blocked for 30 min with 200 µl of blocking solution (Bovine serum albumin, BSA in PBS) before being washed again (x 3) with suitable wash buffer. Once thawed, all clear saliva samples were vortexed and then diluted (1:500) with sample diluent (1% BSA 0.05% Tween 20 in PBS, pH 7.4). A standard curve was constructed using known concentrations of human serum IgA (RS10-110, Bethyl Labs; range 7.8 ng/ml to 500 ng/ml). Sample diluent was used as zero. Internal laboratory controls were used to assess between assay variability. Samples, standards and controls (100 µl) were then added to each well and the plate was
incubated for a further 60 min at room temperature. The plate was washed (x 5) before addition (100 µl) of goat anti-human IgA-HRP conjugate (A80-102P, Bethyl Labs), diluted (1:70000) in sample diluent. The plate was then incubated for 60 minutes prior to being washed again (x 5). Tetramethylbezidine (100µl TMB, KPL, Maryland, USA) was then added to each well as substrate and the plate was incubated (in dark) for 20 min. The colorimetric reaction was stopped via addition of 100µl of sulphuric acid. The absorbance of solution in each well was subsequently determined at 450 nm within 10 min of adding stop solution using a standard spectrophotometer (Dynex Technologies, VA, USA). All samples and standards were assayed in duplicate. Saliva from a known IgA deficient individual was included as a negative control in the IgA ELISA (Francis et al., 2005). Results for s-IgA concentration are reported as µg.ml⁻¹. Intra-assay co-efficient of variation was 6.3%.

5.4.2 Determination of s-Lys
Lysozyme was determined in saliva using a commercial sandwich Human Lysozyme EIA kit (Biomedical Technologies Inc, Stoughton, MA, USA). On the day of analysis, thawed samples were centrifuged (1500 g x 15 min) before diluting (1/10000) with PBS. Appropriate volumes (100 µl) of reconstituted standards, blank (wash buffer), sample and controls were then added to a 96-well microtitre plate (pre-coated with lysozyme specific monoclonal antibody) in duplicate. After incubation with the sample (2 h), the plate was washed (x 3) before further incubation (1 h) with anti-human lysozyme (100 µl sheep polyclonal). The plate was then washed (x 3) and incubated with 100 µl of donkey anti-sheep IgG HRP conjugate for 1 hour at room temperature. After further washing (x 3) and addition of substrate mix (100 ul TMB and Hydrogen peroxide, 1:1 mix) detection was achieved following 15 min of incubation (in dark, RT). Following incubation, stop solution (100 µl, sulphuric acid) was added to all wells and absorbance was read at 450 nm. Concentration of each unknown was determined from standard curve after subtraction of absorbance for blank wells. Intra-assay co-efficient of variation was 5.7%. Assay sensitivity was 0.78 ng/ml, range 0.78-50 ng/ml.

5.4.3 Determination of saliva Cortisol (C) and Testosterone (T)
On the day of analysis, thawed samples were centrifuged (1500 g x 15 min) and the clear supernatant was aspirated into 1.5 ml pre-labelled eppendorf tubes for separate C and T analysis. To avoid repeated freeze-thawing, samples were analysed for both analytes on the same day using commercial high-sensitivity expanded range salivary C and T ELISA kits (Salimetrics LLC, PA, USA). Samples were analysed in duplicate along with appropriate standards (0.012-3.0µg/dL, cortisol; 6.1-600 pg/dL, testosterone) and controls from the same
company. Samples in the same batch were analysed together to avoid between assay variation. For analysis, 25 µl of standards and clear samples were added to an already pre coated 96-well microtitre plate. Assay diluent (25 µl) was also added to selected wells acting as a blank and for non-specific binding (NSB) wells. Upon re-constitution of enzyme conjugate (horseradish peroxidise), 200 µl (150 µl for testosterone) of diluted solution (1:1600, cortisol; 1:1000, testosterone) was added to all wells. The plate was mixed for 5 min (500 rpm) before incubating for a further 55 min, and then washed (x 4) before addition of substrate (TMB; 200 µl) to each well. The plate was then mixed (5 min, 500 rpm) before incubation (25 min in dark, RT). After incubation, the colorimetric reaction was stopped via addition of stop solution (50 µl; 2-M sulphuric acid) and read at 450 nm (reference wavelength, 620 nm) within 10 min. Intra/inter assay variations were 4.6% and 6.2% for C and 5.4% and 8.0% for T.

5.4.4 Blood Contamination Assay (Transferrin)

Since saliva C and T values are known to be influenced by blood plasma leakage (Kivlighan et al. 2004) into the oral musosa, samples were screened for possible blood contamination. Following C and T determination, thawed cooled samples were analysed the following day using a standard blood contamination EIA kit (Salimetrics LLC, PA, USA). This determines transferrin levels and since this component is only found in blood, acts as a measure of contamination. Standards (in duplicate) and clear samples (20 µl; singleton) were added to a pre-coated 96-well microtitre plate. Assay diluent was added to selected wells acting as zero (20 µl) and non-specific binding (NSB; 70µl); both duplicate. Upon re-constitution of enzyme conjugate (horseradish peroxidise), 50 µl of diluted solution (1:400, 20 µl conjugate to 8 ml diluent) was added to all wells. In turn, 50 µl of antiserum (rabbit anti-human transferrin antibody) was added to all wells (except NSB wells) before mixing (5 min, 500 rpm) and incubated (55 min). Following incubation the plate was washed (x 4) before addition of substrate (100 µl TMB). The plate was then mixed (5 min, 500 rpm) before incubation (in dark, 10 min, RT). The colorimetric reaction was then stopped via addition of stop solution (100 µl 2-M sulphuric acid) and read at 450 nm (correction, 620 nm). Values greater then 2 mg/dL were considered for exclusion (both T and C). This was based on published literature (Kivlighan et al. 2004; Schwartz and Granger, 2004; Gleeson et al., 2007) and on current study calculations.

5.4.5 Saliva Osmolality

Saliva osmolality was measured using the freezing point depression method via an Osmometer (Model 3300 MO, Advanced Instruments, Massachusetts). Prior to analysis, the instrument
was calibrated with 3 calibration standards and a control of known concentrations (NaCl 50-800 mOsm-kg H2O). Mixed samples (20 µl) were analysed in duplicate. Intra and inter sample CV’s were 4% and 6% respectively.

5.5 **BLOOD SAMPLING AND ANALYSIS**

Venous blood samples were taken from an antecubital vein by venepuncture, and collected into three vacutainer tubes (Becton Dickinson, Oxford, UK). Blood collected in K3EDTA vacutainers (4 ml) were kept at room temperature and used for standard haematological analysis. After analysis, the remainder of the samples were separated immediately by centrifugation (1800 g x 15 min) for collection of plasma. In addition, whole blood (7 ml) was collected into sterile lithium-heparin vacutainer tubes. After collection, the blood was centrifuged (1500 g x 10 min) and plasma was obtained for future analysis of glutamine and glutamate (detailed below). For serum measurements (hs-CRP and CK; detailed below), blood (7 ml) was collected into vacutainer tubes containing a clot activator (SST; Becton Dickinson, Oxford, UK). Following centrifugation (1500 g x10 min) serum was aspirated into appropriate aliquots and stored at -80ºC for future analysis.

5.5.1 **Determination of Creatine Kinase and high sensitive C-reactive protein**

See section 3.3.5 for details.

5.5.2 **Determination of plasma Glutamine and Glutamate**

Enzymatic assays were used for calculation of glutamine and glutamate using lithium-heparin plasma following storage at -80 degrees.

**Sample deproteinisation**

After thawing [on ice], plasma samples were centrifuged at (16000 rpm for 30 s; desktop centrifuge) to remove fatty deposits and deproteinised according to methods described previously (Bernt and Bergmeyer, 1974). Following centrifugation, 300 µl of plasma was dispensed into a labelled eppendorf tube (A) along with 300 µl of perchloric acid (PCA, 10%). Prepared samples were subsequently vortexed (immediately) and centrifuged (13000 rpm x 2 min) before aspiration of 460 µl [clear supernatant] into a second pre-labelled eppendorf tube (B). In order to determine changes in sample pH, 15 µl of pH Universal Indicator (BHD labs, Poole, England) was added to eppendorf tube B along with 50 µl of triethanolamine buffer (TEA, 0.5M; 637-39-8 Sigma-Aldrich) and 95 µl of potassium hydroxide (20% KOH; Sigma-Aldrich) to neutralize acids. Following preparation, the sample pH was adjusted, *if necessary*
via further addition of KOH and/or sample supernatant to eppendorf tube B. The final sample pH was noted via visual inspection (pH 7.0-7.5, colour: light green) and extra KOH/supernatant additions were noted and used for future calculations. Deproteinised samples (eppendorf tube B) were subsequently centrifuged and the supernatant was aspirated from its pellet into another labelled eppendorf tube (C). This was then stored (-80º) until future analysis of glutamine and glutamate.

**Plasma Glutamine Analysis**

Plasma glutamine concentrations are subject to variability in analytical procedures (Castell, 2003). Hence, cross comparisons of values between studies should not be made if analytical techniques are different between investigations. Plasma concentrations using previously reported spectrophotometric procedures (Rennie et al., 1981; Lund, 1985; Castell et al., 1995), as in the present study are lower than those in other studies (900-1200 µmol.l⁻¹, Kargotich et al., 2007; Rowbottom et al., 1996) using the E coli method (Keast et al., 1998). In the current study, plasma glutamine was analysed using a modified enzymatic assay as described previously (Windmuller and Spaeth, 1974). The asparaginase assay was chosen in preference to the glutaminase assay (Lund, 1974); personal communication (L. Castell, Nuffield Dept. Anaesthetics, University of Oxford, UK).

Prior to analysis, liquid asparaginase (L-asparaginase 1187, Fluka BioChemika) was dialysed for 24 h against two changes of prepared potassium dihydrogen orthophosphate assay buffer (80 mM KH₂PO₄, pH 6.6; Fisher Scientific). This buffer was subsequently calibrated to pH 8. The glutamine assay mix was the prepared via addition of appropriate concentrations and

\[
\begin{align*}
\text{Glutamine} + \text{H}_2\text{O} + \text{Asparaginase} & \rightarrow \text{Glutamate} \\
\text{Glutamine is hydrolysed with asparaginase to produce glutamate}
\end{align*}
\]

\[
\begin{align*}
\text{Glutamate} + \text{NAD}^+ & \rightarrow \text{NADH} + \text{H}^+ \\
\text{Glutamate dehydrogenase [GDH]} & \rightarrow \alpha\text{-ketoglutarate} + \text{NH}_4^+
\end{align*}
\]

The Glutamate concentration is determined indirectly by the hydrolysis of glutamate to \(\alpha\)-ketoglutarate with GDH. Oxidised NAD⁺ is converted to its reduced form, NADH + H⁺ during this reaction which is detected spectrophotometrically at 340 nm.

**Figure 5.1:** Enzymatic reactions involved in production of glutamine and glutamate.
volumes of NADH (172 µM; Roche Diagnostics, 128023.), glutamate dehydrogenase (0.5 mg GDH; Sigma Aldrich) and α-ketoglutarate (3.6mM, Sigma-Aldrich K-3752) to the pre-prepared assay buffer (9 ml) and distilled H₂O. Glycerol (50%) and BSA (0.05%; Sigma-Aldrich A7030) were also added to final solution to protect assay enzymes from denaturation. The above mixture provided enough assay solution for 10 samples in duplicate.

To limit errors, fresh assay mix was prepared on each day of analysis. Known standards were prepared from standard glutamine concentrations (L-Glutamic dehydrogenase, G2626, Sigma-Aldrich) and mixed with varying volumes of distilled H₂O to provide final glutamine concentrations of 300, 400, 500 and 600 µl. On day of analysis, 100 µl of thawed (on ice) samples and standards were added to 960 µl of assay mix in semi-micro clear walled cuvettes (1.6 ml, Fisher Scientific FB 55147). Assay mix (960 µl) and 100 µl of distilled H₂O served as assay control while 1 ml of distilled H₂O acted as zero. The spectrophotometer was subsequently calibrated using the zero sample. All test samples and standards were prepared in duplicate and upon reconstitution, mixed immediately via inversion. After mixing, the samples, standards and control were left sit for exactly 30 min (room temperature) before being read at 340 nm using a standard spectrophotometer (Pharmacia, Biotech Novaspec II). Following this, asparaginase (15 µl) was added to all samples (incl. control) to start the enzymatic reaction (hydrolyze the glutamine). All samples were then vortexed before being left for a further 60 min (room temperature). Following this samples were read again and glutamine was determined from the reduction in absorbance at 340 nm. Absorbance changes were corrected for parallel changes in the reagent blank.

Plasma glutamate
Plasma glutamate was analysed from thawed deproteinized samples according to the methods described by Bernt and Bergmeyer, 1974. Fresh assay buffer was prepared using distilled H₂O, Trizma base (Sigma-Aldrich T1503), EDTA diaminooethanetetra-acetic acid disodium salt (Fisons Scientific, Loughborough, England) and hydrazine hydrate (BDH labs, Poole, England). After preparation, the assay buffer was calibrated to pH = 9. Assay mix was subsequently made up via mixture of appropriate concentrations/volumes of prepared assay buffer, β nicotinamide adenine dinucleotide (NAD, Sigma-Aldrich 6522) and adenosine-5-diphosphate (ADP, Biochemika Fluka 01905).

Similar to assay preparation for glutamine analysis, the spectrophotometer was initially calibrated using a zero standard (1 ml distilled H₂O). Following this, assay standards, samples
and controls were prepared in cuvettes via addition of 980 µl of assay mix with 150 µl of sample, standard or control solutions. In the case of control sample, 150 µl of distilled H2O was used. After mixing, all cuvettes were read immediately at 340 nm before addition of GDH (22.5 µl) to all samples (except zero). Samples were then vortexed before being left to sit at room temperature for 60 minutes. Following incubation, all samples were re-read at 340 nm for possible changes in glutamate concentration.

5.5.3 Urine Osmolality

Urine osmolality was measured using the same procedure as discussed for saliva osmolality. Intra and inter urine sample CV’s were 2% and 5% respectively.

5.6 Physical activity and Training Load (TL) assessment

Monitoring of TL and daily physical activity was carried out using a web-based training diary program. The diary was developed in HTML-format and PHP-scripting language (as internet web pages) that could be used on computers with operating system Windows ‘98 and higher. Updates and administration of the program was possible via logging into the diary by affiliated fitness staff. The system was set up 2-months prior to study commencement so that the majority of players were familiar with the system requirements. Players were provided with an individual user-name and password and software prevented entry of existing name files. Any new player entering the club was provided with user training on how to operate and submit training-related data. Players were given the opportunity to practice data entry during the initial preliminary testing week (wk-1) and to resolve any queries relating to data submission. In order to avoid recall bias, players were required to submit data on a daily basis whilst at squad training headquarters. Where possible, players were encouraged to submit training data on club laptops after training sessions. This normally occurred after cessation of morning training sessions and prior to mid-afternoon meal times. If a player undertook any training activity outside that prescribed by the trainer, they were instructed to input the type, duration and perceived intensity for that exercise activity on the web-diary. In the event of a weekend game, data was submitted the following morning. Players were also instructed to submit diary data while on international duty or absent from the team training base due to injury/illness.

Data input was in the form of drop down menus. Once activity type was selected, players were asked to manually enter the duration for that activity. Aside from registration of daily activity levels (training type, intensity, duration), players were presented with a series of accessory
items (fatigue ratings, illness reports, body weight) that required submission of data upon diary log-in. Completed data for each link was submitted via clicking a ‘submit data’ icon and saved to the server before logging out. A file containing player name, date of entry and time of entry was also stored on the system. A training summary for each player was available to associated fitness staff for each day, week or month of interest. Staff could select data summary sheets as per selected player or for the squad as a whole. The program was set up so that player-squad responses could be compared across time (weekly, monthly). This was in the form of graphical readouts. Raw data was exportable to Microsoft excel (Microsoft Corporation, USA) for further analysis. No attempt was made by investigators to alter TL’s or activity levels during the course of the investigation. Average weekly TL’s were quantified for each player and for the squad as a whole using a previously established method using rating of perceived exertion (Foster, 1998). Validation of this method in rugby can be seen in Case study 3. Weekly TL represented the sum of all sessions carried out in that week (see Appendix F). Monthly TL’s were calculated by summation of weekly TL.

Players were asked to rate session intensity on how they felt about the whole session rather than the most recent exercise intensity. Players were also required to submit data relating to participation in ‘contact’ training sessions. Contact was defined as any session involving player-player contact or contact ‘bag’ work. Defence sessions and scrum sessions were included in this category. An exploratory contact intensity scale (0-10), similar to the one used in perception of exercise intensity, was used in an attempt to help grade weekly training sessions and in determining the extent of ‘contact’ training on observed recovery. In addition to self-reporting by players, coaches rated training days based on levels of set volume and/or intensity and this data was also recorded throughout the season. To cross check if participants did not over-report training volume, coaching staff also submitted training related data for each training day using the diary. This was available to study investigators at all times. Fitness staff uploaded daily and weekly training plans onto the diary in advance to maintain logical ordering of activities performed. Once training-illness data was submitted it was not possible to change or re-submit data. Players could not submit training-illness data for future training days. The diary was in a standard order format so that players could not neglect certain sections of the diary. Players were unable to view summary sheets or other team-mates data on the diary. Registration (diary) data for each player was available to selected staff. In the event of a player failing to enter training or illness data, an ‘auto-alert’ system was employed. This alerted the player in question and fitness staff of data omission upon future diary log-in. Diary compliance throughout the 48 week period of investigation was 81%.
Chapter 6

Effects of Chronic Exercise on Imunoendocrine Markers and Illness Incidence in Professional Rugby

Study Three: Investigation of player attitudes to contemporary demands in professional rugby union

SUMMARY

Few studies have documented player attitudes to current demands within rugby union at the elite level. In light of this, player welfare questionnaires were distributed to professional players within Wales. In total, responses were obtained from 65 players. Of these, 20 players formed part of the current national squad (NAT) and 45 were participating within the regional club structure (REG). Questionnaire data revealed that current season length and structure, as opposed to game number, is a more pertinent issue regarding player welfare. The majority of players in both groups (80%) felt that time ‘away’ from rugby was not sufficiently long enough. Players (73%) favoured a mid-season break, lasting ~2 wks in duration and felt most fatigued/in need of a break around Christmas time. Players (80%) agreed/strongly agreed that game demands are increasing and that inadequate seasonal structure and intensified competition periods contribute to feelings of fatigue. Insufficient recovery between games was allocated the main reason for feelings of fatigue amongst NAT group. The majority of players in both groups (65%, NAT; 81% REG) fear that current game demands will impact on future health and that injury severity is increasing. In conclusion, the current study reveals that elite rugby union is increasingly demanding on players and that current seasonal structure contributes to feelings of vulnerability and fatigue amongst its playing population.

6.1 INTRODUCTION

Recent research has suggested that rugby union is now Britain’s second most popular sport (Ipsos MORI, 2003). In Wales, rugby union is considered the national sport and is played by an estimated 59,900 players (http://www.irb.com, 2003). Of these, ~150 players participate on a full-time professional basis, in a game considered one of the most physically demanding in the world (Mashiko et al., 2004). This physicality of rugby union has been reflected in recent studies which have documented large incidence rates of injury in the sport. In a recent study on elite players participating in the 2007 world cup, incidence of injuries was recorded as 83.9/1000 player-match hours and 3.5/1000 player-training hours (Fuller et al., 2008). These current injury statistics appear worrying considering evolutionary trends within the game. Players have become bigger and faster over time and are involved in more physical contact and collisions during game play (Duthie et al, 2003; Quarrie and Hopkins, 2007). Such changes in
game demands have been mirrored by issues such as increasing injury incidence (Bathgate et al., 2002; Garraway et al., 2000; Brooks and Kemp, 2008), player burnout (Cresswell, and Eklund, 2005) and general player unrest (Ipsos-MORI, 2006). With respect to injury rates, a rise of 57% has been shown in elite Australian rugby following the introduction of professionalism (Bathgate et al., 2002). Similar findings have been published for Scottish rugby (Garraway et al., 2000). Together, the evidence would suggest that game demands are increasing with time, and that rugby at the professional level, creates more frequent and powerful player impacts which result in high injury rates. Aside from issues such as game trends and intensity, other important issues relating to overall player welfare include those such as amount of rugby/training exposure, length of playing season and off-season time. Currently the professional rugby union season (northern hemisphere) runs between the months of August and May in a non-world cup year, with teams normally beginning pre-season training during the month of July. In addition to this, players who represent their country at international level are often required to play during the summer months; a time period which would be normally set aside as recovery from the previous season. In Wales, a professional player may be required to participate in anything up to 36 games over a 49 week season.

The issue of excessive player demands has been the subject of recent attention and highlighted in an RFU commissioned study on player welfare (Ipsos MORI, 2006). In this study, it was found that the majority of players thought that they played too much rugby, the season was too long and the severity of injuries was increasing. Research was based on initial findings showing that players in the professional rugby structure in England exhibited two to three times the level of exhaustion compared to their New Zealand counterparts (Cresswell, 2005). These findings were substantiated in a more recent study leading into the autumn internationals (2006) where half of the England squad showed signs of mental and physical exhaustion (Ipsos MORI, 2006). Such research is significant both from an evolutionary point of view, but perhaps more importantly, with respect to participation levels and safeguarding the health of future playing populations. In view of the above, the aim of the present study was to examine player opinions on current game demands in Wales. Subsequent to findings, future studies will attempt to explore the clinical significance of current game demands in rugby union using more objective markers of fatigue and overreaching across a given rugby year.

6.2 METHODS
An exploratory player welfare questionnaire was distributed to players [age 25.0 yr, n = 65 (31 backs, 34 forwards)] contracted to two of the professional regions in Wales. Questionnaires
were distributed to each of the regions at different time points during the season so as to avoid possible seasonal bias or acute effects of training history on observed player response. Additionally, questionnaires were distributed to players who were currently part of the Wales national squad. In light of this it was hoped that player responses to game demands could be ranked based on club and international competition demands.

Statistics
Data in this study is of a descriptive nature only and expressed as percentages.

6.3 RESULTS

In total, out of 80 questionnaires distributed to the two regions, 65 were completed resulting in an 81% questionnaire response rate. The majority of players surveyed reported that current season was ‘too long’ (55%), ‘poorly structured’ (56%) and that game demands were increasing with time (52%), n = 65. Between group (national: NAT; regional: REG) comparisons revealed that a greater percentage of respondents at regional level reported current season length as being ‘too long’ (Fig. 6.1). Conversely, a greater percentage of the national squad reported current seasonal structure as being ‘poor’ (60% NAT; 44% REG). The majority of players in both groups (80%) felt that time ‘away’ from rugby was not sufficiently long enough (Fig. 6.7).

A greater percentage of respondents within the NAT group ‘strongly agreed’ that game demands are increasing (65% NAT, 50% REG; Fig 6.4). Both player groups deemed that current ‘game number’ during the season as ‘appropriate’ and enjoy the challenge of training hard between games. The majority of players (78%) felt that current preseason was ‘sufficiently long enough’ albeit players were in favour of a mid-season break (75% REG; 85% NAT) and deemed a break within the region of 2 week’s duration as appropriate (Fig. 6.6b). Players in both groups felt most fatigued or in need of a break around the Christmas period of the season, 45% NAT and 29% REG; Fig. 6.5(a). A combination of insufficient recovery between games, existence of mid-week games, too many games and carrying an existing injury were the reported reasons behind this. Both groups fear that collisions experienced while playing rugby may impact on future health. This was most evident at regional level (81%; Fig 6.9b). 80% of players either ‘strongly agreed’ or ‘tended to agree’ that injury severity was increasing across time. All regional players and 65% of national players had considered their future careers after participation within professional rugby.
**SECTION 1: PLAYER INFORMATION**

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<th>PLAYER AGE</th>
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**SECTION 2: Views on CURRENT seasonal structure in Wales**

1. Current season LENGTH is:  
   - Too-Long  
   - Appropriate  
   - Too-short  
   - No-Opinion  

2. Current NUMBER of Games:  
   - Too-Many  
   - Appropriate  
   - Too-Few  
   - No-Opinion  

3. Seasonal STRUCTURE:  
   - Well structured  
   - Appropriate  
   - Poorly-Structured  
   - No-Opinion  

**SECTION 3: Views on TRENDS within Professional Rugby**

4. To what extent, if at all, do you agree or disagree that player demands in rugby are increasing?  
   - Strongly agree  
   - Tend to agree  
   - Neither/Nor  
   - Tend to disagree  
   - Don’t know  

5. To what extent, if at all, do you agree or disagree that player injuries are increasing in severity?  
   - Strongly agree  
   - Tend to agree  
   - Neither/Nor  
   - Tend to disagree  
   - Don’t know  

6. Would you consider retiring from rugby if demands of the sport became too much?  
   - Strongly agree  
   - Tend to agree  
   - Neither/Nor  
   - Tend to disagree  
   - Don’t know  

**SECTION 4: Views on Management Structures**

7. Have you ever been put under pressure by your club/region/country to play whist not fully fit or recovering from injury?  
   - Yes, frequently  
   - Yes, occasionally  
   - Rarely  
   - No, never  
   - Don’t know  

8. Do you think that current training methods allow for sufficient recovery in rugby?  
   - Strongly agree  
   - Tend to agree  
   - Neither/Nor  
   - Tend to disagree  
   - Don’t know  

9. Do you think current injury management procedures are sufficient at your club/region?  
   - Strongly agree  
   - Tend to agree  
   - Neither/Nor  
   - Tend to disagree  
   - Don’t know  

**SECTION 5: Apportionment of STRESS within Rugby**

11(a): At what stage(s), if at all, in the season to you feel most fatigued/need of break?  
   - Comments here please (you may wish to leave blank).  

11(b): If yes, why do you think this occurs? (If you commented on above)  
   - PLEASE TICK APPROPRIATE BOX(S) WHICH BEST APPLIES TO YOU!  
   - Inadequate Conditioning  
   - Too many games  
   - Insufficient recovery between games  
   - Carrying a Chronic Injury  
   - Overlap of competitions  
   - Mid Week Games  
   - Training too hard between games  
   - Carrying an Acute Injury  

12. Would you be in favour of a mid season break?  
   - YES  
   - NO  

13. Do you think time AWAY from rugby is sufficiently long enough?  
   - YES  
   - NO  

14. Do you think your current preseason is sufficiently long enough?  
   - YES  
   - NO  

15. At what stage(s), if at all, during the season do you feel most vulnerable to Injury?  
   - Comments here please e.g. preseason, within season etc (you may wish to leave blank).  

**SECTION 6: OTHER**

16. Do you fear that collisions you experience in rugby will affect your future health?  
   - YES  
   - NO  

17. Have you considered your future career after rugby?  
   - YES  
   - NO
View on increasing player demands

View on current number of games

View on current season length

View on current seasonal structure

Figure 6.1: Graph showing opinions of players on current season length, regional and national.

Figure 6.2: Graph showing opinions of players on current number of games per given season, regional and national.

Figure 6.3: Graph showing opinions of players on current seasonal structure, regional and national.

Figure 6.4: Graph showing opinions of players on current demands, regional and national.
At what stage during season do you feel most fatigued or in need of a break?

![Graph showing opinions of players on demands during specific time points, regional and national.]

Why do you think this occurs?

![Graph relating to cause of fatigue throughout the season, regional and national.]

Would you be in favour of a mid-season break?

![Graph showing player opinions on mid-season break, regional and national.]

If 'Yes', how long would you recommend?

![Graph showing player opinions on duration of break, regional and national.]

Figure 6.5(a): Graph showing opinions of players on demands during specific time points, regional and national.

Figure 6.5(b): Graph relating to cause of fatigue throughout the season, regional and national.

Figure 6.6(a): Graph showing player opinions on mid-season break, regional and national.

Figure 6.6(b): Graph showing player opinions on duration of break, regional and national.
Do you think time away from rugby is sufficiently long enough?

<table>
<thead>
<tr>
<th></th>
<th>Regional</th>
<th>National</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>81.25%</td>
<td>80.00%</td>
</tr>
<tr>
<td>No</td>
<td>18.75%</td>
<td>15.00%</td>
</tr>
<tr>
<td>No answer</td>
<td>0.00%</td>
<td>5.00%</td>
</tr>
</tbody>
</table>

Figure 6.7: Graph showing player opinions on time-away from rugby, regional and national.

Do you think current preseason is sufficiently long enough?

<table>
<thead>
<tr>
<th></th>
<th>Regional</th>
<th>National</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>65.00%</td>
<td>75.00%</td>
</tr>
<tr>
<td>No</td>
<td>30.00%</td>
<td>25.00%</td>
</tr>
<tr>
<td>No answer</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

Figure 6.8: Graph showing player opinions on current length of preseason, regional and national.

Have you considered your career after rugby?

<table>
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<tr>
<th></th>
<th>Regional</th>
<th>National</th>
</tr>
</thead>
<tbody>
<tr>
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<td>100.00%</td>
<td>65.00%</td>
</tr>
<tr>
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<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>No answer</td>
<td>0.00%</td>
<td>35.00%</td>
</tr>
</tbody>
</table>

Figure 6.9(a): Graph showing player response to consideration on future career, regional and national.

Do you fear that collisions you experience in rugby will affect your future health?

<table>
<thead>
<tr>
<th></th>
<th>Regional</th>
<th>National</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>81.25%</td>
<td>65.00%</td>
</tr>
<tr>
<td>No</td>
<td>18.75%</td>
<td>30.00%</td>
</tr>
<tr>
<td>No answer</td>
<td>0.00%</td>
<td>5.00%</td>
</tr>
</tbody>
</table>

Figure 6.9(b): Graph showing player opinions on future health, regional and national.
6.4 DISCUSSION

The aim of this investigation was to document current player opinions on game demands and trends within professional rugby union. It was hoped that these findings may aid in development of future areas of investigation and help in tackling issues such as player-welfare and game management.

Results of this study largely confirm that of previous unpublished research carried out on professional players participating in England (Ipsos MORI, 2006). Similar to the players in England, the current player group (65 elite players, Wales) believe that the current rugby season is ‘too long’, ‘poorly structured’ and that overall game demands are increasing. The majority of players in the current study (80%) felt that time ‘away’ from rugby was not sufficiently long enough and favoured the introduction of a mid-season break. Players did not view current game number as a major debilitating factor within this overall stress paradigm. Instead, players tended to view ‘inadequate’ or ‘poor’ seasonal structure rather than ‘too many games’ as a more important area of concern within professional rugby. These findings may have important implications with respect to overall player welfare and are something which needs to be considered at governing body level, in particular, the amount of time players spend training in between fixtures. Repeated exposure to daily training without sufficient rest or breaks may lead to training monotony (Foster, 1998), overtraining syndrome, sports burnout, increased susceptibility to illness, psychological disturbances, and performance decrements (Fry et al., 1991). With respect to seasonal demands, the majority of players in the current study were in favour of a mid-season break (Fig. 6.6a). Players felt this should last in the region of 2-3 weeks duration. In light of this, and from findings when players were asked to define time periods they felt ‘most fatigued or in need of a break’ (Fig. 6.5a), implementation of a recovery period during the month of December would seem pertinent. However, changes in game structure during this time period would be difficult as anecdotal evidence would suggest that game fixtures during the Christmas period in particular, represents a time of increased revenue for participating professional clubs (personal communication, WRU). Interestingly, a number of players themselves felt the need to document these views on paper during testing and some players suggested that any changes in game structure should be made with prior consideration of these financial implications.

To the author’s knowledge, few published studies, if any, have documented areas of stress within the game as viewed through current player attitudes and opinions. While previous research has shown increasing trends in injury incidence across time (Garraway et al., 2000;
Brooks and Kemp, 2008), few have documented opinions of players themselves on evolutionary trends and apportionment of stresses within the game. Worryingly, in the current study, 80% of players either ‘strongly agreed’ or ‘tended to agree’ that injury severity is increasing across time. Disturbingly, 8% of the sample population ‘strongly agreed’ with the statement that they would ‘consider retiring from the sport due to increasing game demands’. Furthermore, the majority of players fear that the collisions they experience during the game will affect their health at some point in the future (80% REG; 65% NAT). This is an obvious area of concern and is something that needs to be investigated further at governing body level. It is feasible that the lower percentage observed within the NAT group is because players who compete regularly at international level receive better and more intensive injury treatment and/or medical care. This finding would tend to agree with previous findings in English rugby where it has been shown that despite a higher chance of injury occurring whilst playing international matches; the average number of days absent is generally less (Ipsos MORI, 2006). Although speculative, such findings infer greater medical treatment once an injury has occurred at international level. Owing to the lack of specific and objective knowledge on the adverse effects of regular rugby participation on future health, it is difficult to devise recommendations for minimum medical assistance and requirements at a given club. Despite these observations, players, in the main, ‘tended to agree’ (41%) or ‘strongly agreed’ (34%) that current injury management structures are sufficient at their respective club/international base. However, 39% and 17% of players reported that they felt that they have ‘occasionally’ or ‘frequently’ been placed under pressure by management structures to play whilst ‘not fully fit or recovering from injury’. This type of data is essential because professional sports clubs have a legal obligation and a duty of care responsibility to their players to document and implement suitable management strategies to minimise injury risks (Fuller, 1995). Any form of coercion and/or pressure on players to play when not fully fit would contravene this duty.

Despite the heavy demands placed upon the contemporary rugby player, the majority of players felt that they enjoyed the stimulus of training hard (80%). Player opinions were divided when asked if they ‘would consider retiring from the sport if the demands became too much’. However, a greater percentage (30%) of players ‘tended to disagree’ as opposed to agreeing (25%) with this statement. Therefore, overall it would seem that players are prepared to tolerate the contemporary demands despite reporting adverse consequences.
Conclusion

In summary the current study substantiates previous findings suggesting that season length and structure rather than game number are more pertinent issues to the overall stress paradigm in elite rugby union. Player’s opinions support existing scientific opinion that game demands are increasing while players fear that injuries/collisions picked up during their playing career may impact on future health. Although present findings remain, to a large degree, anecdotal in nature, they do highlight the need for further investigation into game demands and stress paradigms in elite rugby union. In this study, a larger database of player responses would have enabled cross comparisons between player position and player experience within the game. Future research is needed to investigate the possible relationship between perceptions of physical load and stress through more objective measures of stress such as changes in immune/hormonal variables across the rugby season. Knowledge of such will help in safeguarding the health of future playing population.
Study Four: Changes in mucosal immunity and infectious incidence within elite rugby union players over a competitive season

SUMMARY

Previous studies have shown that intense exercise results in suppression of host immunity. This may predispose athletes to an increased risk of developing upper respiratory illnesses (URI’s). Few studies have investigated changes in mucosal immunity and URI development within multi-stressor team environments over prolonged periods. The purpose of this study was to assess the effects of chronic exercise exposure on markers of mucosal immunity and URI incidence in elite rugby union players (n = 30). Timed resting morning saliva samples were taken from players at pre-selected time points over the course of a competitive playing season (11 months) for determination of changes in stress and mucosal immunity (s-Cortisol, s-IgA, s-Lys). Weekly self-reported player illness data was obtained using an ‘internet’ based data logging system in addition to data recorded by affiliated medical staff. Daily records of training load and physical activity were obtained using the same system. Players reported on average, 4 URI episodes (min = 0; max = 8) over the 48 wk period. Peaks in URI’s observed during the months December and March were preceded by increases in training volume and intensity. Decreases in s-IgA and s-Lys during these periods were associated with increases in s-Cortisol. Recorded number of URI episodes differed for player position [mean ± SEM: 3.4 (0.47) vs 4.3 (0.51)], for forwards and backs respectively. Backs also displayed lower resting s-IgA and s-Lys concentrations and secretion rates when compared to forwards across the study period. Regular monitoring of s-IgA and s-Lys may be useful in the assessment of both exercise stress and URI risk status in elite rugby union. A combination of alterations in training intensity and seasonal influence are likely contributors to observed peaks in URI incidence. It is probable that stress induced changes in cortisol release may contribute to reductions in mucosal immunity which when lowered predisposes elite rugby union players to increased URI incidence.

6.5 INTRODUCTION

The incidence of banal infections is thought to be related to chronic or acute heavy physical exertion (Foster and Lehmann, 1997; Lehmann et al., 1993., Peters and Bateman, 1983) and may act as an early marker of overtraining in athletes (Foster and Lehmann, 1997; Budgett, 1998). Furthermore, training loads over and above that of individuals coping response and/or training thresholds have been associated with increased susceptibility to colds or upper respiratory illnesses (URI) (Nieman and Pedersen, 1999; Fry et al., 1991; Foster, 1998). This increased susceptibility to URI’s is thought to be largely dependent on immune depression originating from a variety of stressors including physiological, psychological, environmental and behavioural (Meeusen et al., 2006; Gleeson, 2007; Pyne et al., 2000).

More than 90% of all infections involve the mucosae with regard to microbial colonization or entry into the body (Brandtzaeg, 2003). Therefore changes in mucosal immunity have been suggested to play and important role in predisposition to illness (Mackinnon, 1999). Along with protecting the oral mucosa through a mechanical washing effect, mucosal secretions play an important role in innate (natural or nonspecific) immunity and act as the first line of defence
Chapter 6  
Illness Incidence and Mucosal Immunity

against potential pathogens (Bishop et al., 2000). Immunoglobulin A (IgA) is the predominant antibody in saliva where it acts to prevent viral replication and inhibit viral and bacterial attachment to the mucosal epithelium of the mouth, throat and upper respiratory tract (Mackinnon, 1996). However, a direct link between changes in saliva IgA and increased illness incidence remains somewhat equivocal. This may be due in part to the time lag between possible s-IgA decreases and development of URI’s. Few studies have monitored s-IgA before and after development of a URI (Nakamura et al., 2006; Mackinnon et al., 1993). In the latter study, hockey players who developed symptoms of URI were shown to have reductions in salivary immunoglobulin A (s-IgA) of 22% and 23% within 2 days of symptom onset. In comparison, players who remained healthy displayed no change or a slight increase in this immune marker. More recently, a relative decline in s-IgA was shown to occur in the 3-weeks preceding a typical URI in professional yachtmen (Neville et al., 2008). Together these findings seem to highlight the contributory role of s-IgA in oral protection from illness.

While the majority of studies have focused on the role of s-IgA as a biomarker for mucosal immunity; few have investigated the possible role of antimicrobial peptides (AMP’s) in illness development. This is important since defence against microbes is mediated by the early reactions of innate immunity and the later response of adaptive immunity (Kmiliauskis et al., 2005). The presence of AMP’s in secretions occurs without the need for prior exposure to infectious agents where they display activity against a wide range of pathogens (West et al., 2006). Recently, it has been shown that concentrations of AMP’s can be modified through exercise (Allgrove et al., 2008; West et al, 2008). In the latter study, authors concluded that lower concentrations of these proteins may be indicative of an impairment of innate protection of the upper respiratory tract. One such AMP, lysozyme represents an important enzyme of the non-specific salivary immune defence (Meyer and Zechel, 2001) and is present in most mucosal secretions, including saliva (Pruitt et al., 1999). It exerts its immunological action via its enzymatic effects on the peptidoglycan layer of gram positive bacterial walls (Kmiliauskis et al., 2005). Here it hydrolyses the bonds (beta 1-4 glucosidic linkages) ultimately causing cell lysis and eventual death (Tenovuo, 1989). Other characterised functions of lysozyme include stimulation of neutrophils and macrophages and antimicrobial effects carried out in conjunction with immunoglobulins (Germaine and Tellefson, 1979). It has been suggested that saliva lysozyme (s-Lys) may be a promising marker in investigating the effect of stress (Perera et al., 1997) and exercise (Koutedakis et al., 1996, West et al., 2006) on non-specific immunity. Therefore, it is feasible that by combining markers of mucosal immunity, greater mechanistic information on observed changes with exercise and illness may be observed.
Traditionally, studies have investigated temporary exercise related immune disturbances and clinical outcomes over days/weeks (Novas et al., 2003; Tiollier et al., 2005; Pacque et al., 2007; Gomez-Merino et al., 2005; Libicz et al; 2006). Few studies have monitored the occurrence of illness with changes in immune function in elite athletes regularly over extended observational periods (Neville et al., 2008; Gleeson et al., 1999; Fricker et al., 2005; Spence et al., 2007). Alternatively, in studies which have investigated links between depression in immune function and illness, limited or no data on the training history of the athletes involved is provided (Fahlman and Engels, 2005) which in turn, can provide difficulties when determining causation of observed illness. This dearth of knowledge becomes more apparent for athletes involved in team sports. Despite previous investigation on the role of exercise stress on immunity in swimmers (Gleeson et al., 1999; 2000), runners (Pacque et al., 1997; Nieman et al., 2006) and triathletes (Steerenberg et al., 1997; Libicz et al., 2006), very few have investigated the impact of weekly competition and training on mucosal function in team sports (Tharp, 1991; Walsh et al., 1999). This may be partly because of difficulties in quantifying the diverse range of training activities such as running based conditioning, resistance training, skill drills and team drills (Gleeson et al., 2004). To the author’s knowledge, no studies have attempted to monitor changes in illness incidence and immune function in rugby union, a collision field sport involving a large amount of physical activity during 80 minutes of game play.

Infections can impair performance, prevent athletes from competing altogether or interfere with training (Gleeson, 2000). In an elite team setting where athletes are competing on a regular basis, transmission of a virus between squad members could stretch playing resources and cause considerable set backs. As development of URI’s have been linked with immuno-suppression (Gleeson et al., 1999), it is possible that clusters of illness could occur during periods of increasing competition, a time when playing squads need to be at their full potential. In the Northern Hemisphere a typical professional rugby union season may last up to 48 weeks, during which time players may have repeated involvement in intense physical training and weekly game involvement. The close physical contact between team members in conjunction with the trauma inherent in playing rugby may facilitate easier transmission of a virus during clusters of infection. Previous studies have shown that skin infections, both bacterial and viral, are more prevalent in contact sports like rugby union (White and Grant-Kels, 1984). In the latter study, forwards were more likely to contact herpes simplex virus suggesting physical contact between team members as a primary route of virus acquisition. Furthermore, aerosol
and direct body contact have been shown to be important in transmission of viruses normally associated with the common cold (Gwaltney et al., 1978; Dick et al., 1987).

With the above in mind, evaluation of illness potential and mucosal immune responses in a professional rugby union team over a competitive season was the main focus of this investigation. It was hoped that by close evaluation of daily exercise load, greater clarity on this apparent exercise-immune-illness relationship may be obtained. This will aid in the overall management of individual players and between (forwards vs backs) player groups.

### 6.6 METHODS

Training load and illness data was collected prospectively over the course of a competitive rugby union season (2005-2006) totalling 48 weeks in duration. Player characteristics are summarised in Table 6.1. The study was approved by the Research Ethics committee of the University of Glamorgan (section 3.1).

<table>
<thead>
<tr>
<th></th>
<th>Forwards</th>
<th>Backs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>26.8 ± 0.9</td>
<td>25.9 ± 0.9</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>112 ± 2.6</td>
<td>91 ± 2.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>188.3 ± 1.7</td>
<td>182.6 ± 2.4</td>
</tr>
<tr>
<td>VO_{2peak} (ml.kg^{-1}.min^{-1})</td>
<td>50.4 ± 1.3</td>
<td>55.2 ± 1.4</td>
</tr>
<tr>
<td>HRmax (beats.min^{-1})</td>
<td>184 ± 1.7</td>
<td>186 ± 2.5</td>
</tr>
</tbody>
</table>

Before entry into the study, all players were asked to complete a health-screen questionnaire (appendix C). This was to determine the history of known allergies or exercise induced bronchoconstriction which may have effected interpretation of reported URI’s. Players were also asked to disclose information on the existence of URI’s which occurred in the 4 weeks leading up to study commencement. In the event of a player reporting URI symptoms, they were asked in detail as to the nature of the cold/flu and perceived cessation. This was to ensure that all subjects were free of URI’s upon study entry.

**Criterion for URI**

Players were asked to report the weekly presence or absence of flu-like symptoms (fever, headache, joints aches and pains) and cold-like symptoms (runny nose, catarrh in the throat,
sore painful throat, repetitive sneezing and coughing) across the study duration. Criterion used in defining presence/absence of a URI, including duration of symptoms is described in case study 4. Other general symptoms common to both (loss of sleep, general weakness/fatigue, muscle soreness or tenderness > than 8 hours) were also required. Players were asked to report non-URI related complaints during the investigation. These typically included medical episodes such as gastrointestinal symptoms, bone, joint, or muscular ailments without adjoining upper respiratory symptoms. All illness and complaint data was submitted using an electronic illness log contained in an ‘online’ training diary (see Case study 4 for more detail). Additionally, players were asked to report presence/absences of illness symptoms to medical staff throughout the study period. As the present investigation did not employ any clinical method in determination of underlying pathogenic cause, the presence of an underlying infection could not be established with certainty. Therefore, the term upper respiratory illnesses (URI) rather than upper respiratory tract infection (URTI) was used when interpreting data. This decision was made in light of recent findings showing that a significant contribution of all URI’s occur without an identifiable infectious agent or remain undetected due to current laboratory methods (Spence et al., 2007). Players did not have influenza vaccination on study entry. Medications taken by players for the treatment of URI’s were also noted.

Mucosal immunity
Saliva was collected at designated time points throughout the study (Figure 6.10) and analysed to determine the effects of training on stress responses (saliva cortisol) and mucosal immunity (s-IgA, s-Lys). Collection was standardised throughout the study duration (see methods section 4.1). For all sample points, saliva was taken between 8-9 am at the start of the week and after a minimum of 48 h rest from last game/intense exercise. For determination of s-IgA, samples were collected at the beginning of each month, and in the case of s-Lys and cortisol, at selected time points throughout the competitive season. The sampling points were chosen so as to incorporate periods of high/low training loads, high/low contact phases as well intense competition periods. These pre-determined collection points were not altered during the season and at no point were training programs altered to accommodate the experimental study. In the case of s-IgA, samples were collected using a cotton based salivette swab as used in previous investigations (Whitham et al., 2006; Walsh et al., 2004; Pacque et al., 2007; Nakamura et al., 2006; Brenner et al., 2000; Phillips et al., 2006; Klentrou et al., 2002; Ring et al., 2005; Akimoto et al., 2003). It has been reported that the use of absorbent cotton materials to collect saliva has been observed to produce lower s-
IgA concentrations (Shirtcliff et al., 2001); however, it is reasonable to consider that any such reduction would be a constant error (Phillips et al., 2006). Following initial preliminary findings on appropriate collection technique for determination of s-Lys (Case study 3), a manual expectoration ‘passive drool’ method was used. Saliva cortisol was also collected in this manner. After collection, all samples were placed on ice and stored at -80°C until future analysis. All saliva variables are expressed as absolute concentrations. In the case of s-IgA, values are also expressed relative to changes in flow (IgA secretion rate) and to osmolality (Blannin et al., 1998; Mackinnon et al., 1993).

**Figure 6.10:** Schematic representation of sampling/testing points for saliva IgA (n = 11), s-Lys and s-cortisol (n = 7) across the season (n = 48 weeks).

**Monitoring of Training load and Physical activity**

Measurement of training load and physical training was achieved via use of an ‘online’ player diary system (see section 5.6 and Case study 4 for more detail). Average weekly TL’s were quantified for each player and for the squad as a whole using a previously established method (Foster, 1998). Good correlations (r = 0.85-0.94) between this RPE-based TL assessment and other heart-rate based methods were observed on a subset of players from the same squad of players investigated in the current study (Case study 3). In addition to self-reporting by players, fitness coaches rated training days based on levels of set volume and/or intensity. These were also reported using the ‘online’ diary. All data were checked for reliability against data entry by coaching staff. Training loads and volume varied per week.
and month depending on individual session goals, the training cycle or competition involvement (Figure 6.17). For a description of the type of physical activity undertaken by the squad and for TL calculation, see Appendix F.

Table 6.2 Description of season layout. ITP; intensified competition period.

<table>
<thead>
<tr>
<th>Training week</th>
<th>Description of training phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk-1</td>
<td>Start of preseason: after 4 weeks of ‘rest’ away from club training base</td>
</tr>
<tr>
<td>Wks 1-10</td>
<td>Preseason (no games): Strength and conditioning type work, basic fitness</td>
</tr>
<tr>
<td>Wks 11-20</td>
<td>Mid-season: 10 games inc. 6 x Celtic-league, 2 x Anglo-Welsh cup, 1x European cup</td>
</tr>
<tr>
<td>Wks 21-25</td>
<td>Mid-season break (non-contact). Less competitive period</td>
</tr>
<tr>
<td>Wks 26-34</td>
<td>ITP. 9 games inc. 1x Anglo-Welsh cup, 4 x European cup, 4 x Celtic league</td>
</tr>
<tr>
<td>Wks 35-39</td>
<td>Less competitive period. 2 games inc. 1 x Celtic-league &amp; 1 x Anglo-Welsh cup</td>
</tr>
<tr>
<td>Wks 40-48</td>
<td>Competitive period. 10 games inc. 1 x Anglo-Welsh cup &amp; 9 x Celtic-league</td>
</tr>
</tbody>
</table>

Interpretation of data and statistical analysis

Mucosal markers and Physical activity

Data are expressed as mean ± SEM. To determine the effects of training over time on squad immune parameters, a one-way repeated measures analysis of variance (ANOVA) was used. For between player group (forwards vs backs) comparisons, a two-way mixed [group x time] ANOVA, with ‘time’ as a repeated factor and player ‘group’ as the between factor, was used. Due to the number of analyses, a Bonferroni correction factor was applied. The level of significance was set at P < 0.05 and follow up analysis on the main effects were performed using Bonferroni’s post hoc test. Diagnostic checks (Shapiro-Wilks test of normality and Levene’s homogeneity of variance test) were performed on all dependent variables prior to employment of parametric statistics. Where appropriate, adjustments to degrees of freedom were made using the Greenhouse-Giesser method of correction. Relative variability of IgA within players was calculated from the coefficient of variation (CV). Where warranted, independent samples t-test was used to identify differences in the overall group means of selected saliva variables between player’s groups (backs vs forwards). Given the non-normality of distribution, s-Lys data was firstly log transformed prior to statistical analysis (West et al., 2008). All training load and physical activity data are as described in chapter 3. Pearson’s product moment correlations were used to determine the strength of relationships between variables of interest. In order to investigate the possible association between changes in IgA concentration and URI symptomology, mean monthly IgA concentrations in
players reporting symptoms of URI (infected) were compared to concentrations in players not reporting URI symptoms (non-infected). If saliva sampling took place on the same week when a player reported URI symptoms, IgA data for that individual was not included in mean monthly analysis. For comparative purposes, s-IgA concentrations in those individuals with 0-3 URI’s were compared to those with 4 or more URI’s. All analyses was performed using SPSS v14.0 for windows.

**Illness data**

Data are reported as percentage of subjects reporting symptoms of upper respiratory illness as well as mean and total number of URI’s per week and month. The duration and severity of each illness was also noted.
Table 6.3: Shows breakdown of training volume and activity undertaken by players throughout the period of investigation (n = 48 wks)

<table>
<thead>
<tr>
<th>Training week</th>
<th>1-4</th>
<th>5-8</th>
<th>9-12</th>
<th>13-16</th>
<th>17-20</th>
<th>21-24</th>
<th>25-28</th>
<th>29-32</th>
<th>33-36</th>
<th>37-40</th>
<th>41-44</th>
<th>45-48</th>
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</tr>
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<tbody>
<tr>
<td>Month of season</td>
<td>Jun</td>
<td>Jul</td>
<td>Aug</td>
<td>Sept</td>
<td>Oct</td>
<td>Nov</td>
<td>Dec</td>
<td>Jan</td>
<td>Feb</td>
<td>Mar</td>
<td>Apr</td>
<td>May</td>
<td>Total</td>
</tr>
<tr>
<td>Monthly Training Hours</td>
<td>37</td>
<td>43</td>
<td>28.5</td>
<td>23</td>
<td>24</td>
<td>32</td>
<td>25</td>
<td>21</td>
<td>29</td>
<td>36.5</td>
<td>25</td>
<td>21</td>
<td>345</td>
</tr>
<tr>
<td>Monthly Training Days</td>
<td>19.5</td>
<td>20.5</td>
<td>13</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>160</td>
</tr>
<tr>
<td>Games Per Month</td>
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<td>8</td>
<td>7</td>
<td>6</td>
<td>9</td>
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<td>7</td>
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<td>4</td>
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<td>Monthly Training Sessions</td>
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<td>21</td>
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<td>22</td>
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<td>Rest Days</td>
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<td>4.5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>†Formalised contact training sessions</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>4.5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>5</td>
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<td>55</td>
</tr>
<tr>
<td>*Contact session number (inc games)</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>4.5</td>
<td>4</td>
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<td>5</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>55</td>
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<td>3.9</td>
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<td>4.0</td>
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<td>7.9</td>
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<td>7.1</td>
<td>5.9</td>
<td>3.3</td>
<td>67.0</td>
</tr>
<tr>
<td>Backs</td>
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<td>4.0</td>
<td>4.0</td>
<td>1.4</td>
<td>6.0</td>
<td>2.3</td>
<td>6.8</td>
<td>8.1</td>
<td>4.6</td>
<td>6.4</td>
<td>5.7</td>
<td>2.1</td>
<td>52.4</td>
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</table>

Monthly data are representative of mean values over 4 week blocks. †As outlined by coach [included defence sessions, scrum sessions and pad work]. *Reported by players and included formalised sessions and games. Note: number of training hours excludes recovery training time and time involved in game participation. Players absent due to illness and medical conditions were not included in assessment of training data.

<table>
<thead>
<tr>
<th>% Activity Time</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
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<tr>
<td>% of time spent conditioning</td>
<td>80</td>
<td>81</td>
<td>54</td>
<td>40</td>
<td>48</td>
<td>69</td>
<td>46</td>
<td>49</td>
<td>65</td>
<td>48</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>% time playing rugby</td>
<td>19</td>
<td>18</td>
<td>41</td>
<td>52</td>
<td>46</td>
<td>28</td>
<td>52</td>
<td>49</td>
<td>33</td>
<td>50</td>
<td>50</td>
<td>47</td>
</tr>
<tr>
<td>Ratio rugby/conditioning</td>
<td>0.23</td>
<td>0.22</td>
<td>0.78</td>
<td>1.31</td>
<td>0.95</td>
<td>0.41</td>
<td>1.13</td>
<td>0.99</td>
<td>0.51</td>
<td>1.05</td>
<td>1.07</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Table 6.4: Breakdown of time (%) spent performing conditioning and rugby activity. Conditioning activity = all non-rugby specific training activity.
6.7 RESULTS

Saliva collection and analysis

Saliva IgA

Saliva for determination of (s-IgA) was collected and analysed on 11 occasions across the study duration. Mean resting squad values were $81.5 \pm 5.6$ mg.L$^{-1}$ (95% CI: 70.5-92.6 mg.L$^{-1}$). Analysis of data revealed significant main effects for both group ($P < 0.01$), and time ($P < 0.01$), although no significant group x time interaction was found. Higher overall mean s-IgA concentration was observed in the forward playing group ($94.6 \pm 8.4$ mg.L$^{-1}$; 95% CI: 78.2-110.0 mg.L$^{-1}$) compared to the backs ($69.1 \pm 6.7$; 95% CI: 56.1-82.3 mg.L$^{-1}$) across the study period ($P < 0.05$). This finding was consistent for each month investigated, with post hoc analysis revealing significantly lower s-IgA concentrations for backs during the months June, July, October, December, February and April (Figure 6.11b). S-IgA concentrations varied considerably across the season. Aside from a general trend for increasing IgA levels during the season, notable decreases were observed for the months August, December and February. Lowest IgA concentrations were observed for December; levels significantly lower ($P < 0.05$) from the previous month (November) and following two months (January and February); Figure 6.13a.

<table>
<thead>
<tr>
<th>Testing (T1-T7)</th>
<th>Training week (1-48)</th>
<th>Description of training phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Wk-1</td>
<td>Start of season after 4 weeks of ‘rest’.</td>
</tr>
<tr>
<td>T2</td>
<td>Wk 9</td>
<td>End of preseason (no games) immediately after pre-season training camp.</td>
</tr>
<tr>
<td>T3</td>
<td>Wk 19</td>
<td>Mid-season after start of European cup.</td>
</tr>
<tr>
<td>T4</td>
<td>Wk 24</td>
<td>End of mid-season break (non-contact). Less competitive period.</td>
</tr>
<tr>
<td>T5</td>
<td>Wk 34</td>
<td>After important period of intense competition. European &amp; domestic.</td>
</tr>
<tr>
<td>T6</td>
<td>Wk 45</td>
<td>Penultimate stage of season.</td>
</tr>
<tr>
<td>T7</td>
<td>Wk 1</td>
<td>Before start of following season</td>
</tr>
</tbody>
</table>

Salivary IgA concentration was highly variable within-players (CV = 41%). There was a trend for lower s-IgA concentrations among players who reported incidences of URI’s during each month (mean: $66.5 \pm 14.0$ mg.L$^{-1}$; 95% CI: 39.0-94.1mg.L$^{-1}$) than those who were symptom free (mean: $83.4 \pm 13.6$; 95% CI: 56.6-110.1 mg.L$^{-1}$), although this finding was non-significant. No relationship existed between a players overall mean s-IgA (across
season) and the total number of reported URI episodes. Furthermore, no difference in IgA concentration was observed in those players with 4+ URI’s compared to those with 0-3 URI’s. In one player, no detectable IgA levels were observed throughout the study period. Values for this player were subsequently excluded from analysis.

No main effects for time, group or interaction (time x group) were observed for saliva flow at any stage across the season. Similar findings were observed for saliva osmolality. Analysis of IgA secretion rate across the season revealed no main effect for player group or interaction effect [group x time]. Similar findings were observed for IgA:Osmolality although a main group effect approached significance (P = 0.055). Significant main effects for time were observed for both measures (P < 0.05). Comparison of overall s-IgA secretion rate values between forwards (mean: 68.3 µg.min⁻¹ ± 8.9; 95% CI: 51.0-85.7 µg.min⁻¹) and backs (mean: 50.9 µg.min⁻¹ ± 6.8; 95% CI: 37.6-64.2 µg.min⁻¹) did not reveal statistical significance (P > 0.05). Comparable findings were observed for s-IgA:osmolality with between group differences approaching significance (P = 0.059). Saliva IgA secretion rates were significantly higher in forwards vs backs for the months of February (P < 0.05) and April (P < 0.05), with values non-significant for December (P = 0.08); Figure 6.12b. In turn, between group differences for s-IgA:Osmolality were significant for the months June, August, February and April (P < 0.05), with values non-significant for October (P = 0.08).

Similar to IgA concentration, IgA secretion rate varied considerably across the season. Notable decreases were observed during August, December and February. Lowest IgA secretion rates were recorded during August; values significantly lower (P < 0.05) than November, January and March. Highest values were reported during January (Figure 6.12a). Similar findings were observed for s-IgA:Osmolality across the season.
Figure 6.11(a): Mean ± SEM values for squad IgA concentration. * Lower than months November, January and February (P < 0.05).

Figure 6.11(b): Mean ± SEM values for IgA concentration; Forwards vs Backs. * Months where concentrations were lower in backs (P < 0.05).

Figure 6.12(a): Mean ± SEM values for squad IgA secretion rate. * Lower than months November, January and March (P < 0.05).

Figure 6.12(b): Mean ± SEM values for IgA secretion rate; Forwards vs Backs. * Values significantly (P < 0.05) lower in backs.
Saliva Lysozyme and Cortisol

In total, s-Lys and cortisol were analysed 7 times across the study period. Mean resting squad s-Lys concentrations were 12.8 ± 1.7 mg.L\(^{-1}\) (95% CI: 10.9-14.6 mg.L\(^{-1}\)). Overall s-Lys concentrations appeared higher in forwards (15.2 ± 3.0 mg.L\(^{-1}\); 95% CI: 9.3-21.1 mg.L\(^{-1}\)) compared to backs (10.5 ± 1.7 mg.L\(^{-1}\); 95% CI: 7.7-13.2 mg.L\(^{-1}\)), although these differences were non-significant (P > 0.05); Figure 6.13b. There was a significant main effect for time (P < 0.05), although no significant [group x time] interaction effect or group effect was observed.

**Figure 6.13a:** Mean ± SEM values for squad s-lysozyme. * Significantly lower (P < 0.05) than pre-season (June) values.

**Figure 6.13b:** Mean ± SEM values for s-lysozyme; Forwards vs Backs.
Compared to pre-season values, s-Lys values decreased significantly during the months November (P < 0.05) and February (P < 0.01) before gradually returning to pre-season values again the following June (Figure 6.13a).

![Figure 6.14a](image1)

**Figure 6.14a:** Mean ± SEM values for squad s-Cortisol. * Higher than pre-season (June) values (P < 0.05).

![Figure 6.14b](image2)

**Figure 6.14b:** Mean ± SEM values for s-Cortisol; Forwards vs Backs.

* Significant difference between playing groups; p < 0.05.

Mean resting s-cortisol values were 11.0 ± 2.6 nmol.L⁻¹ (95% CI: 10.0-11.8 nmol.L⁻¹) for the squad throughout the study period. No between group [forwards backs] differences were observed (P > 0.05). However, within season effects were evident; with a greater rise in values observed for backs (Figure 6.14b). This was particularly evident during November (P < 0.05) and February (P > 0.05). Conversely, s-Cortisol values were higher in the forward player group at the end of pre-season (August; P < 0.05). No significant main effects were
observed for player group or interaction effects. Main effects for time and interaction [group x player] were observed (P < 0.05). Subsequent analysis revealed a gradual rise in cortisol values across time; significantly higher within season values (November, February and April) compared to those at the beginning of season (June and August, all P < 0.05); Figure 6.14a.

**Training Load and Physical Activity**

A significant effect of time was observed for group training load (TL; P < 0.001) across the season (Fig 6.16). Monthly comparisons revealed that TL values for June were significantly lower than all other months (P < 0.001) except August and May. An increase in reported squad TL was observed for July; significantly higher than the preceding month (June) and months of August and September (P < 0.05). Values then increased steadily throughout the season until the month of March. Peak TL values were reported for February (10914 ± 831 AU) before decreasing again until the close of the season (May, 5177 ± 369 AU). Forwards, in general, reported higher training loads compared to the backs throughout the investigation (Figure 6.16). This was particularly evident during the penultimate stage of the season (February, March, April); values reaching significance (P < 0.05) during March (11021 ± 716 forwards; 8926 ± 573 backs). Conversely, higher training loads were reported by the backs during the month of November. Despite higher overall mean monthly TL values, no main effect for group (8081 ± 415 AU forwards; 7609 ± 434 AU backs, P > 0.05) or interaction effect (time x group) was found.

A main effect of time was observed for monthly training intensity (P < 0.001). Notable increases were reported by players during July and November; peak values observed during February. These increases corresponded to times of reduced game activity (Table 6.4) and in the case of November and February, during times when formalised rest days were reduced (Figure 6.17). Higher overall mean intensity ratings were reported by forwards (5.7 ± 0.19 AU) compared to backs (4.9 ± 0.19 AU); P < 0.05). Between group differences reached significance during August, September, October, December and March (P < 0.05). Conversely, intensity values were higher in backs during the month of November, although this was non-significant.
Table 6.6: Changes in selected saliva variables across time. Values displayed as mean (SEM).

<table>
<thead>
<tr>
<th>Training week</th>
<th>1</th>
<th>6</th>
<th>9</th>
<th>14</th>
<th>19</th>
<th>24</th>
<th>28</th>
<th>34</th>
<th>38</th>
<th>41</th>
<th>43</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA concentration (mg.L⁻¹)</td>
<td>77.4 (7.6)</td>
<td>82.2 (7.1)</td>
<td>67.2 (6.1)</td>
<td>74.5 (7.2)</td>
<td>85.7 (9.2)</td>
<td>104.1 (10.4)</td>
<td>61.1 (7.0)</td>
<td>104.8 (11.4)</td>
<td>81.5 (8.8)</td>
<td>99.6 (8.6)</td>
<td>83.1 (8.2)</td>
</tr>
<tr>
<td>Saliva Flow (ml.min⁻¹)</td>
<td>0.73 (0.06)</td>
<td>0.79 (0.06)</td>
<td>0.69 (0.06)</td>
<td>0.72 (0.05)</td>
<td>0.68 (0.07)</td>
<td>0.68 (0.05)</td>
<td>0.76 (0.05)</td>
<td>0.69 (0.06)</td>
<td>0.73 (0.05)</td>
<td>0.75 (0.05)</td>
<td>0.63 (0.05)</td>
</tr>
<tr>
<td>IgA secretion (µg.min⁻¹)</td>
<td>59.9 (8.1)</td>
<td>60.1 (6.5)</td>
<td>43.7 (5.4)</td>
<td>58.5 (8.1)</td>
<td>63.1 (8.9)</td>
<td>72.1 (8.5)</td>
<td>45.7 (7.0)</td>
<td>79.8 (11.5)</td>
<td>60.1 (8.5)</td>
<td>75.6 (9.6)</td>
<td>51.5 (5.8)</td>
</tr>
<tr>
<td>Osmolality (mOsmol.kg⁻¹)</td>
<td>68.5 (2.5)</td>
<td>79.0 (3.9)</td>
<td>68.7 (3.3)</td>
<td>74.1 (2.2)</td>
<td>78.7 (3.8)</td>
<td>79.2 (3.4)</td>
<td>67.6 (3.4)</td>
<td>68.8 (2.2)</td>
<td>65.4 (2.5)</td>
<td>76.9 (3.2)</td>
<td>72.6 (2.4)</td>
</tr>
<tr>
<td>IgA:Osmol ratio (mg.mOsmol⁻¹)</td>
<td>1.11 (0.11)</td>
<td>0.99 (0.07)</td>
<td>0.94 (0.07)</td>
<td>1.00 (0.10)</td>
<td>1.07 (0.09)</td>
<td>1.33 (0.12)</td>
<td>0.84 (0.07)</td>
<td>1.58 (0.16)</td>
<td>1.19 (0.10)</td>
<td>1.25 (0.10)</td>
<td>1.14 (0.11)</td>
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<table>
<thead>
<tr>
<th>Training week</th>
<th>1</th>
<th>9</th>
<th>19</th>
<th>24</th>
<th>34</th>
<th>45</th>
<th>1</th>
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</thead>
<tbody>
<tr>
<td>Cortisol (nmol.L⁻¹)</td>
<td>7.76 (0.73)</td>
<td>8.21 (0.69)</td>
<td>11.08 (0.80)</td>
<td>13.62 (1.10)</td>
<td>13.90 (1.61)</td>
<td>13.91 (0.81)</td>
<td>11.06 (1.05)</td>
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<tr>
<td>Cortisol secretion (nmol.L⁻¹)</td>
<td>5.46 (0.92)</td>
<td>6.33 (0.66)</td>
<td>8.10 (0.98)</td>
<td>13.37 (2.11)</td>
<td>15.43 (3.19)</td>
<td>11.13 (1.04)</td>
<td>8.77 (0.93)</td>
</tr>
<tr>
<td>Lysozyme concentration (mg.L⁻¹)</td>
<td>12.63 (1.85)</td>
<td>11.27 (2.54)</td>
<td>12.25 (2.26)</td>
<td>8.67 (1.16)</td>
<td>7.58 (0.93)</td>
<td>11.76 (2.91)</td>
<td>14.33 (3.27)</td>
</tr>
</tbody>
</table>
Illness data

Compliance rate in the reporting of illness data was 80% using the online diary. Overall, 92% of players experienced at least one URI over the season of investigation [total = 123 URI’s]. This resulted in a mean incidence of 4 [min = 0; max = 8] URI’s per player. Recorded number of URI episodes differed for playing position [mean ± SEM: 3.4 (0.47) vs 4.3 (0.51)], forwards and backs respectively. The average duration of reported URI’s lasted 4.7 days (min = 2; max = 33). Duration of URI’s were greater for backs (4.9 ± 0.7 days) than forwards (4.1 ± 0.32 days) although this finding was non-significant. Of the total number of URI episodes, 13% lasted longer than 7 days, 32% between 4-7 days while 55% lasted between 1-3 days. The percentage of players reporting presence of upper respiratory symptoms varied throughout the season. Number of reported URI’s increased during the months of August and March, with a large peak during the month of December (Figure 6.15). On any given week throughout the season, 17% of players reported the presence of a URI. The highest proportion of the sample reporting a URI in a single month was 25%. This occurred during December (30% during week 27). In 23% of all URI incidences, players reported that presence of the illness either reduced activity (14.4%) or felt the need to go to bed (8.6%). These figures were matched by illness severity ratings. Players reported the use of medication in 40% of all reported URI episodes. In those who took this medication, treatments ranged from use of analgesics such as paracetamol (43.9%) and NSAIDS (10.6%), over the counter cold/flu remedies (21.2%) and oral antibiotics (24.2%).

A total of 25 GI complaints were reported over the study investigation. This resulted in a mean incidence of 0.8 [min = 0; max = 3] gastrointestinal illnesses (GI’s) per player while 40% of players reported at least one GI complaint over the investigation. Just 2% of the squad reported presence of GI on any given month over the season. Peak incidence of GI illness occurred during the month of February (6% of squad reporting symptoms).
Figure 6.15: Weekly and monthly number of self-reported illnesses [URI] across the season.

Figure 6.16: Mean ± SEM values [AU] for monthly training load [duration x intensity] over the season. # Forwards significantly different from backs; P < 0.05.

Figure 6.17: Number of low volume/intense training days and/or formalised rest days [set by fitness-staff] and self-reported player training intensity.
6.8 DISCUSSION

This is the first large scale prospective monitoring study to assess occurrence of upper respiratory illnesses [URI’s] in team sport athletes with corresponding changes in mucosal immunity and detailed quantification of physical activity. The main findings are: (a) clusters of infections seem to occur following periods of reduced game time and increased training loads; (b) observed differences between playing position suggests that ‘backs’ are more susceptible to infection and (c) decreases in innate mucosal immunity appear to be linked with predisposition to illness and is in part, mediated through increases in cortisol secretion.

A large variation in susceptibility to upper respiratory infections has been previously reported among healthy individuals (Gwaltney, 2002). In the current study, professional rugby players were shown to experience an average of four URI’s over a given year (Northern hemisphere), with 92% of all players experiencing at least one illness during this period. These findings concur with others suggesting that up to four URTI’s are normal per year in clinical practice (Gleeson et al., 1999b) but higher than those in a similar longitudinal study on elite yachtsmen (2.7 URI’s, 50-weeks) (Neville et al., 2008). It is possible that differences in methodology of illness recording may, in part, have contributed to observed discrepancies. In the current study, analysis of illness incidence was achieved through medical staff recording and self-reporting by players using a novel web-based illness log. It is probable that lower illness rates are recorded when reporting is achieved through medical staff recording alone or when illness rates are calculated from days when an athlete is unable to compete in formalized training activity. Using the current method, athletes provided more information on banal infections which would otherwise have gone undetected using previous methodologies (see case study 3). Furthermore, subjects are more honest when reporting behaviour to a computer than on paper (Millstein, 1987; Skinner and Allen, 1983), something which may help in identifying athletes with low severity but persistent infections.

Few studies have attempted to quantify the impact of illness on athletic performance, a factor which is difficult in intermittent team sports like rugby. In 23% of all URI incidences, players reported that the presence of an illness either reduced activity (14.4%) or felt the need to go to bed (8.6%). In terms of squad preparation, this could impact significantly on player availability, their ability to perform if selected or indeed in terms of player welfare. Clusters of infections were apparent following intense training periods, in particular during the months August, December and March. The highest incidence of URI’s occurred during December (25% of squad); a month traditionally deemed a critical time in the Northern hemisphere rugby
union calendar. The average duration of reported URI’s across the season lasted 4.7 days, values which are similar to those observed in army recruits (Whitham et al., 2006) but lower than those in triathletes using more objective methods of illness assessment (Spence et al., 2007). Although current findings suggest that elite rugby union players experience similar illness rates to those within the normal population, considerable variation (0-8 URI’s) did exist amongst players as noted previously (Fricker et al., 2000; Gleeson et al., 1999). In view of this, weekly characterization of illness patterns may have important implications in terms of identifying ‘at risk’ individuals and those showing signs of further complications. A recent study has shown that athletes suffering from persistent fatigue and/or recurrent infections, 68% had an underlying clinical condition such as partial humoral immune deficiency (28%) or unresolved viral infections (27%); Reid et al., 2004. Prolonged illness presence was evident in two players showing symptoms of URI for 33 days despite their failure to report full duration of symptoms to medical staff. In such instances, early identification of complications may have helped in avoiding further exacerbation of symptoms that could otherwise have been treated medically or through simple tapering of training.

The current study found no relationship between individual players overall mean s-IgA (across season) and the total number of reported URI episodes as previously shown (Neville et al., 2008). Similar findings were noted for saliva lysozyme. Exploratory analysis revealed that s-IgA concentrations in players whom reported incidences of URI’s during each month (66.5 mg.L\(^{-1}\) ± 14.0) appeared lower than those who were symptom free (83.4 mg.L\(^{-1}\)± 13.6). However, on further investigation, this finding was non-significant and suggestive that resting s-IgA concentration per se is not predictive of upper respiratory illness and is contrary to previous studies (Gleeson et al., 2001; Fahlman and Engels, 2005). This observed lack of significance may in part, be due to the high within athlete s-IgA variability (CV: 41%) as noted previously (Neville et al., 2008; Francis et al., 2005). A larger sample number may have provided greater clarity on this matter. However, decreases in both s-IgA and s-Lys concentrations were evident at certain time periods throughout the season. Incidentally, drops in both markers seemed to occur during/after preceding periods of intense conditioning type work (end of pre-season: August; break in game fixtures: November and February). Notable decreases in the ratio of rugby to conditioning was observed during November and February, while a decrease in the number of moderate and light training days was also observed (Tables 6.3 and 6.4; figure 6.17). Observed decreases in mucosal immunity, in particular s-IgA, seemed to be followed by corresponding increases in illness incidence after approximately 2-3 weeks (December and March); Figs 6.11a and 6.15. Although difficult to establish an exact cause-
effect between increases in training load and intensity with decreases in mucosal protection and increased illness; these observed trends do point to the role of heavy training on diminished host protection. With reference to December, the fact that peak illness levels occurred during a time where the greatest drop in s-IgA levels does point to the role of this marker in pathogenic protection. Seasonal variation in illness incidence may have contributed in part to this observation. However, it would be difficult to reconcile the large decrease for this saliva parameter in December with seasonal variation alone given the dramatic drop. Nevertheless, failure to employ the use of a control group is an obvious limitation of this study and would have provided greater clarity on this immune-illness relationship.

It is unknown what mechanisms are responsible for observed decreases in mucosal protection during December. It is possible that elevated cortisol levels following heavy November training may have directly affected IgA synthesis. It is also feasible that through repeated stimulation, secretion of IgA entered a refractory state after which levels returned to normal the following month. However, the above reasons remain speculative only. Repeated stress has been previously shown to be associated with reduced secretion of salivary lysozyme (Koh et al., 2002). It is unlikely that observed decreases in s-IgA concentration were a result of changes in saliva flow, known to vary across seasons (Kavanagh et al., 1998), as similar changes were observed for both s-IgA secretion and s-IgA:osmolality. These factors are not often considered in longitudinal investigations and could in effect contribute to any observed decreases in s-IgA concentration. Further longitudinal studies are needed to investigate the possible mechanisms involved in decreased mucosal protection.

The mean monthly s-IgA concentration in the current athletic group (66.5 mg.L⁻¹ ± 14.0) is similar to that reported in other studies (Cox et al., 2004; Francis et al., 2005) although considerable variation exists within the literature. In turn, resting concentrations of saliva lysozyme (12.8 ± 1.7 mg.L⁻¹) are moderately higher than those shown observed in recent investigations (Allgrove et al., 2008; West et al., 2008). One of the main findings of the current study was the difference in concentrations of both markers between player groups. Throughout the study, s-IgA concentrations were lower in backs (27%) compared to forwards (P < 0.05). This was consistent for each month investigated. Furthermore, aside from the month of October, overall mean concentrations of s-Lys were also lower in this player group (31%). Therefore, it appears from observed results that absolute concentrations of innate protective agents are lower in rugby union backs than forwards. It is unknown as to why this may occur, although differing fitness levels and positional requirements should not be discounted. Recent findings have shown lower (~50%) innate mucosal factors in elite athletes compared to
sedentary individuals (West et al., 2008). Alternatively it is also possible that the greater increases in saliva cortisol observed in ‘backs’ may have contributed to these findings (Figure 6.14b). Interestingly, illness rates in this player group were also higher [3.4 (0.47) vs 4.3 (0.51) URI’s] when compared to their forward counterparts.

The fact that higher illness rates were observed in the back playing group goes against our initial thinking. It has been suggested that the most common mode of transmission of virus outbreaks in sport is direct, person-person contact (Tuberville et al., 2006). With the above in mind, one would have expected a greater illness incidence in forwards given their positional requirements (close physical contact and trauma due tackling). While it is difficult to ascertain as to why this may have occurred, it is possible that since backs spend a greater portion of time running and sprinting, larger ventilatory volumes and/or upper respiratory airway damage could have been partly responsible for observed URI findings. Investigation of game demands between forwards and backs using GPS software in the current study does confirm higher intensity running and greater running distances for this player group (Case study 4). Furthermore, contemporary backs are now involved more in plays such as rucks and mauls that were traditionally the domain of forwards (Quarrie and Hopkins, 2007). Therefore, it is feasible that exercise at higher intensities in both training and competition may have contributed, in part, to observed findings. It is also feasible that greater activation of the hypothalamic-pituitary-adrenal axis and the secretion of glucocorticoids occurred as a result of time spent undergoing exercise at these greater exercise intensities. Higher concentrations of these neuropeptides have been associated with a reduction in both s-Lys (Allgrove et al., 2008; Perara et al., 1997) and s-IgA (Hucklebridge et al., 1998). Incidentally, higher cortisol values were observed in this player group during times of reduced mucosal protection.

Conclusion
This is the first study to comprehensively examine the role of exercise load on illness rates and mucosal immunity in professional rugby players across time. Additional strengths of this investigation include the use of a novel computerised training diary in the collection of daily activity and weekly illness data on players across an entire rugby year. Furthermore great methodological care was employed in the collection and standardisation of data collection. Although comparisons of illness data to recreational controls was not the intended focus of this investigation, employment of a control group would have provided greater clarity on observed changes in mucosal immunity and illness expression across time. This is an obvious limitation
of this study. It is possible that normal seasonal effects may have contributed to observed
trends in investigated markers (Walker et al., 1997; Nelson, 2004; Eccles, 2002) and this
should be taken into consideration in future longitudinal investigations on team athletes.
Nevertheless, the study does emphasize the role of innate mucosal protection in upper
respiratory illness development and provides clarity on the effects of intensified training
periods in decreasing host protection. The study highlights important time points during a
typical rugby season when players are at greater risk of opportunistic infection, something
which should be taken into account during planning of training phases.
Study Five: Biochemical and endocrine responses to training load during a competitive elite rugby union season

SUMMARY

Few studies have monitored changes in immunoendocrine and hormonal markers with training load (TL) over time in rugby. In this study, TL and biochemical data was collected on a squad of elite rugby players over a competitive rugby year (48-weeks). Weekly training related complaints were reported by players using an ‘online’ training diary while blood and saliva samples were obtained at selected time points throughout the season (n = 7). First testing (T1) took place before the start of the season (week 1); the second (T2) at the end of pre-season (week 9), while other took place on weeks 19 (T3), 24 (T4), 34 (T5), 45 (T6) and again before the start of the following season (T7). In comparison to backs, forwards reported higher TL’s during the penultimate stage of the season while higher TL’s and intensity values were reported by backs during November. A gradual rise in squad cortisol (C) was evident across the season with concentrations at T1 and T2 lower than all other remaining sampling points (P < 0.05). A greater increase in mid-season C values (T4, T5) was evident in backs, with concentrations at T4 (November) ~31% greater than their forward counterparts; P < 0.05. Conversely, higher cortisol (~35%) was observed in forwards at the end of pre-season (T2) compared to the backs. Reported training intensity ratings were higher in forwards throughout the season as were mean concentrations of hs-CRP (1.5 ± 0.3 vs 0.8 ± 0.3 mg.L⁻¹) and CK (371 ± 38 U.L⁻¹ forwards vs 296 ± 36 U.L⁻¹ backs); all P < 0.05.

A significant effect of time was observed for squad CRP, where values decreased across time. This decrease coincided with a fall in the number of training related complaints. Squad T/C ratio also decreased with T/C values at points T2-T7 lower than those at the start of the season; P < 0.05. Decreases in plasma glutamate were observed at T4 and T5 while an increase in plasma Glu/Glu ratio was evident at T4 (November; P < 0.05). Together, these findings provide evidence for positional related variations in hormonal and biochemical responses to TL in rugby union. Forwards appear to have greater low grade inflammation and muscle damage than backs, possibly as a result of positional requirements and differences in body characteristics. Decreases in residual inflammation were coupled with a corresponding decrease in the number of training complaints. This is possible evidence for a ‘repeated-bout’ effect in rugby union.

6.9 INTRODUCTION

Rugby union is considered to be one of the most intense and physically demanding field sports in the world (Mashiko et al., 2004). Since its emergence as a professional game, it appears to have become a faster, ruck dominated game that contains more phases of play (Eaves and Hughes, 2003). While previous studies have investigated the intense physiological demands of acute game play (Mashiko et al., 2004; Takarada, 2003); few, if any, have assessed the effects of repeated daily rugby activity on physiological function over a longitudinal setting. In northern hemisphere rugby, a competitive season may last up to 48 weeks during which time players are exposed to a variety of physical and psychological stressors from practice and competition. In view of these heavy demands, appropriate management of exercise load represents an important component in the training process and in the prevention of fatigue, injury and overtraining.
Optimal training results in exercise-recovery balance (Filaire et al., 2003), while heavy training and overreaching has been shown to result in changed physiological and biochemical states in athletes (Halson et al., 2003; Rietjens et al., 2005). Furthermore, the ability of bodily systems (e.g. neuromuscular system and endocrine system) to recover and regenerate following composite training stresses including strenuous activity, psychological stress of practice and competition, etc., have been shown to influence physical performance (Kraemer et al., 2004). In view of this, the monitoring of athletes using neuroendocrine and biochemical markers across the training process is intriguing. A few studies have attempted to examine biochemical or hormonal changes over long study durations in rugby league (Coutts et al., 2007), soccer (Elloumi et al., 2003; Filaire et al., 2001; 2003; Naessens et al., 2000), American football (Hoffman et al., 2005), Australian rules football (Cormack et al., 2008) and rugby union (Finaud et al., 2006). Kraemer et al (2004) have previously shown that soccer players entering a competition with low plasma testosterone concentrations in conjunction with elevations in plasma cortisol experienced significant performance reductions. Furthermore, a recent study has shown that changes plasma testosterone to cortisol ratio, plasma glutamate, glutamine to glutamate ratio and creatine kinase correspond with changes in muscular performance following an overload training period in rugby league (Coutts et al., 2007). However despite the use of biochemical assessment of training state across time, studies have often failed to provide detailed information on preceding training activity or loads undertaken by the players/athletes (Banfi et al., 2006).

In a sport like elite rugby union, it is possible that physical trauma received by certain player groups (e.g. forwards) may place an additional burden on muscle regenerative capacity. This may be particularly evident if sustained in the absence of adequate recovery. With the above in mind, the purpose of this study was to define the response pattern of selected biochemical and hormonal variables to training load throughout a competitive rugby season. Additional study aims were to (a) establish time periods of decreased recovery (b) discover if variations in training stress and recovery occur as a result of playing position.

6.10 METHODS

Data was collected on a squad of professional rugby union players (n = 30) over a 12 month period (2005-2006 season). The club competed at European level competition which is the highest level of play for regional teams in northern hemisphere rugby. Player characteristics are summarised in Table 6.7. Measurements were taken following detailed explanation to all
players via visual presentation and study information sheets. The study was approved by the Research Ethics committee of the University of Glamorgan (section 3.1).

Table 6.7: Mean (SEM) data for subject characteristics (n = 30).

<table>
<thead>
<tr>
<th></th>
<th>Forwards</th>
<th>Backs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26.8 ± 0.9</td>
<td>25.9 ± 0.9</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>112 ± 2.6</td>
<td>91 ± 2.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>188.3 ± 1.7</td>
<td>182.6 ± 2.4</td>
</tr>
<tr>
<td>∑Skin folds (mm)</td>
<td>101 ± 7.1</td>
<td>66 ± 5.0</td>
</tr>
<tr>
<td>HRmax (beats.min⁻¹)</td>
<td>184 ± 1.7</td>
<td>186 ± 2.5</td>
</tr>
<tr>
<td>2peakO₂ (ml.kg⁻¹.min⁻¹)</td>
<td>50.4 ± 1.3</td>
<td>55.2 ± 1.4</td>
</tr>
<tr>
<td>Peak Power (w)</td>
<td>1515 ± 59</td>
<td>1352 ± 44</td>
</tr>
<tr>
<td>Relative power (w.kg⁻¹)</td>
<td>13.4 ± 0.3</td>
<td>15.0 ± 0.2</td>
</tr>
<tr>
<td>10 m running sprint (m.sec⁻¹)</td>
<td>1.74 ± 0.02</td>
<td>1.67 ± 0.02</td>
</tr>
<tr>
<td>40 m running sprint (m.sec⁻¹)</td>
<td>5.38 ± 0.06</td>
<td>5.16 ± 0.06</td>
</tr>
</tbody>
</table>

VO₂peak: Peak oxygen uptake expressed per kilogram body mass. HRmax: maximum heart rate. Skinfolds: sum of nine skinfolds (suprasinale, pectoralis, bicep, tricep, thigh, abdominal, subcapular, suprailliac, medial-calf).

Dietary Control and Monitoring
All haematological and saliva measures were obtained from players following an overnight fast. Furthermore, all morning collection times were standardized throughout the study period (8-9 am) so as to avoid any compounding dietary and circadian influences on measured markers. Players were instructed to remain fasted from 11 pm the previous night and consume 500 ml of water on waking. All players were instructed to consume their ‘typical’ diet in the two days prior to sampling and asked to avoid alcohol and caffeine beverages 24 hours before each sample point. All testing was performed on a Tuesday morning so as to avoid any ‘abnormal’ dietary practices which may have occurred over the preceding weekend. This was kept consistent throughout the duration of the study and also served as a means to ensure a minimum of 48 h rest from any previous game/training activity. Players were asked to record all food consumed during the 24 hrs prior to initial testing [T1] and asked ‘where possible’ to replicate this for all remaining sample points. Dietary records were taken again at T5 to monitor consistency of dietary habits across the study period. Distribution of testing points [T1-T7] throughout the season were based on previous questionnaire data obtained from players in relation to periods of perceived fatigue, recovery or intensive competition (Study 3, chapter 6). Location of when these testing points occurred with reference to seasonal
training/competition calendar can be viewed below (Fig. 6.18b). Analysis of macronutrient intake was performed using commercial dietary analysis software (CompEat). No player reported the consumption of glutamine supplements at any stage throughout the study.

![Figure 6.18: Schematic representation of haematological and saliva testing points across the season.](image)

**Testing points and competition structure**

Measurements were taken from players over a competitive rugby year; seven testing points in total. Sampling points were chosen in an attempt to incorporate periods of high/low training loads, high/low contact phases, and intense competition periods. These pre-determined collection points were not altered during the season and at no point were training programs altered to accommodate the experimental study. A schematic representation of testing schedule across the season is shown in figure 6.18 and a full description of season layout is described in table 6.8.

Elite Welsh players (this study) are normally required to participate in 3 competitions over a given season. Detailed description of this competitive structure is as follows:

**Celtic league:** Minimum of 20 games consisting of home and away fixtures between three countries (Wales, Scotland, Ireland). This competition made up the vast majority of games during the reviewed season.

**Anglo-Welsh cup:** Competition consisting of teams from the Welsh Regions and English Rugby Premiership. Minimum of 3 games consisting of group stages, semi-final and final. The investigated squad reached the final of this competition and therefore played 5 games in total.

**European Cup:** Competition consisting of top teams from Ireland, Wales, England, France and Italy and is considered the pinnacle in elite European club rugby. Minimum of 4 games arising from group stages with tournament progression depending on final group placing. The investigated squad failed to advance beyond the group stages.
Monitoring of Training Load [TL] and Physical Activity

Given the playing level of the investigated rugby squad, a number of players were also required to participate in international games over the study period. With this in mind, measurement of training load and physical training was achieved via use of an ‘online’ player diary system (see case study 4). Average weekly TL’s were quantified for each player and for the squad as a whole using a previously established method (Foster, 1998). Good correlations (r = 0.85-0.94) between this RPE-based TL assessment and other heart-rate based methods was observed on a subset of players from the same squad investigated in the current study (case study 2). In addition to self-reporting by players, fitness coaches rated training days based on levels of set volume and/or intensity. These were also reported using the ‘online’ diary. All activity data entered by players was checked for reliability against data entry by coaching staff. Training loads and volume varied per week and month depending on individual session goals, the training cycle or competition involvement. For a description of the type of physical activity undertaken by the squad and for TL calculation, see Appendix F.

In order to investigate responses to TL, players were asked to note the existence and severity of persistent muscle soreness/tenderness (> 8 hr), joint aches and pains and sleep disturbances using a weekly medical diary. Players were instructed to note these complaint symptoms only in the absence of a known injury. Furthermore, complaint scores were only recorded in the absence of other reported of cold/flu like symptoms. This was to eliminate the possible interference of systemic fever (sometimes associated with viral illness) on muscular complaints and sleep. Total amount of training complaints were noted each week and a mean complaint score was recorded for each month. In order to determine the impact of these complaints on performance, a general complaint index (GC index) was devised. Once the

<table>
<thead>
<tr>
<th>Training week (1-48)</th>
<th>Description of training phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk-1</td>
<td>Start of preseason: after 4 weeks of ‘rest’ away from club training base</td>
</tr>
<tr>
<td>Wks 1-10</td>
<td>Preseason (no games): Strength and conditioning type work, basic fitness</td>
</tr>
<tr>
<td>Wks 11-20</td>
<td>Mid-season: 10 games inc. 6 x Celtic-league, 2 x Anglo-Welsh cup, 1x European cup</td>
</tr>
<tr>
<td>Wks 21-25</td>
<td>Mid-season fixture break. Conditioning type work (non-contact)</td>
</tr>
<tr>
<td>Wks 26-34</td>
<td>ITP. 9 games inc. 1x Anglo-Welsh cup, 4 x European cup, 4 x Celtic league</td>
</tr>
<tr>
<td>Wks 35-39</td>
<td>Less competitive period. 2 games inc. 1 x Celtic-league &amp; 1 x Anglo-Welsh cup</td>
</tr>
<tr>
<td>Wks 40-48</td>
<td>Competitive period. 10 games inc. 1 x Anglo-Welsh cup &amp; 9 x Celtic-league</td>
</tr>
</tbody>
</table>
presence of a complaint was noted, the player then recorded the duration and perceived impact of this complaint on daily activity (1 = normal; 2 = reduced; 3 = went to bed). Following multiplication of complaint duration x activity rating, the scores were summed to give an overall GC score. Following summation of GC scores (Total), average monthly GC scores were calculated and divided by the number of complaints to normalise data forming a GC index.

Team performance was assessed by computing the winning percentage (win/total number of games) between testing points (Filaire et al., 2001).

Table 6.9. Results of games played throughout the sampling period.

<table>
<thead>
<tr>
<th>Type of game</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Games played</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Wins</td>
<td>-</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pre-season friendly</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>European cup</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Domestic league</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Anglo-Welsh cup</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Winning percentage</td>
<td>-</td>
<td>100</td>
<td>58.3</td>
<td>100</td>
<td>55.6</td>
<td>42.8</td>
<td>60</td>
</tr>
</tbody>
</table>

Sample collection

In order to check for possible changes in hydration status between testing points, players were asked to provide morning urine samples in 20 ml universal tubes (Sterilin, UK). For haematological variables, venous blood samples were taken from the antecubital vein on the crease of the arm by a trained phlebotomist. All samples were taken while players were in a supine position and testing procedures were kept consistent throughout study duration. Whole blood was collected into sterile 4 ml K$_3$EDTA and 7 ml lithium-heparin vacutainer tubes (Becton Dickinson, UK) for standard haematological analysis and plasma glutamine/glutamate concentrations. For serum measurements (hs-CRP and CK), blood (7 ml) was collected into vacutainer tubes containing a clot activator (SST; Becton Dickinson, UK). Detailed description of blood handling procedures as well as separation and analysis of plasma/serum can be seen in section 5.5. Saliva was collected from players using the ‘passive drool’ method for determination of testosterone and cortisol. See sections 5.3 and 5.4 for full description of collection method and determination of these analytes.
Statistical analysis

Data are expressed as mean ± SEM. To determine the effects of training on plasma and mucosal markers over time, a one-way repeated measures analysis of variance (ANOVA) was used. Independent t tests were used to compare means of measured parameters between playing groups [forwards vs backs]. For between player group (forwards vs backs) comparisons across time, a two-way mixed [group x time] ANOVA, with ‘time’ as a repeated factor and player ‘group’ as the between factor, was used. Due to the number of analyses, a Bonferroni correction factor was applied. The level of significance was set at P < 0.05 and follow up analysis on the main effects were performed using Bonferroni’s post hoc test. Diagnostic checks (Shapiro-Wilks test of normality and Levene’s homogeneity of variance test) were performed on all dependent variables prior to employment of parametric statistics. Where appropriate, adjustments to the degrees of freedom were made using the Greenhouse-Giesser method of correction. All analyses was performed using SPSS v14.0 for windows.
Table 6.10: Shows breakdown of training volume and activity undertaken by players throughout the period of investigation (n = 48 wks)

<table>
<thead>
<tr>
<th>Training week</th>
<th>1-4</th>
<th>5-8</th>
<th>9-12</th>
<th>13-16</th>
<th>17-20</th>
<th>21-24</th>
<th>25-28</th>
<th>29-32</th>
<th>33-36</th>
<th>37-40</th>
<th>41-44</th>
<th>45-48</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month of season</td>
<td>Jun</td>
<td>Jul</td>
<td>Aug</td>
<td>Sept</td>
<td>Oct</td>
<td>Nov</td>
<td>Dec</td>
<td>Jan</td>
<td>Feb</td>
<td>Mar</td>
<td>Apr</td>
<td>May</td>
<td>Total</td>
</tr>
<tr>
<td>Monthly Training Hours</td>
<td>37</td>
<td>43</td>
<td>28.5</td>
<td>23</td>
<td>24</td>
<td>32</td>
<td>25</td>
<td>21</td>
<td>29</td>
<td>36.5</td>
<td>25</td>
<td>21</td>
<td>345</td>
</tr>
<tr>
<td>Monthly Training Days</td>
<td>19.5</td>
<td>20.5</td>
<td>13</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>160</td>
</tr>
<tr>
<td>Games Per Month</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>High Vol/intense days</td>
<td>7</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>11</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>93</td>
</tr>
<tr>
<td>Moderate Days</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td>Light Days</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>56</td>
</tr>
<tr>
<td>Monthly Training Sessions</td>
<td>32</td>
<td>35</td>
<td>21</td>
<td>18</td>
<td>20</td>
<td>27</td>
<td>21</td>
<td>17</td>
<td>21</td>
<td>22</td>
<td>16</td>
<td>15</td>
<td>265</td>
</tr>
<tr>
<td>Rest Days</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>14</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>10</td>
<td>138</td>
</tr>
<tr>
<td>Formalised contact training sessions</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>4.5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>55</td>
</tr>
</tbody>
</table>

*Contact session number (inc games)
†Formalised contact training sessions

Monthly data are representative of mean values over 4 week blocks. †As outlined by coach [included defence sessions, scrum sessions and pad work]. *Reported by players and included formalised sessions and games. Note: number of training hours excludes recovery training time and time involved in game participation. Players absent due to illness and medical conditions were not included in assessment of training data.

Table 6.11: Breakdown of time (%) spent performing conditioning and rugby activity. Conditioning activity = all non-rugby specific training activity.
Figure 6.19a: Changes in reported training load [duration x intensity] across the season. Values; mean ± SEM. *P < 0.05 from previous month; # P < 0.05 Jun, Aug, Apr, May.

Figure 6.19b: Changes in reported training intensity and game number across the season. Values; mean ± SEM. * p<0.05 from previous month; # p<0.05 Jun, Aug, Dec.

Figure 6.20a: Changes in reported number of muscle/joint/sleep complaints across the season. Total number of complaints by players per month/no training weeks = mean no. complaints.

Figure 6.20b: Changes in general complaint [GC] index across the season.
6.11 RESULTS

Physical training

A significant effect of time was observed for group training load (TL; P < 0.001) across the season (Fig 6.19a). An increase in reported squad TL was observed for July; values significantly higher than the preceding month (June) and following two months (August and September; P < 0.05). Values then increased steadily throughout the season until the month of March. Peak TL values were reported for February (10914 ± 831 AU) before decreasing again until the close of the season (May, 5177 ± 369 AU). In comparison to backs, the forwards reported higher TL values over the penultimate stage of the season (February, March, April); P < 0.05 for March (AU 11021 ± 716 forwards; 8926 ± 573 backs). Conversely, higher TL’s were reported by backs during November. Despite higher mean monthly TL values in forwards, no main effect for group (8081 ± 415 AU forwards; 7609 ± 434 AU backs, P > 0.05) or interaction effect (time x group) was found.

A main effect of time was observed for monthly training intensity (P < 0.001). Notable increases were reported during July and November with peak values during February (Fig. 6.19b). These increases corresponded to times of reduced game activity (Fig. 6.19b) and in the case of November and February, alongside times of reduced rest day number (Table 6.11). Higher overall mean intensity ratings were reported for forwards (5.7 ± 0.19 AU) compared to backs (4.9 ± 0.19 AU); P < 0.05). Between group differences were evident for August, September, October, December, and March; all p < 0.05). Conversely intensity values were higher in backs during the month of November, although between group differences were non-significant. After an initial high number of reported training related complaints during June and July (pre-season training), complaint values decreased thereafter as the season progressed. In contrast mean GC index values varied throughout the season and appeared to resemble changes in reported TL (Figure 6.20b).

Biochemistry

Players were found to consume similar diets when assessed at both dietary sampling points (T1 and T5). Analysis revealed that players consumed diets with a mean macronutrient composition of 46 ± 9.5% carbohydrate, 29 ± 5.0% protein and 25 ± 5.3% fat. No significant changes in hydration status were observed across sampling points with mean resting morning urine osmolality values of 693 ± 53 mosmol.kg⁻¹ recorded (Table 6.15).
No changes in resting blood leukocytes or leukocyte subsets were observed in the playing squad across the rugby year (Table 6.14). Mean resting squad hs-CRP concentrations of 1.19 ± 0.25 mg.L⁻¹ (95% CI: 0.94-1.44 mg.L⁻¹) were recorded throughout the study period. Significant between group differences were observed with forwards displaying higher overall concentrations of hs-CRP than backs (1.5 ± 0.3 vs 0.8 ± 0.3 mg.L⁻¹); P < 0.05.

These findings were consistent throughout the study period and significant group differences were observed at T1, T3, T4 and T7 (Figure 6.21). A significant effect of time was also observed (P < 0.05), where with the exception of T6, CRP values decreased across time. CRP concentrations at T6 were significantly higher than preceding values at T5.

Figure 6.21: Changes in resting concentrations of hs-CRP across rugby year. *Significant between group differences; values mean ± SEM.

Figure 6.22: Changes in resting concentration of CK across rugby year. $\ P < 0.05$ between groups; # $P < 0.05$ from than all other sampling points; * $P < 0.05$ from T4 and T5. Values mean ± SEM.
Mean resting squad CK levels of 338 ± 24 U.L\(^{-1}\) (95% CI: 291-386 U.L\(^{-1}\)) were recorded across the study period (P < 0.05). Similar to CRP findings, concentrations of CK were higher in forwards compared to backs (371 ± 38 U.L\(^{-1}\) forwards; 296 ± 36 U.L\(^{-1}\) backs; P < 0.05). A significant effect of time was also observed (P < 0.05) with values increasing towards the end of the rugby year. Peak concentrations of CK were observed at T6 with values significantly higher than all previous testing points (Table 6.12).

### Table 6.12: Mean ± SEM values for plasma Glutamine, Glutamate, Gln/Glu ratio, hs-CRP, and CK in playing squad across the rugby year.

<table>
<thead>
<tr>
<th>Testing week</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine [µM]</td>
<td>614 ± 15</td>
<td>609 ± 13</td>
<td>590 ± 12</td>
<td>630 ± 11</td>
<td>616 ± 11</td>
<td>614 ± 11</td>
<td>550 ± 10*</td>
</tr>
<tr>
<td>Glutamate [µM]</td>
<td>84.6 ± 6.5</td>
<td>90.0 ± 3.6</td>
<td>87.4 ± 4.5</td>
<td>81.4 ± 4.4</td>
<td>80.8 ± 3.2†</td>
<td>88.7 ± 3.9</td>
<td>83.6 ± 3.6</td>
</tr>
<tr>
<td>Gln/Glu ratio</td>
<td>7.8 ± 0.7</td>
<td>7.1 ± 0.3</td>
<td>7.3 ± 0.4</td>
<td>8.6 ± 0.6#</td>
<td>7.9 ± 0.3</td>
<td>7.5 ± 0.5</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>hs-CRP [mg.L(^{-1})]</td>
<td>1.5 ± 0.4$</td>
<td>1.4 ± 0.3</td>
<td>1.2 ± 0.2$</td>
<td>1.0 ± 0.2$</td>
<td>0.7 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>0.9 ± 0.2$</td>
</tr>
<tr>
<td>CK [U.L(^{-1})]</td>
<td>297 ± 37$</td>
<td>269 ± 24</td>
<td>327 ± 51</td>
<td>268 ± 34</td>
<td>271 ± 33$</td>
<td>563 ± 52φ</td>
<td>376 ± 38φ</td>
</tr>
</tbody>
</table>

$ P < 0.05 between groups. * P < 0.05 from T1, T4, T5 and T7. † P < 0.05 from T2 and T3. # P < 0.05 from T3 and T7. φ P < 0.05 from all other testing points.

Mean squad glutamine and glutamate concentrations of 596 ± 9 µM (95% CI: 582-609 µM) and 85 ± 4 µM (95% CI: 82-89 µM) were observed across the study period. Despite variations in both plasma markers across time, no between group effects were observed. However, concentrations of glutamine were significantly lower at T7 compared to values at T1, T4, T5 and T6. Reductions in plasma glutamate were observed at T4 and T5; with concentrations at T5 significantly lower than T2 and T3 (Figure 6.24). An increase in plasma Gln/Glu ratio was observed at T4 (November) with values significantly higher than those recorded at T3 and T7 (Table 6.12). No between group differences were observed.

### Table 6.13: Mean ± SEM values for saliva testosterone, cortisol and testosterone/cortisol ratio (T/C ratio) in playing squad across the rugby year.

<table>
<thead>
<tr>
<th>Testing week</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone [pmol.L(^{-1})]</td>
<td>593 ± 21</td>
<td>486 ± 43$</td>
<td>578 ± 21</td>
<td>648 ± 3</td>
<td>730 ± 52$</td>
<td>710 ± 44$</td>
<td>579 ± 30$</td>
</tr>
<tr>
<td>Cortisol [nmol.L(^{-1})]</td>
<td>7.8 ± 0.7*</td>
<td>8.2 ± 0.7$*</td>
<td>11.1 ± 0.8</td>
<td>13.6 ± 1.1$</td>
<td>13.9 ± 1.6</td>
<td>13.9 ± 0.8</td>
<td>11.1 ± 1.0</td>
</tr>
<tr>
<td>T/C ratio</td>
<td>78 ± 11*</td>
<td>61 ± 8</td>
<td>54 ± 5</td>
<td>49 ± 11</td>
<td>54 ± 11$</td>
<td>53 ± 11</td>
<td>55 ± 5</td>
</tr>
</tbody>
</table>

$ P < 0.05 between group effect. † P < 0.05 from T2. * P < 0.05 from all remaining test points.
Mean squad resting s-cortisol concentrations of 11.0 ± 2.6 nmol.L⁻¹ (95% CI: 10.0-11.8 nmol.L⁻¹) were observed across the study period. Main effects for time and interaction [group x player] were observed (P < 0.05). A gradual rise in cortisol (C) values was evident with concentrations at T1 and T2 lower than all other remaining testing points (all P < 0.05); Table 6.13. Between group differences were observed at certain time points throughout the season. A greater increase in mid-season values (T4 and T5) was observed in backs, with concentrations at T4 significantly higher (~31%) than their forward counterparts (11.57 vs 16.48 nmol.L⁻¹). Conversely, C was higher (~35%) in the forward player group at the end of pre-season (T2) compared to the backs; P < 0.05.

A main effect of time was also observed for saliva testosterone (Figure 6.23b) with mean resting concentrations of 608 ± 18 pmol.L⁻¹ (95% CI: 574-642 pmol.L⁻¹) recorded in the playing group. Significantly lower concentrations of testosterone were observed in backs compared to forwards (~30%) at T2. Conversely, higher concentrations (~24%) were observed in this player group at T7; P < 0.05. Squad values tended to increase with season progression before decreasing again towards the seasons end (T7). Significantly higher squad testosterone concentrations were found at T5 and T6 compared to end of preseason values (T2). The T/C ratio was found to decrease across the season such that significantly lower values were observed for all sampling points compared to those at the start of the season (T1); table 6.13. Higher T/C values were observed in the forward playing group at T5 compared to backs (P<0.05); figure 6.23c.
Figure 6.23b: Mean ± SEM values for saliva testosterone; Forwards vs Backs. * P < 0.05 between groups.

Figure 6.23c: Mean ± SEM values for saliva T/C ratio; Forwards vs Backs. * P < 0.05 between groups.

Figure 6.24: Changes in resting plasma glutamate; # P < 0.05 from T3. Values mean ± SEM.
<table>
<thead>
<tr>
<th>Testing point</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Leukocytes ($\cdot 10^9\text{L}^{-1}$)</td>
<td>6.19 (0.28)</td>
<td>5.91 (0.23)</td>
<td>5.60 (0.23)</td>
<td>5.89 (0.26)</td>
<td>6.00 (0.23)</td>
<td>5.42 (0.17)</td>
<td>5.22 (0.17)</td>
</tr>
<tr>
<td>Neutrophils ($\cdot 10^9\text{L}^{-1}$)</td>
<td>3.33 (0.19)</td>
<td>3.06 (0.16)</td>
<td>2.92 (0.17)</td>
<td>3.22 (0.19)</td>
<td>3.25 (0.18)</td>
<td>2.89 (0.13)</td>
<td>2.74 (0.12)</td>
</tr>
<tr>
<td>Lymphocytes ($\cdot 10^9\text{L}^{-1}$)</td>
<td>2.05 (0.20)</td>
<td>2.12 (0.10)</td>
<td>1.98 (0.07)</td>
<td>1.91 (0.09)</td>
<td>2.02 (0.10)</td>
<td>1.83 (0.06)</td>
<td>1.81 (0.08)</td>
</tr>
<tr>
<td>Neut:Lymp ratio</td>
<td>1.87 (0.14)</td>
<td>1.53 (0.09)</td>
<td>1.52 (0.09)</td>
<td>1.75 (0.12)</td>
<td>1.68 (0.12)</td>
<td>1.62 (0.09)</td>
<td>1.59 (0.09)</td>
</tr>
<tr>
<td>Monocytes ($\cdot 10^9\text{L}^{-1}$)</td>
<td>0.52 (0.03)</td>
<td>0.49 (0.03)</td>
<td>0.47 (0.02)</td>
<td>0.47 (0.02)</td>
<td>0.50 (0.03)</td>
<td>0.46 (0.02)</td>
<td>0.46 (0.02)</td>
</tr>
<tr>
<td>Eosinophils ($\cdot 10^9\text{L}^{-1}$)</td>
<td>0.25 (0.05)</td>
<td>0.22 (0.03)</td>
<td>0.21 (0.03)</td>
<td>0.26 (0.04)</td>
<td>0.21 (0.02)</td>
<td>0.22 (0.04)</td>
<td>0.18 (0.02)</td>
</tr>
<tr>
<td>Basophils ($\cdot 10^9\text{L}^{-1}$)</td>
<td>0.020 (0.008)</td>
<td>0.038 (0.002)</td>
<td>0.036 (0.004)</td>
<td>0.030 (0.010)</td>
<td>0.030 (0.016)</td>
<td>0.020 (0.010)</td>
<td>0.012 (0.006)</td>
</tr>
<tr>
<td>Platelets ($\cdot 10^9\text{L}^{-1}$)</td>
<td>215.5 (8.10)</td>
<td>218.0 (7.81)</td>
<td>233.3 (7.29)</td>
<td>218.0 (8.72)</td>
<td>219.5 (10.33)</td>
<td>215.6 (7.97)</td>
<td>228.7 (7.31)</td>
</tr>
<tr>
<td>RBC's($\cdot 10^{12}\text{L}^{-1}$)</td>
<td>5.05 (0.06)</td>
<td>5.15 (0.07)</td>
<td>4.86 (0.04)</td>
<td>4.98 (0.06)</td>
<td>5.06 (0.05)</td>
<td>4.80 (0.05)</td>
<td>4.95 (0.04)</td>
</tr>
<tr>
<td>Haemoglobin (g.dL$^{-1}$)</td>
<td>14.99 (0.12)</td>
<td>14.60 (0.10)</td>
<td>14.84 (0.12)</td>
<td>15.12 (0.13)</td>
<td>15.48 (0.12)</td>
<td>14.58 (0.15)</td>
<td>15.12 (0.11)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>44.44 (0.35)</td>
<td>43.84 (0.33)</td>
<td>42.66 (0.32)</td>
<td>44.22 (0.43)</td>
<td>45.03 (0.35)</td>
<td>42.62 (0.38)</td>
<td>44.17 (0.32)</td>
</tr>
</tbody>
</table>

Table 6.14: Haematological and immunological variables in players across the rugby year; values mean (SEM).

<table>
<thead>
<tr>
<th>Testing point</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Osmolality (mosmol.kg$^{-1}$)</td>
<td>595.3 (45.5)</td>
<td>699.9 (47.8)</td>
<td>709.3 (50.0)</td>
<td>743.5 (57.0)</td>
<td>742.2 (54.0)</td>
<td>680.6 (61.0)</td>
<td>581.0 (52.7)</td>
</tr>
</tbody>
</table>

Table 6.15: Resting morning hydration status (urine osmolality) in players across testing points; values mean (SEM).
6.12 DISCUSSION

Previous research in elite rugby union has primarily focused on injury analysis (Brooks et al., 2008; Fuller et al., 2008), game analysis (Roberts et al., 2008; Deutsch et al., 2007) and conditioning type aspects of player preparation (Hartwig et al., 2008; Duthie et al., 2006). While the acute physiological effects of rugby play has been previously shown (Mashiko et al., 2004; Takarada, 2003), few studies have focused on physiological and biochemical changes over long study durations (Banfi et al., 2006; Finaud et al., 2006).

To the author’s knowledge, this was the first study to document detailed training data in conjunction with changes in biochemical and hormonal markers across a competitive rugby year. Following an initial increase in squad TL’s during preseason, values decreased during August before rising steadily with season progression (Fig 6.19a). On further investigation it was found that forwards reported higher TL’s during the penultimate stage of the season (February, March, April) while conversely, higher TL’s and intensity values were reported by backs during the month of November. With respect to forwards, it is likely that the greater physical requirements of forward play, coupled with the intensified competition that took place during these months may have had an accumulative loading effect on this player group. In contrast, higher TL values reported by backs during the month of November may have been the result of increased conditioning type activity that took place during a break in competitive fixtures (Table 6.11). Similar to findings for TL, increased concentrations of saliva cortisol (C) were also observed across time. Although speculative, it is possible that the cumulative training stress experienced by players may have resulted in this observed C response. These findings are similar to those recorded in American footballers, where lower C values were observed at the start and end of preseason in comparison to rest of season values (Hoffman et al., 2005). In the current study, cortisol concentrations at T1 and T2 were significantly lower than all other remaining testing points. Chronic stress has been suggested to result in increased catecholaminergic input to CRF-containing cells, which in turn leads to increased ACTH and glucocorticoid levels (Pruessner et al., 1999). However, it is also possible that the elevated cortisol levels seen with season progression may have been related to the anti-inflammatory process of training (Kirwan et al., 1988) or indeed, an artefact of natural seasonal variation (King et al., 2000). A combination of the above factors represents the most likely explanation for these findings.

Interestingly, a greater increase in mid-season cortisol values was observed in the back player group, with concentrations at T4 significantly higher (~31%) than their forward counterparts. It is difficult to ascertain as to why such findings occurred although it is likely that the sharp
increase in conditioning type activity undertaken at this time may have elicited a greater stress response in this player group. With respect to T4, the time point coincided with a break in club fixtures due to autumn internationals; suggesting that training type activity and not competition per se exerts greater physiological stress in backs. Conversely, higher C concentrations (~35%) were observed in the forward player group at the end of pre-season (T2). It is likely that sudden increases in conditioning type activity which occurred during preseason may have accounted for this augmented stress response in forwards when compared to their backs counterparts. Pre-season training generally consisted of squad conditioning type activity and running drills without positional differentiation of drill type that would normally occur ‘in season’. It is therefore possible that increased conditioning type work, coupled with the extra body mass forwards carry during locomotion could have resulted in this augmented stress response.

The T/C ratio provides information about the recovery ability of the athlete and the athlete’s ability to synthesise protein and maintain muscle mass (Fry et al., 1991). In this study, a large decrease in squad T/C values was observed following ten weeks of preseason training (T2) when compared to values at the beginning of season (T1) (P < 0.05). This data is indicative of decreased anabolic state, possibly as a result of a sudden increase in TL during pre-season. These findings are in agreement with others where decreases in T/C ratio have been observed in athletes undergoing progressive overload training (Coutts et al., 2007; Filaire et al., 2001; Steinacker et al., 1993; Hoogeveen and Zonderland, 1996). Despite this initial decline in anabolic state, further reductions were non significant for the remainder of the season. It is possible that a reduction in training volume during August seemed to halt any further declines in squad T/C ratio. Furthermore, although squad T/C ratio values were lower for all other remaining testing points (compared to T1); increases in cortisol were counteracted by corresponding increases in testosterone levels. This indicates a possible readjustment of player hormonal state with repeated training stimulus. However, on further analysis of positional data, a continuous decline in T/C values were evident in the back playing group up to and including T4. This was a result of the higher cortisol values (catabolic) present during this time (Fig 6.23a), which indicates decreased recovery in this player group. Although associations between T/C ratio and team winning percentage have been previously made in soccer (Filaire et al., 2001), attempts to link hormonal changes with performance were not attempted in the current study. This was deemed prudent given the diverse nature of rugby union play.
Previous authors have suggested that subjective assessment of disturbances in sleep quality and muscle soreness may indicate early signs of fatigue and overtraining in athletes (Hooper et al., 1995; Meeusen et al., 2006). In this study, player ratings of complaints, including muscle soreness/tenderness (>8hr), joint aches/pains and loss of sleep, was achieved using an online diary. Once a complaint was registered, the duration and severity of each complaint was also noted and a ‘General complaints (GC) Index’ was subsequently calculated. It was hoped that by doing this, a greater understanding of training stress and its impact on the ability to perform could be achieved. As seen figure 6.20b, GC index values appeared to increase with season progression and the data seemed to closely resemble changes in TL. These findings indicate that resolution of training stress was reduced in certain players as the season progressed and also the possibility of cumulative fatigue. Also, as a crude assessment of squad adaptation to TL, weekly totals of training related complaints were registered and a subsequent calculation of mean monthly totals was made. This, like the GC index is something which has not been outlined previously in other longitudinal studies. However, following an initial high number of complaint values over the first two months of the season; numbers of training related complaints decreased progressively with time unlike values for GC index (Figure 6.20a). Taken together these findings demonstrate a reduced number of training related complaints with season progression but a diminished resolution/increased severity of these complaints in certain players. It has been suggested that not all athletes respond similarly to training (Chatard et al., 2002); some positively adapt while others exhibit signs of overreaching (Mackinnon et al., 1997). Therefore, this data possibly highlights the individual nature of training responsiveness and the existence of players unable to tolerate training loads as well as others. With respect to decreased numbers of training related complaints across the season, it is possible that increased training tolerance and the ‘repeated bout effect’ (Clarkson et al. 1987) contributed to these findings.

Training is thought to allow muscles to become more resistant to damage by exercise (Takarada, 2003) and also allows muscles to repair at a faster rate (Paul et al., 1989; Clarkson et al., 1988; Vincent and Vincent, 1997). Furthermore, the reactions to stress depend on the permanent and consumptive resources, which can offer a person some resistance to stress (Schonpflug, 1983). It is therefore possible that training stress and tissue trauma which occurred following sudden increases in TL early in the season (June and July) conferred additional musclo-skeletal protection as the season progressed. Interestingly a significant effect of time was also observed for CRP, where with the exception of T6, values decreased across the season. This finding may also be suggestive of a ‘repeated bout effect’ and
desensitisation of the acute phase response. Of note, a similar ‘contact adaptation’ effect has been suggested in a similar longitudinal study on collegiate American footballers (Hoffman et al., 2005). Further clarification of this effect is required in longitudinal studies using frequent sampling points of other inflammatory/anti-inflammatory markers such as cytokines.

Despite decreases in CRP levels, resting concentrations of CK remained relatively stable up to T6, where values were shown to increase. It is unknown why an increase occurred at this time. However, given that a corresponding elevation in CRP values was noted, residual physical trauma and inflammation from an intensified competition period is the most likely scenario. This time point was located in the middle of an intense period of game fixtures, primarily because of previous fixture cancellations and resultant mid-week games. Although testing occurred after a minimum of 48 hr rest, it is likely that residual muscle damage from this intensified competition period remained. Of note, resting CK and CRP values were consistently higher in forwards than backs over the season (Figs 6.21 and 6.22). It is possible that this finding is reflective of the greater amount of physical contact that forms part of rugby forward play and resultant tissue trauma associated with game collisions (Smart et al., 2008). Although lower CK values have been shown in more trained individuals (Vincent and Vincent, 1997), differences in training state would not have accounted for these observations given the highly trained nature of both sets of players in the current study.

As previously observed in Italian rugby players (Banfi et al., 2006), no significant changes in WBC’s or their subclasses were found to occur in the present study. However it should be noted that abnormal blood counts were observed in three players who were subsequently referred to their clinician for further assessment. One of these players was excluded from all remaining testing points. Despite previous studies showing decreases in plasma glutamine (Gln):glutamate (Glu) ratio in rugby league players with intensified training (Coutts et al., 2007), no significant decreases in Gln:Glu ratio were observed in the present study. Conversely, Gln:Glu ratio values were found to increase at T4 (November). This increase was as a result of increased levels of plasma glutamine and corresponding decreased levels of plasma glutamate. Interestingly, decreases in plasma glutamate were evident at both T4 and T5; time periods of high TL and intensity. This finding is in contrast to other studies where elevations in glutamate have been observed in athletes undergoing intensified training (Halson et al., 2003; Coutts et al., 2007; Smith and Norris, 2000). It is difficult to explain why this decrease occurred especially when considering that both time points were considered intense training periods. This was also evident from the high cortisol and reported TL and intensity values at these times. Glutamate along with glutamine has a role in acid-base balance, _de novo_
synthesis of nucleotides, and is also an important regulator of protein synthesis and degradation (Rowbottom et al., 1996). Increased transport and uptake of glutamate into peripheral muscle may have occurred for repair and regenerative purposes as a result of heavy conditioning type training. Furthermore, glutamine is synthesized from ammonia and glutamate by glutamine synthesase (Halson et al., 2003). In the event of increased training stress, the necessity to preserve and support glutamine levels may have impacted on corresponding glutamate levels. Indeed, despite the high level of competition in the current study, concentration of plasma glutamine remained either stable or increased during intense training/competition period. Stable or increased levels of plasma glutamine have been previously shown with heavy intensified training (Kargotich et al., 2006). Alternatively decreases in plasma glutamate may have been an artefact of impaired muscle repair processes or de novo glutamate synthesis itself. Further investigation into the role of chronic exercise and exercise type on plasma glutamate and glutamine levels is therefore required.

Lower values for plasma glutamate [85 ± 4 µM] were observed in this study comparison to those recorded in rugby league players (Coutts et al., 2007) and endurance trained cyclists (Halson et al., 2003) undergoing heavy training. It is possible that variations in methodology of measurement and differences in study population could have accounted for these differences. Unlike previous investigations, the current study employed detailed assessment of daily activity undertaken by players. Future studies should also employ similar protocols for measurement of training load over longitudinal investigations, thus providing greater clarity into causative factors pertaining to any changes in observed biochemical variables across time. Noticeably, like CRP and CK values, mean glutamate concentrations were higher in forwards than backs. As previously discussed, it is possible that this observation may be related to the increased muscle damage resultant from game collisions and the repair processes required after this damage.

**Conclusion**

Unlike other studies, the current study employed detailed documentation of training activity and loads undertaken by players during the season of investigation. Analysis of biochemical and hormonal changes in conjunction with subjective assessment of physical complaints revealed the possible existence of ‘contact adaptation’ in elite rugby union and individual player responsiveness to training load. Future studies should attempt to determine the effects of observed changes in biochemical markers with clearly documented training load data as well as simple field fields that are reflective of muscular performance.
Study Six: Training related psychological stress in professional rugby union players over a competitive season

SUMMARY

This purpose of this study was to investigate changes in the perceived stress-recovery balance across a competitive rugby season. Thirty elite rugby players filled out the Recovery-Stress questionnaire for Athletes (REST-Q Sport) every 4 weeks over a 48 week rugby year. Data revealed significant changes in both stress and recovery scales across time. Between group comparisons showed significantly lower scores for social recovery in the forwards during week 14 (September), week 19 (October) and week 24 (November) in comparison to backs; P < 0.05. Additionally, higher scores (P < 0.05) for emotional exhaustion were observed in the forwards when compared to backs during week 19 (October) and week 38 (February). Gradual increases in General Stress were apparent in both groups over time (P < 0.05). Peaks in total stress scores appeared to occur following periods of intensified training and on player resumption to normal game activity. Present results indicate the existence of critical periods of stress and recovery across a typical rugby season. Frequent monitoring of these indices using the RESTQ may help coaches in identifying possible imbalances in the training process and aid in the diagnosis of underperformance.

6.13 INTRODUCTION

Psychological stress in athletes has been suggested to originate from a variety of sources including anxieties associated with training performance, competition and/or some lifestyle stresses which are unrelated to sport (Hong, 2000). Indeed, disturbances in mood have also been shown to increase with step-wise increases in training load and recede again with concurrent training load reductions in a dose-dependent manner (Morgan et al., 1987). With the above in mind, close monitoring of stress and recovery may therefore have important ramifications in terms of training prescription of the athlete. Furthermore, measurement of both psychological stress and recovery has been reported to reflect well the clinical state of the athlete (Filaire et al., 2001; Kellmann et al., 1997; Morgan et al., 1987). Perturbations in mood state resultant from heavy training loads over and above that of an athletes coping response (O’Connor et al., 1989; Flynn et al., 1994) are thought to influence a number of negative aspects within the training process. Changes in mood are thought to represent some of the first signs of staleness and overtraining in athletes (Armstrong and Vanheest, 2002; Foster and Lehman 1997; McKenzie, 1999; Morgan et al., 1987; Stone et al., 1991, Urhausen and Kinderman, 2002) while acute and chronic psychological stress has also been implicated with decreased immunity (Bosch et al., 2002) and increased susceptibility to the common cold (Cohen et al., 1991).

In the sport of rugby union, research has suggested that injuries, mental errors and physical errors are the most reported stressors among this group of athletes (Nicholls et al., 2008). It is
also conceivable that issues such as team selection, player form, contractual issues as well as overlap of competition (international and domestic) could place additional psychological strain on this athletic group (at elite level). Existing research examining the possible link between player burnout and motivation in rugby players have shown changes in burnout related categories to be associated with playing position, injury, selection and starting status over a 30 week season in this group (Cresswell and Eklund, 2006). In such studies however, measurements are often made in the absence of sufficient detailing of training load data (Raedeke and Smith, 2001; Cresswell and Eklund, 2006). This can therefore provide difficulties in the interpretation of data and any underlying causative agents of stress-recovery balance in the athlete.

To date, the majority of studies using psychometric monitoring of athletes has focused on stress related scales using the Profile of Mood States (POMS, McNair et al., 1992). However, use of this questionnaire in the athletic arena has been the subject of debate, given its limitations in differing personality traits between athletes of similar levels of ability (Renger, 1993). Furthermore, it has been suggested that since this questionnaire attempts to explore current stress processes only, limitations exist given its vague reflection of the recovery processes (Kellmann and Günther, 2000). An alternative approach training monitoring is to measure the athlete’s perception of stress and recovery concurrently, since restricting analysis to the stress dimension alone is insufficient when dealing with elite athletes (Kellmann et al., 1997; Steinacker et al., 2000). Few studies have examined the role of training load on parameters of psychological stress in high performing team athletes over long study durations. With the above in mind, the purpose of this investigation was to evaluate the effect of changes in training load and intensity on perceptions of stress and recovery in elite rugby union players over a competitive season.

6.14 METHODS

Evaluation of changes in player stress and recovery were made using the RESTQ-52 sport questionnaire (Kellman and Kallus, 2001). Players (n = 30) were asked to complete the 52 item questionnaire every 4 weeks throughout the study period (48 weeks). Standardized instructions were given to all participants on administration of the questionnaire once prior written informed consent had been obtained. The questionnaire was issued to players at the start of each month and after a minimum of 48 hours rest from previous exercise activity. The study was approved by the University of Glamorgan, research ethics committee (Section 3.1).
Daily activity data, including training load and intensity, were self-reported by players over the entire training season. This was achieved using an online training diary (see case study 4). Formalised training session number and type of activity undertaken was also noted by club fitness staff using the same system.

**Figure 6.25:** Schematic representation of RESTQ analysis and time intervals.

**REST-Q 52 Sport**

The REST-Q 52 Sport was used over that of the REST-Q 76 form, since this model of the questionnaire has been stated to be more robust in longitudinal designs or when information in the general scales are not the focus of interest (Kellman and Kallus, 2001). It consists of 12 basic scales, with seven additional sports specific scales which using a self-report approach, attempts to evaluate physical, subjective, behavioural and social aspects of stress and recovery. Description of scales can be seen in Appendix X. The RESTQ has been shown to display consistently high short-term stability with test-retest reliability reported at $r = 0.51-0.81$ (Kellmann and Kallus, 2001). Considerable correlations have been shown between RESTQ-sport and the POMS (Kellman et al 2001; Kallus and Kellman, 2000), despite that fact that both questionnaires differ in terms of scales.

**Scoring and statistics**

Within each scale, the player responded to a series of items, which were rated according to their frequency on a Likert-like rating scale ranging from 0 (never) to 6 (always). In all cases, the scale values were calculated by taking the mean of the item values. In the case of the sleep quality scale, item scores were inverted (Kellmann and Kallus, 2001). A mean score for scales
1-7 was taken as representing ‘general stress score’ while a mean value for scales 8-12 taken as those representing ‘general recovery’. The same procedure was applied for ‘sport specific’ stress (mean of scales 13-15) and ‘sport specific recovery’ (mean of scales 16-19).

In turn, the general and sport specific stress and recovery scales were combined to give a total stress and recovery score. These two scores were then subtracted to provide a general indicator of stress-recovery balance (Coutts et al., 2007). In general, low scores in the stress related areas and high scores in the recovery related areas are positively labelled and vice versa (Kellman and Kallus, 2001). Comparison of group mean scores for each scalar variable across time were analysed using a repeated measures ANOVA. Between player group comparisons (Forwards vs Backs), across time, were analysed using a two factor (time x group) repeated measures ANOVA. Data displayed in tables are presented as mean ± SD. For purposes of clarity, data in figures are presented as mean ± SEM.

6.15 RESULTS

Training load and physical activity data is as described in section 6.11. Table 6.19 shows the results for the 19 scales of the REST-Q sport. There was a significant main effect of time for the stress scales ‘General Stress’, ‘Conflicts/Pressure’, ‘Lack of Energy’, and ‘Emotional Exhaustion’.
A significant effect of time was also observed for the recovery related scales such as ‘Success’, ‘General well-being’ and ‘Self-Efficacy’. An overall increase in ‘General Stress’ was observed across the season with significantly higher values observed for training weeks 28 (December), 34 (January), 41 (February) and 45 (April) compared to those taken at the start of the season, week 0 (June); figure 6.28. No between group differences (forwards v backs) were observed. Scores for ‘Lack of energy’ and ‘Conflicts/Pressure’ also increased with season progression but were non significant. Pairwise comparisons revealed significantly lower scores (P < 0.05) for ‘Physical Complaints’ during week 24 (November) when compared to week 14 (September) and week 34 (January); figure 6.29.
Table 6.17: Shows breakdown of training volume and activity undertaken by players throughout the period of investigation (n = 48 wks)

<table>
<thead>
<tr>
<th>Training week</th>
<th>1-4</th>
<th>5-8</th>
<th>9-12</th>
<th>13-16</th>
<th>17-20</th>
<th>21-24</th>
<th>25-28</th>
<th>29-32</th>
<th>33-36</th>
<th>37-40</th>
<th>41-44</th>
<th>45-48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month of season</td>
<td>Jun</td>
<td>Jul</td>
<td>Aug</td>
<td>Sept</td>
<td>Oct</td>
<td>Nov</td>
<td>Dec</td>
<td>Jan</td>
<td>Feb</td>
<td>Mar</td>
<td>Apr</td>
<td>May</td>
</tr>
<tr>
<td>Monthly Training Hours</td>
<td>37</td>
<td>43</td>
<td>28.5</td>
<td>23</td>
<td>24</td>
<td>32</td>
<td>25</td>
<td>21</td>
<td>29</td>
<td>36.5</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>Monthly Training Days</td>
<td>19.5</td>
<td>20.5</td>
<td>13</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>9</td>
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<tr>
<td>Games Per Month</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>High Vol/intense days</td>
<td>7</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>11</td>
<td>9</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Moderate Days</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td>Light Days</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Monthly Training Sessions</td>
<td>32</td>
<td>35</td>
<td>21</td>
<td>18</td>
<td>20</td>
<td>27</td>
<td>21</td>
<td>17</td>
<td>21</td>
<td>22</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Rest Days</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>†Formalised contact sessions</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>4.5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>*Contact session number (inc games)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forwards</td>
<td>1.0</td>
<td>6.0</td>
<td>8.0</td>
<td>3.9</td>
<td>6.6</td>
<td>4.0</td>
<td>7.5</td>
<td>7.9</td>
<td>5.8</td>
<td>7.1</td>
<td>5.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Backs</td>
<td>1.0</td>
<td>4.0</td>
<td>4.0</td>
<td>1.4</td>
<td>6.0</td>
<td>2.3</td>
<td>6.8</td>
<td>8.1</td>
<td>4.6</td>
<td>6.4</td>
<td>5.7</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Monthly data are representative of mean values over 4 week blocks. †As outlined by coach [included defence sessions, scrum sessions and pad work].
*Reported by players and included formalised sessions and games. Note: number of training hours excludes recovery training time and time involved in game participation. Players absent due to illness and medical conditions were not included in assessment of training data.

<table>
<thead>
<tr>
<th>% Activity Time</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of time spent conditioning</td>
<td>80</td>
<td>81</td>
<td>54</td>
<td>40</td>
<td>48</td>
<td>69</td>
<td>46</td>
<td>49</td>
<td>65</td>
<td>48</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>% time playing rugby</td>
<td>19</td>
<td>18</td>
<td>41</td>
<td>52</td>
<td>46</td>
<td>28</td>
<td>52</td>
<td>49</td>
<td>33</td>
<td>50</td>
<td>50</td>
<td>47</td>
</tr>
<tr>
<td>Ratio rugby/conditioning</td>
<td>0.23</td>
<td>0.22</td>
<td>0.78</td>
<td>1.31</td>
<td>0.95</td>
<td>0.41</td>
<td>1.13</td>
<td>0.99</td>
<td>0.51</td>
<td>1.05</td>
<td>1.07</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Table 6.18: Breakdown of time (%) spent performing conditioning and rugby activity. Conditioning activity = all non-rugby specific training activity.
Between group comparisons revealed significantly lower scores for ‘Social Recovery’ in the forward player group during week 14 (September), week 19 (October) and week 24 (November) in comparison to backs; P < 0.05 (Figure 6.30). Conversely, higher scores (P < 0.05) for ‘Emotional Exhaustion’ were observed in the forwards when compared to backs for week 19 (October) and week 38 (February); figure 6.31. Higher scores for ‘Self-efficacy’ were observed in both player groups during week 24 (November), week 28 (December) week 34 (January) and week 41 (March) in comparison to start of the season values (June, week 0).

Both player groups reported highest scores for injury during at the end of the season (week 45); values were significantly higher than week 24 (November), week 28 (December) and week 34 (January). Between group differences were also observed with higher values noted in forwards than backs during week 0 (June), week 24 (November) and week 41 (March).

An overall increase in total stress scores (Σ10 stress subscales) were observed during week 28 (December); values were significantly higher than all other preceding weeks. Total stress scores were higher (P < 0.05) in backs compared to forwards during weeks 14 (September), 38 (February) and 41 (March). Accordingly, total recovery (Σ9 recovery subscales) scores were lower in backs (P < 0.05) compared to forwards during week 38 (February). Aside from weeks 28 (December) and 38 (February), a gradual decrease in stress-recovery balance (total stress-total recovery) was observed across the season; values indicative of improved recovery.
Table 6.19: RESTQ-52 Sport subscales of stress and recovery (mean ± SD) from playing squad across rugby season.

<table>
<thead>
<tr>
<th>Month of season Training week</th>
<th>June 1</th>
<th>July 6</th>
<th>Aug 9</th>
<th>Sept 14</th>
<th>Oct 19</th>
<th>Nov 24</th>
<th>Dec 28</th>
<th>Jan 34</th>
<th>Feb 38</th>
<th>March 41</th>
<th>April 45</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stress Subscales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Stress†</td>
<td>1.0 ± 0.6</td>
<td>1.2 ± 1.0</td>
<td>1.2 ± 0.9</td>
<td>1.3 ± 0.9</td>
<td>1.4 ± 0.9</td>
<td>1.2 ± 0.8</td>
<td>1.5 ± 0.9*</td>
<td>2.0 ± 0.9*</td>
<td>1.3 ± 0.8</td>
<td>1.8 ± 1.2*</td>
<td>2.2 ± 1.2*</td>
</tr>
<tr>
<td>Emotional Stress</td>
<td>1.4 ± 0.7</td>
<td>1.6 ± 0.9</td>
<td>1.5 ± 0.7</td>
<td>1.7 ± 0.7</td>
<td>1.8 ± 0.6</td>
<td>1.6 ± 0.8</td>
<td>1.7 ± 1.0</td>
<td>1.7 ± 1.0</td>
<td>1.6 ± 0.7</td>
<td>2.0 ± 1.1</td>
<td>1.9 ± 1.1</td>
</tr>
<tr>
<td>Social Stress</td>
<td>1.2 ± 0.8</td>
<td>1.4 ± 1.1</td>
<td>1.4 ± 0.8</td>
<td>1.5 ± 0.9</td>
<td>1.4 ± 0.8</td>
<td>1.3 ± 0.9</td>
<td>1.4 ± 0.8</td>
<td>1.5 ± 0.8</td>
<td>1.4 ± 1.0</td>
<td>1.8 ± 1.2</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td>Conflicts/Pressure†</td>
<td>1.7 ± 1.0</td>
<td>2.0 ± 1.0</td>
<td>1.7 ± 0.9</td>
<td>2.0 ± 1.0</td>
<td>2.0 ± 0.8</td>
<td>1.9 ± 1.1</td>
<td>2.0 ± 1.0</td>
<td>1.9 ± 1.0</td>
<td>2.0 ± 1.1</td>
<td>2.1 ± 1.3</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1.8 ± 0.7</td>
<td>2.4 ± 0.8</td>
<td>2.2 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>2.2 ± 0.9</td>
<td>1.9 ± 1.1</td>
<td>2.1 ± 1.0</td>
<td>2.6 ± 1.0</td>
<td>1.9 ± 1.3</td>
<td>1.9 ± 1.2</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>Lack of Energy†</td>
<td>1.2 ± 0.7</td>
<td>1.4 ± 0.9</td>
<td>1.2 ± 0.7</td>
<td>1.4 ± 0.8</td>
<td>1.2 ± 0.7</td>
<td>1.4 ± 0.9</td>
<td>1.6 ± 0.8</td>
<td>1.6 ± 1.2</td>
<td>1.4 ± 1.2</td>
<td>1.9 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Physical Complaints</td>
<td>1.3 ± 0.9</td>
<td>1.2 ± 1.1</td>
<td>1.2 ± 0.7</td>
<td>1.5 ± 0.7</td>
<td>1.3 ± 0.9</td>
<td>1.1 ± 0.9</td>
<td>1.4 ± 0.9</td>
<td>1.8 ± 0.9</td>
<td>1.1 ± 1.1</td>
<td>1.6 ± 1.0</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>Disturbed Breaks</td>
<td>1.2 ± 1.2</td>
<td>1.4 ± 1.1</td>
<td>1.3 ± 1.2</td>
<td>1.2 ± 0.8</td>
<td>1.3 ± 0.9</td>
<td>1.2 ± 0.8</td>
<td>1.4 ± 0.8</td>
<td>1.5 ± 0.8</td>
<td>1.3 ± 1.1</td>
<td>1.6 ± 1.0</td>
<td>1.5 ± 1.2</td>
</tr>
<tr>
<td>Emotional exhaustion†</td>
<td>1.2 ± 1.1</td>
<td>1.2 ± 0.9</td>
<td>1.1 ± 1.1</td>
<td>1.4 ± 0.8*</td>
<td>1.5 ± 0.7$</td>
<td>1.4 ± 0.7*</td>
<td>1.5 ± 0.9*</td>
<td>1.5 ± 1.0</td>
<td>1.4 ± 0.9$</td>
<td>1.6 ± 0.9</td>
<td>1.6 ± 1.0*</td>
</tr>
<tr>
<td>Injury</td>
<td>2.3 ± 0.9$</td>
<td>2.7 ± 1.0</td>
<td>2.7 ± 0.8</td>
<td>2.7 ± 0.9</td>
<td>2.6 ± 0.8</td>
<td>2.4 ± 0.9$</td>
<td>2.4 ± 0.7</td>
<td>3.2 ± 0.7</td>
<td>2.4 ± 1.0</td>
<td>2.4 ± 1.0$</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td><strong>Recovery Subscales</strong></td>
<td></td>
<td></td>
<td></td>
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<td>4.1 ± 1.1$</td>
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†Significant effect of time (P < 0.05). *Significantly different from baseline scores (week 0); P < 0.05. $Significant difference between playing groups (forwards vs backs); P < 0.05
**Figure 6.28:** Means ± SD of RESTQ-scale for General Stress across season. * Higher than week 0 (June); P < 0.05.

**Figure 6.29:** Means ± SD of RESTQ-scale for Physical Complaints across season. * Lower than weeks 14 and 24; P < 0.05.

**Figure 6.30:** Positional differences in Social Recovery scores across season. * Lower in forwards compared to backs; P < 0.05.

**Figure 6.31:** Positional differences in Emotional Exhaustion scores across season. * Higher in forwards compared to backs; P < 0.05.
6.16 DISCUSSION

The purpose of this study was to examine the effects of regular competitive rugby union on perceptions of stress and recovery in elite rugby union players. Despite large variability in stress and recovery scores between players, the present data does highlight the role of increases in training load on stress-recovery balance in this player group.

Large variability in scores on the psychometric subscales was found with some scales increasing and others decreasing at certain time points during the season. However, scores for general stress did show uniformity across player groups with values increasing significantly across the training season (Figure 6.28). Although speculative, it is possible that such changes may have represented signs of mental stress and fatigue, feelings of which intensified as the season progressed. Despite this, scores for other stress related scales did not increase across time, possibly reflecting the specific nature of stress related paradigms and sensitivity to training load. A notable peak in scores for total stress were observed for all players during week 28, values which were significantly higher than all other preceding weeks. It is difficult to ascertain exactly why peak stress values occurred at this time. However, it is possible that increased training volume and intensity observed 2-3 weeks prior to this period may have contributed to these delayed feelings of mental and physical exhaustion. In the current study, a break in game fixtures was evident during the month of November because of competing international rugby over this time period. It is feasible that the increased training loads and intensity which occurred during this time period (Tables 6.17 and 6.18) could have contributed to such a heightened stress response. However, it is also possible that a sudden increase in game fixtures during December exacerbated this stress reaction, culminating in peak values at the end of the month (week 28). Of note, this week represents one of the most intensified competitive periods in the fixture calendar and occurs around the Christmas period. It is also a time when family members and the normal working population have a break in work related activity which along with the above, may have contributed to increased levels of mental strain and irritability.

Interestingly, despite the increase in training load and intensity which occurred during breaks in game fixtures (November and February), a corresponding decrease in scores for physical complaints was noted (Figure 6.29). Both of these time points reflected instances of reduced game activity and increased conditioning type activity (Table 6.18), pointing to the fact the game related activity rather than training, exerts greater physiological stress on this player group. Considerable variation was also observed for both stress and recovery scales.
between players of differing playing position. Forwards displayed significantly higher values for ‘emotional exhaustion’ and lower scores for ‘social recovery’ than backs at regular times throughout the season (Figures 6.30 and 6.31). These findings indicate that forwards are more likely to suffer from symptoms such as burnout and display higher values for frustration and mental exhaustion in comparison to their back counterparts. Such data is consistent with that observed in a previous study investigating player burnout in rugby (Cresswell and Eklund, 2006). Similar to current findings, investigators showed that changes in exhaustion over time (rugby year) were dependent on playing position with forwards displaying higher levels of physical exhaustion over the study period. The authors postulated that such findings represent a possible link between perceptions of physical training and playing stress. It is also possible the differences in positional requirements between player groups may have contributed to such findings. Higher overall work-rates have been traditionally observed in the forward player group during game play (Roberts et al., 2008, Deutsch et al., 1998; Cunniffe et al., 2009). Furthermore, forward play is generally more formalised and structured without the same degree of open play normally associated with back playing positions. It is possible that repetitive daily participation in game related technical work coupled with high game related stress could have contributed to these findings. Regardless of such heightened feelings of frustration and emotional exhaustion in the forward playing group, this did not seem to affect their confidence to perform or their self-assessed ability to prepare and train well. This was reflected via increased values for self-efficacy with season progression.

Despite increased levels of ‘emotional exhaustion’, it should be noted that summated stress scores were lower in forwards compared to backs in the second half of the season. This was contrary to our expectations with total stress scores higher (P < 0.05) in backs during weeks 14 (September), 38 (February) and 41 (March). Accordingly, total recovery scores were lower in backs (P < 0.05) during week 38 (February). It is difficult to ascertain as to why this may have occurred given that no distinguishable differences were noted in training activity between groups. It has been previously suggested that in back players, acute mental fatigue is influenced mainly by the degree of energy metabolism, as their major activity during a match is running (Mashiko et al., 2005). Alternatively, in forwards, mental fatigue is likely to be influenced by high frequency of intense muscular impacts in addition to running (Mashiko et al., 2005). Therefore, it is possible that variations in the physical demands of game play, not measured directly in the current study, could have accounted for these observed positional differences. However one cannot discount other potential explanations which may have
supported these findings such as positional differences in appraisal of stress or coping responses to game related stress and pressures.

Aside from notable increases in stress-recovery balance during weeks 28 and 38, a general decrease was observed throughout the season (Figure 6.27). It is possible that this was a result of increased training tolerance or adaptation to training stress across the season. Interestingly, these findings mirror those found in athletes using more objective markers of physical stress and strain with repeated exercise and this concept has been termed the ‘repeated bout effect’ (Clarkson et al. 1987). Although exploratory, it is possible that as long as training monotony is avoided and athlete association with a sport is maintained, a similar scenario may exist in athlete appraisal of stress. With respect to observed increases in stress-recovery balance during weeks 28 and 38, indicative of reduced recovery; these time points occurred at the end of December and February/early March. It is possible that either of two scenarios may have contributed to these findings. Firstly, as mentioned above, both time points occurred on the back of intensified training periods where a break in game fixtures (November and February) was met with a corresponding increase in training activity. Previous studies have shown that increases in training volume are associated with paralleled elevations in stress and lowered levels of recovery (Kellmann et al., 1997; Kellmann and Gunter, 2000). Therefore, present findings may be indicative of residual type stress and fatigue which was not resolved on resumption to normal game activity. Alternatively, both points may have represented acute stress reactions to increased competition around Christmas time and again, in late February/early March. Of interest, the increased stress state observed during week 38 (late February-early March) also corresponded with the clubs participation in the semi-final of the Anglo-Welsh cup for that year. It is therefore likely that a combination of both residual type stress and acute reactions to ongoing game activity scenarios may have occurred.

Previous longitudinal studies have shown that the RESTQ-Sport is a sensitive instrument for monitoring stress and recovery processes in training camps (Kellmann et al., 1997). The instrument is thought to indicate the extent to which someone is physically and/or mentally stressed, as well as whether that person is capable of using individual strategies for recovery (Bouget et al., 2006). To the authors knowledge, this is the first study to attempt to monitor changes in stress and recovery in a group of team sport athletes over a competitive season. Time of questionnaire administration, interval length and recovery from previous exercise (minimum 48 hours) were kept consistent throughout the investigation. However since measurements were only made every four weeks, care should be taken when interpreting
data. RESTQ profiles reflect a relatively short period in an athlete’s life, which may change significantly within a few days. Although an evaluation of 3 days and nights may reflect artefacts of previous training history and provide a reasonable snapshot of monthly stress-recovery balance, future studies should attempt to monitor frequent changes in stress-recovery state over longer periods in an athlete’s life. Conversely, it may be proposed that too frequent exposure of this questionnaire to athletes over a competitive season may have resulted in recall bias and thus confounded study goals. Therefore future administration should be done with both scenarios in mind. Possible changes may also be associated with other extraneous factors within the training environment such as pressures of team selection, loss of form, injury, playing experience and training monotony.
Chapter 7

General Discussion

The aim of this chapter is to integrate findings from all the studies in this thesis. The first section, general discussion, will review and discuss the main findings of the previous chapters. The second section will highlight some of the strengths and limitations of the research studies while sections three and four will attempt to summarise the main study findings and offer some practical recommendations and future research for fitness coaches and administrators working in the game.

7.1 GENERAL DISCUSSION

Findings from study one shows that an acute game of international rugby union elicits considerable increases in markers of both muscle damage and inflammation. As observed previously, the magnitude of this response is associated with blunt traumas and game collisions (tackles, ruck, mauls) received by players during play (Smart et al., 2008). Interestingly, significant decreases in the number of circulating T-cytotoxic (CD4−CD8+) lymphocytes, NK cells and functional neutrophil capacity (LPS-stimulated elastase release per neutrophil) were also observed post-exercise, all of which are indicative of decreased host immune protection. Indeed, current findings appear to support previous data (Takahashi et al., 2007) which show that blood neutrophils are highly mobilised through rugby exercise and as observed in the present study, this cell group has decreased functional capacity following a game. Given the degree of tissue damage (as defined by large increase in CK activity) in rugby and the role of blood neutrophils during inflammation, it is feasible that repeated rugby exposure could deplete the amount of mature blood neutrophils and thereby host protection. Such findings however, require further investigation. Data from both study one and two indicate that a period of 38 h is insufficient to facilitate complete resolution of muscle damage and inflammation from preceding rugby play.

In both studies, significant disturbances in anabolic-catabolic balance were observed as reflected through elevations in plasma cortisol (~40%) and corresponding decreases (~40%) in plasma testosterone. These findings corroborate those of a previous study in top level rugby
union (Elloumi et al., 2003), although increases in C level were observed longer into the recovery period in the present studies. It is possible that differences in playing standard (higher this study) and playing environment contributed to this greater stress response. Participants in studies one and two were current internationals and played against opposition ranked number 1 and 3 in the world at that time. In addition to this, both games were played and in front of over 74,000 spectators. Therefore, the changes recorded in hormonal and immunoendocrine status are likely to be extreme observations in professional team sport. Interestingly, reductions in plasma C below resting values 38 h into recovery were observed; a finding which was matched with corresponding elevated T levels. Together this data resulted in a significant increase in T/C ratio, possibly indicative of some sort of a rebound anabolic stimulus during the recovery period.

Data from both studies also indicate that players entering an international training camp had considerable tissue damage and residual inflammation following European cup rugby involvement (club rugby). In light of the fact that these players were required to play an international game against the team ranked number one in the world inside six days, such conditions were far from ideal. However, affiliated conditioning staff did seem to provide a training environment supportive of increased regeneration and recovery throughout the remaining 20 day period of investigation (study 2). This was reflected by a gradual increase in resting T/C ratio and these changes were commensurate with fitness goals for that tournament. Analysis of CK, IL-6 and blood neutrophil data showed that the magnitude of the observed stress response in rugby appears dependent on the prior degree of physical trauma and number of contact instances occurring during the game.

Data from study three revealed that players perceive the current season length as ‘too long’, ‘poorly structured’, and that these issues, as opposed to game number, are more pertinent regarding player welfare. Indeed, during the course of these investigations players from one club (region) were left in a situation whereby no games were scheduled over a 6 week period because of competing internationals (6 nations) and Anglo-Welsh cup. Consequently, they were required to play 4 games in 10 days due to a subsequent build up in game fixtures. Worryingly, the majority of players fear that the collisions they experience during the game will affect their health at some point in the future although differences were apparent between players of regional (80% REG) and international standard (65% NAT). Despite these observations, players, in the main, ‘tended to agree’ (41%) or ‘strongly agreed’ (34%) that current injury management structures are sufficient at their respective club/international...
base. However, players did report that they felt that they have ‘occasionally’ (39%) or ‘frequently’ (17%) been placed under pressure by management structures to play whilst ‘not fully fit or recovering from injury’. This is an obvious area of concern and is something that needs to be investigated further at governing body level. One of the main findings of study three was that the majority of players in both groups (80%) felt that time ‘away’ from rugby was not sufficiently long enough and would welcome the introduction of mid-season break in the region of 2 weeks duration. With respect to this, the majority of respondents felt in most need of a break leading up to/during the Christmas period.

Interestingly, these perceptions appear to concur with psychological stress-recovery data taken on a subset of thirty players over a competitive season (study 6) where a notable peak in total stress scores was observed during week 28 (end of December). It is possible that a combination of increased training volume/intensity observed 2-3 weeks prior to this period, a sudden increase in game fixtures, and heightened emotional turmoil at not being able to spend time with family and friends over the Christmas period contributed to these delayed feelings of mental and physical exhaustion. Findings from study four revealed that decreases in markers of host mucosal protection (s-IgA and s-Lys) were apparent at certain stages during the season and that these decreases were associated with corresponding increases in saliva cortisol levels. Of note, concentrations of resting saliva IgA and lysozyme were consistently lower in backs compared to their forward counterparts and that backs also displayed higher levels of the stress hormone cortisol. Of note, the mean number of recorded upper respiratory infections (URIs) were also higher in backs compared to forwards [mean ± SEM: 3.4 (0.47) vs. 4.3 (0.51)], data which is suggestive that decreases in mucosal immunity is indicative of increased predisposition to illness.

The use of an online training diary in the current thesis provided a comprehensive analysis and early identification of illness patterns in players. Data also revealed that players under-report incidences of infections (URIs and GIs) to medical staff (case study three), providing future justification for continued use and development of this monitoring system in team sport. Analysis of data also revealed that increased clusters of infections occurred at certain time periods during the season. This was particularly evident for the month of December where 25% of the squad reported the existence of a URI, a time period traditionally considered critical in terms of game number and potential success in the club calendar. Furthermore, in 23% of all URI incidences, players reported that the presence of an illness either reduced activity (14.4%) or required the need to go to bed (8.6%). In terms of squad preparation, this could impact
significantly on player availability or their ability to perform. Therefore early identification of illness and possible causative factors seem pertinent from data presented in study four. One such factor may involve the role that breaks in game fixtures have on immunoendocrine markers and illness occurrence. Analysis of daily physical activity data revealed that breaks in game fixtures during (November and February) were met with corresponding increases in player reported training intensity and conditioning type activity in club players. Incidentally, decreases in markers of mucosal protection (s-IgA and s-Lys) as well as increases in URI’s occurred 2-3 weeks after this time period. Such data point to the role of prior heavy training activity on host immune protection and the role of recovery in alleviating the adverse effects of preceding training stress. In the case of November and February, increases in training intensity were also met with decreases in the number of moderate and light training days, conditions not favourable to physiological recovery.

Data from study six showed that forwards displayed significantly higher values for ‘emotional exhaustion’ and lower scores for ‘social recovery’ than backs at regular times throughout the season. In conjunction with this, reported training intensity ratings were also higher in forwards throughout the season. Similar to previous studies (Cresswell and Eklund, 2006), these data suggest that forwards are more likely to suffer from experiential symptoms such as burnout and display higher values for frustration and mental exhaustion in comparison to their back counterparts. However, despite increased levels of ‘emotional exhaustion’, it should be noted that summated stress scores were lower in forwards when compared to backs in the second half of the season. This finding is substantiated from data in study five (same squad of players) where higher early season cortisol concentrations were observed in forwards while conversely; greater increases in mid-season (November-February) cortisol values were noted in backs. Interestingly, total recovery scores were also lower in backs (P < 0.05) during week 38 (February). Taken together, this data provides evidence that psychometric monitoring of stress-recovery balance in rugby players reflects objective hormonal stress state. This finding has been previously shown in other sports (Purge et al., 2005; Bouget et al., 2006).

Following an initial high number of complaint values over the first two months of the season; data in study five showed that the quantity of training related complaints decreased progressively with time, unlike values for GC index. Taken together, these findings suggest that although the number of training related complaints (muscle soreness, aches/pains, and sleep disturbances) ameliorates with exercise exposure; diminished resolution/increased severity of these complaints also occurs with time in certain players. It has been
suggested that not all athletes respond similarly to training (Chatard et al., 2002); some positively adapt while others exhibit signs of overreaching (Mackinnon et al., 1997). Therefore, this data possibly highlights the individual nature of training responsiveness and the existence of players unable to tolerate training loads as well as others. With respect to decreased numbers of training related complaints across the season, it is possible that increased training tolerance and the ‘repeated bout effect’ (Clarkson et al. 1987) or ‘contact adaptation’ (Hoffman et al., 2005) contributed to these findings. This finding was substantiated from squad CRP data, which was shown to decrease significantly with time.

### 7.2 STRENGTHS AND LIMITATIONS

The physiological determinants of team sport performance are not easily understood and difficulties in carrying out research in team sports athletes have been previously acknowledged (Mujika, 2007). These include issues such as quantification of training, analysis of performance, risk of injury to participants, inaccessibility to players, interindividual variability in responses and often long competitive periods within and between national and international competitions. All of the above combine to provide a challenging environment in which to carry out physiological research. Therefore, it is not surprising that such a paucity of scientific data exists in professional rugby union, something which is compounded by the logistical constraints often imposed during data collection.

With the above in mind, the current body of research can be deemed unique and is aided by the study cohort (international and elite club rugby players), a population traditionally under researched in applied sciences. Data was taken on players before and after international competition and in the case of club players, across competition cycles. This is important since real life competition elicits differing psycho physiological responses than those observed in non-competitive situations e.g. within the laboratory or similar preparatory events (Bonifazi et al., 2000; Carrasco et al., 2007). Furthermore, competition activates the sympathetic nervous (Harrison et al., 2001; Veldhuijzen van zanten et al., 2002), which is known to modulate the immune system (Madden et al., 1995); an area of key focus in the present investigation.

It is very difficult to achieve complete control of extraneous variables considering the ‘field’ based nature of this investigation. Other limitations are also apparent. In the case of the longitudinal studies, employment of a control group would have provided greater clarity
on any exercise mediated effect, as opposed to seasonal variation, on investigated psychological and immunoendocrine related variables. However, a between group factor (forwards vs. backs) was employed and this did provide greater clarity on the influence of training load and playing position on selected variables, irrespective of seasonal effects. Nevertheless, it should be conceded that this was not ideal. It is also acknowledged that analysis of changes in player performance would have helped in the evaluation of current psycho physiological state. Performance evaluation is difficult to quantify in field sports like rugby union given the varied nature of play and differences in positional demands. Additional to this were difficulties in accessing players on a regular weekly basis without causing disruption to normal training routines, time scheduling and recovery. In future, field based tests should be designed to monitor the training process in accordance with changes in player fatigue and stress state. These tests should show positive associations with changes in observed immunoendocrine or psycho-physiological markers and be easily administered on a regular basis.

Control of diet, hydration status, exercise history and training effects in the lead up to all data collection points was carried out and standardised wherever required. In addition to this, daily exercise data and training loads were taken on a squad of elite club players over the course of an entire rugby season, something which has not been done in other similar applied research studies. This, although difficult could only be achieved through continual coordination with affiliated coaches and fitness staff, something which in the authors view, is prerequisite in the success of research data in team settings. With the above in mind, the current collection of studies does allow for ‘meaningful’ data in a truly elite rugby union environment.

7.3 MAIN FINDINGS FROM THESIS:

- Game GPS data from case study one revealed that an elite rugby union player exercises at ~88% of HRmax, 80-85% VO2max, and covers a distance between 6.9-7.2 km. Estimated energy expenditure varies as per playing position, with the back expending ~6.9 MJ in comparison to the forward who expends ~8.2 MJ. Furthermore, the forward is involved in substantially greater number (n = 170) of high level impacts (7g+) than the back (n = 66).

- An acute game of international rugby union significantly decreases host immune
protection and functional neutrophil capacity (degranulation response). A period of 38 h recovery is insufficient in allowing complete resolution of biochemical disturbances resulting from a game of rugby union football.

- The magnitude of muscle damage (CK) and inflammation (CRP, IL-6, blood neutrophilia) appears dependent on the prior degree of collisions and contact instances in a game.

- Acute game play elicits large increases in cortisol (~40%) and corresponding decreases (~40%) in testosterone levels in players. The magnitude of these disturbances appears dependent on the level and intensity of game play. Data from two international games show the existence of a possible rebound anabolic-catabolic stimulus during the recovery period.

- International players entered the November series training camp with residual muscle damage and inflammation following preceding involvement in European cup rugby.

- Following entry into the international camp players showed signs of improved physical recovery and anabolic-catabolic balance (increased T/C ratio) throughout the November international series. Such changes confirm the demanding nature of participation in preceding regional rugby and the importance of modifying training loads to ameliorate residual training stress. International fitness coaches achieved this through reduction in training volume and maintenance of intensity, thereby promoting a more favourable anabolic environment as reflected by progressive increases in team T/C ratio over 3 weeks.

- Players perceive that the current season length and structure, as opposed to game number, are more important in terms of game issues affecting long term player welfare. The majority of players (80%) felt that time ‘away’ from rugby was not sufficiently long enough and would welcome the introduction of mid-season break (~2 weeks duration). Player’s felt they were most fatigued around Christmas time and the end of the season.

- Analysis of psychometric scale data revealed increased squad scores for total stress during week 28 (end of December). It is possible that a combination of preceding increases in training activity, sudden onset of game activity and reduced time with family and friends during the Christmas period contributed to this observed response.
- Forwards displayed significantly higher values for ‘emotional exhaustion’ and lower scores for ‘social recovery’ than backs at regular times throughout the season. Findings indicate that forwards are more likely to suffer from experiential symptoms such as burnout and mental exhaustion in comparison to their back counterparts. This is particularly evident during the first half of the season.

- Rugby forwards display greater low-grade inflammation (CRP) and muscle damage (CK) throughout a season. This is most likely a direct result of positional requirements and number of contact instances during play.

- Lower concentrations of mucosal immune markers, namely s-IgA (27%) and s-Lys (31%) were observed in rugby backs compared to forwards throughout a rugby season. Greater cortisol levels and illness incidence in this player group is suggestive that stress mediated decreases in mucosal immunity is predictive of increased predisposition to URIs.

- Analysis of a seasons training data revealed that breaks in game fixtures during November and February were met with corresponding increases in training intensity, decreased number of moderate and light training days and increases in conditioning type activity. Observed decreases in markers of mucosal immunity and increased URIs 2-3 weeks following this implicate the role of heavy training in illness occurrence.

- Comparison of medical reporting methods revealed that players under-report incidences of illness (GI’s and URI’s) to medical staff and that a web-based training diary permits more comprehensive analysis of illness data in rugby players.

- Squad decreases in residual inflammation across the season were coupled with a corresponding decrease in the number of training related complaints. This is suggestive of a ‘repeated-bout’ effect or ‘contact adaptation’ in rugby union.

7.5 PRACTICAL SUGGESTIONS AND FUTURE RESEARCH

- Early identification of illnesses is crucial in team sport and avoiding further exacerbation of illness symptoms. Use of an online training diary, as used the present investigations significantly helps in identification of ‘at risk’ individuals and avoids under-reporting of medical and training data.
Clusters of infections seem to occur following periods of reduced game time and increased training loads. Greater knowledge of individual player tolerance to training loads and intensity should help in minimising the adverse effects of increased loading on players, in particular during periods such as November and February.

Based on data from studies one and two, players participating in European club competition the weekend prior to entering the international training camp (November) showed signs of residual fatigue, muscle damage and inflammation. Such a scenario is not ideal if players are expected to play an international rugby game inside 7 days. Authorities should look into the feasibility of either limiting game number during the November series or allow players sufficient recovery before joining up for international duty.

Findings from studies one and two indicate that increased anabolic-catabolic balance was evident throughout player’s time in the international set up. This was commensurate with fitness goals and based around decreases in training volume. Such data can reassure coaches that player involvement in international duty is not always detrimental, at least in terms of recovery time and its effect on player’s hormonal state. Futures studies should attempt to model training loads which are supportive of increase anabolic state and ultimate regeneration. The feasibility of maximising a potential rebound anabolic effect of rugby should be further investigated.

Exercise data taken from elite club players across the season revealed that a break in competitive fixtures was met with corresponding increases in training load and intensity. This had adverse effects of psychological, hormonal and illness state. Strategies aimed at ameliorating the adverse effects of increased training loads in this player group should be investigated.

Given the elite level of the players analysed in this thesis, added difficulties were presented in terms of study preparation and data collection. These included the sometimes chaotic atmosphere that surrounded game play, collection of data immediately post games and balancing of study goals with those of players needs (e.g. rehydrating post games, or being attended by medical personal). Together these factors did not promote situations whereby data collection was easily accomplished. However, a unique opportunity of unprecedented
cooperation and genuine interest in study goals was presented to us by the Wales Rugby Union. The opportunity to gain access to elite rugby union players and collect invasive physiological data was one of the main strengths of the current investigation. It is hoped that the studies included in this thesis will help both the Wales Rugby Union and greater rugby fraternity in understanding many of the physiological demands within the sport, of which appears to be vastly changing with time.
Chapter 8
Supporting Case Studies
Case Study One: Determination of an appropriate saliva collection method for analysis of human salivary lysozyme (s-Lys)

SUMMARY
Lysozyme has been identified as a stress biomarker in addition to its function in local immunological defence. With the above in mind, analysis of saliva lysozyme (s-Lys) in athletes may help in the evaluation of training stress and its possible effects on local immune protection following periods of heavy training. The objective of this study was to determine an appropriate saliva collection method for analysis of s-Lys concentration. Six healthy males were asked to produce 10 ml of whole unstimulated saliva via passive ‘drool’ in a resting condition. Expectorated saliva was subsequently divided into pre-weighed vials corresponding to volumes 0.4, 0.7, 1, 2, and 3 ml. The remainder of saliva (3-4 ml) acted as the study control (swab ‘free’ sample). Pre-weighed cotton ‘salivette’ swabs were immersed in each vial [2 min] and mixed [shaker 500 rpm] before being removed and subsequently centrifuged (1500 g x 15 min). Samples were re-weighed to determine saliva release and stored (-80ºC) before subsequent analysis. Exposure of resting whole unstimulated saliva to the cotton based salivette material profoundly affected s-Lys concentration (P < 0.05). Mean s-Lys concentrations using the ‘salivette’ swab method were 77% lower than corresponding values collected via the passive ‘drool’ method. Furthermore, the ability of the swab to release saliva for subsequent analysis was found to be diminished with smaller saliva volumes. In conclusion, collection of saliva for s-Lys determination using cotton based swabs significantly effects resultant s-Lys concentrations. Future use of this collection method should be done so with caution.

INTRODUCTION
Saliva is frequently used as a diagnostic fluid and in many cases, provides a cheaper, less intrusive and more convenient method of assessing biochemical/immunological status in subjects when compared to blood based methods. Indeed, the use of saliva as a diagnostic alternative has increased markedly over the past decade (Streckfus and Bigler 2002; Soo-Quee Koh, and Choon-Huat Koh 2007). However, varying saliva collection methods have been used which can provide difficulties when interpreting data and for cross study comparisons. Traditionally, studies have used either of two collection techniques, (a) manual collection method via expectoration of saliva into a sterile vial (passive ‘drool’ method) (Neville et al., 2008; Allgrove et al., 2008; Koch et al., 2007; Sari-Sarraf et al., 2008; Nieman et al., 2006; Li and Gleeson, 2005; Bishop et al., 2000; Oliver et al., 2007) or (b), use of the cotton swab (‘salivette’ method) (Whitham et al., 2006; Walsh et al., 2004; Pacque et al., 2007; Nakamura et al., 2006; Brenner et al., 2000; Phillips et al., 2006; Klentrou et al., 2002; Ring et al., 2005; Akimoto et al., 2003). When using the ‘salivette’ method, saliva is absorbed into the swab within the subject’s mouth before centrifugation to release saliva for subsequent analysis. Swab saturation may be achieved via chewing on the swab or passively, by placing the swab under the subjects tongue. Both collection methods (passive ‘drool’ and ‘salivette’) may or may not require timing of saliva during collection. This depends on whether the selected variable is knowingly dependent on changes in salivary flow, although standardisation of
collection time is normally considered best practice given the known diurnal variations in saliva secretion (Qvarnstrom et al., 2008).

Generally, collection of saliva via use of the ‘salivette’ represents an easy and convenient method in studies with large subject numbers. However use of this method has been shown to affect certain salivary biomarkers when analysed via immunoassay (Shirtcliff et al., 2001; Minetto et al., 2007). With this in mind, studies have investigated the effect of saliva collection method (passive ‘drool’ and ‘salivette’) on salivary biomarkers such as cortisol (Strazdins et al., 2005; Poll et al., 2007), testosterone (Granger et al., 1994; Shirtcliff et al., 2001), alpha-amylase (DeCaro, 2008), dehydroepiandrosterone (Gallagher et al. 2006), interleukin-6 (Minetto et al., 2007), and s-IgA (Strazdins et al., 2005; Li and Gleeson 2003). In the latter study, it was found that exposure of saliva to the cotton based ‘salivette’ resulted in 24%, 16% and 12% lower concentrations for total protein, s-IgA and amylase in saliva. Conversely, cortisol values were shown to be 33% higher (than in the control sample) (Li and Gleeson 2003).

Lysozyme is one of the main enzymes of the non-specific mucosal immune defence system and is thought to display various antimicrobial properties (Meyer and Zechel, 2001; West et al., 2006). Lysozyme is secreted locally (Wagner and Wagnerova, 1989) and is produced by mucous membranes and mononuclear cells entering the oral cavity through gingival crevices (Perera et al., 1998). Salivary lysozyme (s-Lys) has been previously used as a stress biomarker (Perera et al., 1997; Yang et al., 2002), and may provide an adjunctive marker of mucosal defence along with salivary immunoglobulin A (s-IgA). Serum lysozyme has been implicated in impaired glucose metabolism (Zheng et al., 2001), a contributory factor for endothelial dysfunction. Recently, salivary lysozyme has been associated with coronary heart disease (Janket et al., 2006), endothelial dysfunction (Janket et al., 2008), and in the development of hypertension (Qvarnstrom et al., 2008). Despite the apparent importance of this marker in overall systemic health, to the author’s knowledge, no study to date has examined the effect of using cotton based ‘salivettes’ during collection and analysis of this enzyme. Furthermore, it is not known whether variation in the volume of saliva in contact with the salivette i.e. variation in saliva flow, effects the rate of lysozyme release from the cotton salivette swab. This is something which needs to be considered given that large inter-individual variations in saliva flow-rate have been reported (Walsh et al., 2004). Furthermore, under/oversaturation of the swab may potentially occur due to variation in duration of the saliva collection protocol. With the above in mind, it is feasible that variations in the swab saturation could affect biomarker release and overall concentration of s-Lys present in recovered saliva.
The purpose of the present investigation was to (a) determine the effect of saliva collection method on s-Lys concentration, (b) whether or not changes in salivary volume effects recovery of saliva and s-Lys concentrations indirectly through its effect on ‘salivette’ saturation, and (c) if a change in centrifugation time effects release of saliva from the collection swab. This study was deemed important given the relevant costs involved for s-Lys determination, for future studies and with respect to cross-study comparisons.

MATERIALS AND METHODS

Six recreationally active male subjects (mean ± SEM: age 26.1 ± 0.9 yrs; body mass 74.2 ± 2.9 kg; \( V_{O_{2\text{max}}} \) 50.8 ± 1.7 ml/kg/min) agreed to participate in the experimental trial. Before testing, ethical approval was granted by the University of Glamorgan Research Ethics Committee. All subjects were apparently healthy, non-smokers and not on present medication. In the lead up to the investigation, the subjects were asked to refrain from caffeine consumption and intense exercise for periods of 12 h and 24 h respectively. This was so as to standardise conditions between subjects. At the time of experimentation, all subjects were free of any known colds/flu’s/infectious episodes. Data collection took place between 1-2 pm and no saliva collection lasted longer than 30 min.

Subjects were asked to refrain from food consumption 2 hours before saliva collection. On entry to the laboratory, they provided a single saliva sample via the passive ‘drool’ method after 15 minutes of seated rest. During this time subjects were asked to rinse out their mouths with sterile water prior (~10 min) to collection of saliva. Subjects were also asked to swallow any residual saliva present in the mouth before ‘drooling’ into a pre-weighed plastic sterile vial (30 ml; Sterilin, UK) until they provided approximately 10 ml of saliva. The time taken to produce this saliva volume was recorded by the experimenter (portable stopwatch). Saliva volume was obtained by weighing to the nearest milligram (mg), given that saliva density is assumed to be 1.00 g/ml (Navazesh and Christensen, 1982). From this, saliva flow (ml/min) was determined.

Collected saliva was mixed (via vortex) before being divided into appropriate aliquots (volumes: 3, 2, 1, 0.7, 0.4 ml) and dispensed into pre-labelled vials (5 ml Bijou tubes). The remaining volume of saliva (3-4 ml) was placed into another vial marked as control (swab ‘free’ sample). In order to establish the possible effects of the swab exposure on s-Lys concentration and salivary volume on subsequent release of s-Lys, cotton salivette (Sarstedt Ltd, Leicester, UK) swabs (diameter 1 cm, length 4 cm) were placed in each of the pre-
marked vials (except control). These vials were then placed on a shaker for mixing (500 rpm x 2 min). The swabs where subsequently removed from each vial with a sterile tweezers and placed into the upper drawer of the salivette container (Sarstedt Ltd, Leicester, UK). These tubes (including control) were then centrifuged on two occasions, firstly for 5 min (1500 g, 18ºC) and secondly, for a further 10 min (1500 g, 18ºC; 15 min total duration). This was to determine the possible effect of centrifugation time on release of saliva from the swab (saliva drains from the upper drawer of the salivette container into the bottom holder upon spinning). The control vial and bottom holders of each salivette tube were weighed before and after centrifugation (to determine swab release) before being stored at -80ºC for future analysis.

On the day of analysis all saliva samples were allowed to thaw (1 h, 20ºC) before being centrifuged (1500 g x 5 min) to remove any debris. The clear supernatant was then diluted (1/1000, PBS) before samples were analysed using a commercial sandwich Human Lysozyme EIA kit (Biomedical Technologies Inc, Stoughton, MA, USA). Full details of s-Lys analysis is documented in section 5.4.2. All samples were analysed in duplicate, within the same assay run (intra-assay CV: 5.7%; dynamic range 0.78 - 50 pg/ml) and read at 450 nm.

**Statistical analysis**

Data is presented as mean values and standard errors of mean (± SEM). All data was checked for normality (Shapiro-Wilk test) and homogeneity of variance before statistical analysis. Following assumption of normality, the data were examined using repeated measures one way ANOVA and paired t-tests. Significance was set at P < 0.05 for analyses which were performed using SPSS version 14.0 for Windows.

**RESULTS**

Subjects took 18.25 ± 2.71 min to produce 11.16 ± 0.13 ml of saliva; corresponding to saliva flow rates of 0.54 ± 0.10 ml/min (mean ± SEM). The percentage of saliva not absorbed by the salivette swab was low (< 1% for saliva volumes 0.4-3 ml) while the percentage of saliva not recovered from the swabs was greater with lower initial saliva volumes (Table 8.1). In the case of 0.4 ml, 37% of saliva was not released following centrifugation; no differences observed for centrifuge time. The amount of saliva retained in the swab was not a constant percentage/amount of the initial saliva sample volume. Saliva volumes of 2-3 ml resulted in the greatest recoverability of saliva obtained for analysis (86.4% and 83.3% respectively; values representative of 15 min centrifugation time).
Saliva lysozyme concentration was significantly affected by exposure to the salivette swab (Fig. 8.1a; P < 0.05). Lysozyme concentration in the control (non-swab) sample was significantly higher (8.51 ± 1.85 µg/ml) than all other saliva samples exposed to the swab (mean values for volumes 0.4-3 ml; 1.96 ± 0.17 µg/ml) P < 0.01. There was a trend for lower lysozyme concentrations (non-significant) in samples with lower initial saliva volumes (Fig. 8.1b).

Prolonging centrifuge time resulted in a small (0.017 ml; 1%) but significant (P < 0.05) net increase in saliva release from the salivette swab.
Table 8.1 - Effect of saliva volume and centrifuge time on swab absorbance and swab release (data presented as mean ± SEM).

<table>
<thead>
<tr>
<th>Vial volume (ml)</th>
<th>Saliva not absorbed by swab</th>
<th>Saliva not released from swab</th>
<th>Saliva obtained for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vol (ml)</td>
<td>Percent (%)</td>
<td>5 min</td>
</tr>
<tr>
<td>3 ml</td>
<td>0.017 (0.006)</td>
<td>0.56</td>
<td>0.424 (0.041)</td>
</tr>
<tr>
<td>2 ml</td>
<td>0.007 (0.003)</td>
<td>0.34</td>
<td>0.342 (0.004)</td>
</tr>
<tr>
<td>1 ml</td>
<td>0.013 (0.007)</td>
<td>1.32</td>
<td>0.266 (0.020)</td>
</tr>
<tr>
<td>0.7 ml</td>
<td>0.004 (0.001)</td>
<td>0.61</td>
<td>0.222 (0.016)</td>
</tr>
<tr>
<td>0.4 ml</td>
<td>0.004 (0.000)</td>
<td>0.98</td>
<td>0.149 (0.018)</td>
</tr>
</tbody>
</table>

*Values representative of 15 minute centrifuge time
DISCUSSION

Despite the obvious limitations of low subject number in the current study, the observed findings do provide some insight into the effect of saliva collection method on lysozyme levels in healthy subjects. Significantly lower lysozyme values ($1.96 \pm 0.17 \, \mu\text{g/ml}$) were observed using the ‘salivette’ method when compared to saliva collected via the passive ‘drool’ method ($8.51 \pm 1.85 \, \mu\text{g/ml}$; $P < 0.01$). With this in mind, care should be taken when interpreting saliva lysozyme values from studies using swab ‘salivettes’ during sample collection.

These observed differences between collection methods cannot be resultant from between trial variations in saliva flow since all saliva samples were firstly obtained via passive ‘drool’, after which cotton swabs were immersed in pre-determined saliva volumes. It is possible that the lower concentrations of lysozyme observed using the salivette swab method were the result of interference between lysozyme and the cotton based swab itself. This cotton based interference effect has been previously reported by Shirtcliff et al. (2001) on other salivary biomarkers. Given that there was a trend for greater reductions in lysozyme values in samples with lower saliva volumes (Fig. 81b); it is possible that adhesion of lysozyme to the individual swab fibres occurred. Such findings have been previously observed in a similar study investigating the effect of saliva collection on IL-6 (Minetto et al., 2007), although values were non-significant in the present study. The exact mechanism by which this interference occurs however remains unknown. Conversely, the higher s-Lys values observed in swabs containing larger saliva volumes may have been a result of greater swab saturation. However, the percentage of saliva not absorbed by the swab was not highest in samples with the greatest saliva volume (Table. 8.1). Indeed, the percentage of saliva not absorbed by the salivette ‘swab’ was relatively low across the range of saliva volumes ($<1\%$ for saliva volumes 0.4-3 ml). Therefore, over-saturation of the cotton based swabs was not a contributory factor to this observed trend. One cannot exclude the possibility that salivettes may become over-saturated with higher initial saliva volumes i.e. > 4ml. A larger study population and collection of saliva volumes greater than 3 ml may have provided greater clarity on this issue.

However, many studies attempting to investigate changes in salivary biomarkers have traditionally employed saliva collection protocols of 2-3 minutes in duration (Li and Gleeson, 2005; Bishop et al., 2000; Walsh et al., 2004). Therefore, for values to exceed 3 ml in saliva volume, subject flow-rates would have to exceed 1-1.5 ml/min during saliva collection; values of which would be considered high in a resting healthy individual (Walsh et al., 2004).
Of more importance perhaps is the ability of salivette swabs to release the absorbed saliva for subsequent analysis. In the current study, the lower the initial saliva volume, the less recoverability (percentage released) of saliva from the swab. Indeed, with saliva volumes of 0.4 and 0.7 ml, up to 37% of saliva remained bound within the cotton ‘salivette’ swab. In such instances, recoverability of saliva was somewhat enhanced (0.017 ml; 1%) with extension of centrifuge time from 5 to 15 minutes (Table. 8.1). It is possible that other protein particles/cell debris which may have attached lysozyme, could have been filtered out by the swab with extension of centrifuge duration. Changes in centrifuge speed however were not investigated. With the above in mind, it can be concluded that salivary volumes of 2-3 ml results in the greatest recoverability of saliva with 86.4 and 83.3% respectively obtained for analysis.

To conclude, investigation of mucosal immunity and stress as viewed through saliva lysozyme requires prior consideration of appropriate sample collection technique. Collection and analysis of s-Lys via the ‘salivette’ swab results in considerably lower (77%) concentrations than saliva taken via the passive ‘drool’ method. Future studies with larger study groups need to be explored to investigate the mechanistic reasons for this observed effect. Furthermore, less recoverability of saliva was observed in samples with lower initial volumes, and therefore low saliva flow-rates. These findings are important if absolute lysozyme values are pertinent to the investigation and large variability in saliva flow-rates exist within the study population.
Case Study Two: Cross comparison of varying methods used for calculation of training load in rugby union

SUMMARY
The aim of study was to compare the session-RPE method for quantifying internal training load (TL) with previously established heart-rate (HR) based methods within a variety of training modes in elite rugby union. Session-RPE, HR and duration data was collected on 5 players across 12.0 ± 1.0 training sessions (n = 60) of varying nature and on different days over a 5 week training period. Correlation analysis was used to compare session-RPE TL’s with two other [1: Bannister’s training impulse (TRIMP); 2: Edwards summated-heart-rate-zone (SHRZ)] commonly used HR-based methods for TL assessment. Results showed significant and positive correlations for the session-RPE method with SHRZ (r = 0.88, P < 0.01) and TRIMP (r = 0.91, P < 0.01) methods respectively. Individual correlations ranged between 0.83 and 0.94 for the three TL assessment methods. When expressed relative to training mode, the strongest correlations were reported for training which contained more intense activity (conditioning, rugby play) than game specifics and/or technique work. The results of this case study in rugby support data from previous studies in other field sports that session-RPE TL compares favourably with HR-based methods for quantifying internal TL. Use of this method offers a practical and user friendly alternative for TL quantification in longitudinal investigations.

INTRODUCTION
In longitudinal studies, regular and effective monitoring of the training process has proved difficult. Very few studies have examined team sports partly because of difficulties in quantifying the diverse range of training activities such as running based conditioning, resistance training, skill drills and team drills (Gleeson et al., 2004). Therefore global quantification of training load [TL] from activities which are varied in nature and using a common unit of measurement needs to be achieved. Appropriate documentation and management of TL’s over the course of a season may help alleviate potentially harmful consequences of heavy training (Hartwig et al., 2008). Furthermore, a reliable and effective means of TL assessment in team sport may assist researchers in data collection and assessment of interventions subsequent to preliminary investigation.

Traditionally heart rate (HR) has been employed as a training tool in assessing physiological load within team sports such as soccer (McMillan et al., 2005; Stroyer et al., 2004; Impellizzeri et al., 2005; Coutts et al., 2007) and rugby (Maud, 1983; Gamble, 2004; Duthie et al., 2003; Deutsch et al., 1998). This is because HR demonstrates almost a linear relationship with \( \text{VO}_{2\text{max}} \) over a wide range of steady state sub-maximal intensities (Astrand and Rodahl, 1986). However, HR is thought to be a comparatively poor method when evaluating high intensity exercise such as weight training, intense interval training, sprinting and
plyometric training (Foster et al., 2001). In professional rugby, continual monitoring of HR in large playing squads (up to 40 players) may be costly and time consuming. Additionally players are not allowed to wear communication devices during official match play (Law 4.4. Laws of the game, IRB 2007), an activity component that represents a high percentage of overall weekly TL. Therefore, a frequent and valid measurement of TL is prerequisite in the overall monitoring of players. A simpler means of quantifying TL has been proposed by Foster (1995). This method employs modification of the traditional TRIMP (training impulse) concept developed by Bannister (1975) via use of session RPE as a marker of training intensity (Foster et al., 2001) instead of HR. Using this method, training loads are calculated by multiplying the whole session rating of perceived exertion (Borg et al., 1985) using a category ratio scale (CR10-scale) by session duration; the product of which yields a single number of internal training load in arbitrary units (AU).

The use of RPE in evaluation of physiological load is acceptable since it is scalar and integrates physiological status and homeostatic disturbances (Joesph et al., 2008). Previous research has found session-RPE to be related to the percent of HR reserve (HR<sub>r</sub>) during 30 min of steady state running and to the time spent at different intensities corresponding to HR at lactate thresholds (2.5 and 4 mmol.L<sup>-1</sup>) during 30 min of continuous and interval running (Foster et al., 1995). Other research has shown session-RPE to be significantly correlated with other HR-based methods (Edwards SHRZ method, 1993) for quantifying internal TL in endurance athletes (Foster, 1998; individual correlations, r = 0.75-0.90) and field athletes (Foster et al., 2001). Session-RPE has also been considered a good indicator of global internal load in all training types common to soccer (Impellizzeri et al, 2004; Alexiou and Coutts, 2008). In these latter studies moderate to strong individual session RPE-HR correlations with other HR-based methods range (r = 0.50-0.85) have been shown to occur. This method has also been used in other intermittent sports such as basketball (Coutts et al., 2003), rugby league (Coutts et al., 2007) and tennis (Reid et al., 2008) and appears to provide a viable method for quantifying the intensity of resistance training (Sweet et al., 2004).

No studies have investigated the use of this method in rugby union at the elite level. This is important since rugby union, like league, is a sport where a considerable amount of body load is resultant from non-aerobic exercises such as player-player contact, static exertion and anaerobic(ballistic movements during match play (Roberts et al., 2008; Duthie et al., 2005). It is feasible that estimation of physiological demands through cardiovascular and/or metabolic assessment may not provide a true reflection of global player loading. Therefore,
incorporation of subjective player assessment of bodily load may be of use in overall
calculation and categorisation of TL. With this in mind, the purpose of this study was to verify
if a session of RPE-based method of internal TL quantification provides a suitable alternative
to other HR based methods during various forms of physical activity in elite rugby union.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Session Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Rest</td>
</tr>
<tr>
<td>1</td>
<td>Very, very easy</td>
</tr>
<tr>
<td>2</td>
<td>Easy</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>5</td>
<td>Hard</td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Very Hard</td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Maximal</td>
</tr>
</tbody>
</table>

**Table 8.2:** The Borg Category Ratio-10 Rating of Perceived Exertion Scale

**METHODS**

Session RPE and HR data was collected on 5 players (age: $24.8 \pm 2.2$ yr, height: $189.1 \pm 9.2$
cm, body mass: $102.8 \pm 10.4$ kg, $\bar{VO}_{2\text{peak}} : 53.5 \pm 2.3$ mL·kg$^{-1}$·min$^{-1}$) across 60 different
sessions of varying nature on different days of the week over a 5 week pre-season training
period. Training sessions selected differed in duration and intensity and typically involved
conditioning and rugby-specific training. Conditioning sessions usually involved high-
intensity speed training with repetitive maximal sprinting over 60 m and 100 m. During these
sessions, power development also occurred and usually involved medicine ball throwing, sled
pushing/pulling and/or varying speed agility drills involving short duration 20 m sprints and
plyometrics. These sessions varied in duration depending on fitness goals devised by fitness
staff. Data was not obtained from resistance sessions. Rugby specific drills normally
incorporated general game specific principles such as attacking and clearing of rucks,
tackling, touch rugby or full contact games with protective padding. Data was only taken
from exercise sessions lasting greater than 20 min in duration. Typical conditioning sessions
lasted 20-30 min while rugby sessions lasted between 30-90 min.

Players were asked to rate training intensity 30 min after the session had ended using the CR10-scale modified by Foster et al (1995); table 8.2. All players had been familiarized with this scale before commencement of the study. HR was recorded at 1 sec intervals during each session using standard HR transmitters (Polar Electro, Kempele, Finland). Training load data collected using the session-RPE rating method was compared to Edwards (1993) and Bannisters (1991) HR-based training load methods as used in previous investigations (Impellizzeri et al., 2004; Alexiou and Coutts, 2008). The relationships between the session-RPE TL and these commonly used HR-based TL quantification methods were used to examine the criterion validity of the session-RPE.

Equations used for TL quantification:

(1) Fosters session-RPE method: Training load (AU) = Session RPE rating (0-10) x Session duration (min)

(2) Edwards summated-HR-zones (SHRZ) method: calculated by measuring the product of the accumulated training duration (mins) of 5 HR zones by a coefficient relative to each zone.

\[(\text{duration in zone 1 x 1}) + (\text{duration in zone 2 x 2}) + (\text{duration in zone 3 x 3}) + (\text{duration in zone 4 x 4}) + (\text{duration in zone 5 x 5})\]
\[\text{where zone 1 = 50-60% of HRmax = 1, 60-70% of HRmax = 2, 70-80% of HRmax = 3, 80-90% of HRmax = 4, and 90-100% of HRmax = 5).}\]

(3) Banisters’ training impulse (TRIMP) method: Training impulse = TD·HRR·0.64·e^{1.92·HRR}

where TD is session duration (min), e is the napierian log having a value of 2.712 and HRR (heart rate reserve) is determined by the following:

\[\text{HRR} = \Delta HR_{exercise}/\text{span HR}\]
\[\text{where } \Delta HR_{exercise} = HR_{exercise} - HR_{rest} \text{ and span HR} = HR_{max} - HR_{rest}\]

Prior to commencement of the study, an incremental exercise test to volitional exhaustion was carried out on squad members for estimation of maximal HR. These values were subsequently used as criterion for HRzone training load assessment. This took place before the season commenced.

Players are asked to rate the session based on physiological load and not sensations of pain as would be expected to arise from the many contact elements within the game itself. All
players were injury free through the period of investigation. Please refer to Appendix F for an example of how training load (TL) was calculated in rugby using Fosters RPE method. Before testing, ethical approval was granted by the Research Ethics committee of the University of Glamorgan. It should be noted that the current study was used to substantiate previous existing findings in intermittent sports (Alexiou and Coutts, 2008; Impellizzeri et al., 2005; Coutts et al., 2007) and to see whether similar correlations between session-RPE and HR based TL quantification existed in elite rugby union. With this in mind, a small number of subjects were recruited and so any conclusions made should take this into account.

**Statistical Analysis**

Descriptive analysis of data was used and where relevant, presented as means ± SD. After checking data for normality of distribution and homogeneity of variance, relationships between session-RPE and the HR-based TL were analysed using Pearson’s product moment correlation. Relationships were determined between each of these methods for each player and also the specific type of training completed by the players. Differences between the mean TL for each exercise type was determined using a one-way ANOVA with a post hoc test (Bonferroni) to reveal which methods were significantly different. Statistical significance was set at $P < .05$ and analysed using SPSS statistical software (version 14.0; SPSS Inc., Chicago, USA).

**RESULTS**

Data was taken on players who participated in $12.0 \pm 1.0$ training sessions. Close correlations were observed between the two HR based TL methods ($r = 0.98$, $P < 0.01$). Individual correlations of session-RPE and the two HR-based TL methods are shown in table 8.3, all of which were significant, $P < 0.01$. Strong overall relationships were observed between the session-RPE TL method and the other two ($r = 0.91$, Bannisters TRIMP; $r = 0.88$, Edwards SHRZ) HR-based TL methods. TL values were shown to differ significantly between each monitoring method ($P < 0.01$). Significant differences were also shown to occur between each monitoring method based on training type ($P < 0.01$). Post hoc analysis revealed that rugby playing loads involving player-player contact were significantly higher than game specifics/technical sessions, conditioning and other skill based sessions.
<table>
<thead>
<tr>
<th>Subject</th>
<th>N</th>
<th>RPE</th>
<th>Bannister’s TRIMP</th>
<th>Edwards TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>-----</td>
<td>0.92</td>
<td>0.89</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>-----</td>
<td>0.85</td>
<td>0.86</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>-----</td>
<td>0.94</td>
<td>0.93</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-----</td>
<td>0.90</td>
<td>0.85</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>-----</td>
<td>0.92</td>
<td>0.87</td>
</tr>
</tbody>
</table>

| Mean ± SD | 0.91 ± 0.03 | 0.88 ± 0.03 |
| Range     | 0.85-0.94   | 0.87-0.93   |
| 95% CI    | (0.84-0.97) | (0.83-0.92) |

**Table 8.3:** Mean group correlations between session-RPE TL and HR-based TL assessment. All correlations were significant (P < 0.01).

Higher correlations between session-RPE TL and other HR-based TL were observed for general conditioning work (r = 0.90-0.94) and rugby play (r = 0.68-0.74). The poorest correlations were observed for game specifics and skill sessions (r = 0.21-0.25).

**DISCUSSION**

Any absolute training stimulus has been suggested to pose a higher relative physiological strain on athletes with lower physiological capabilities (Gabbett, 2004). Therefore, within team sports like rugby union, one player’s evaluation of training activity may be substantially different from another’s, despite undergoing the same training stress. With this in mind, the assessment of internal TL, which requires quantification of the intensity and duration of the physiological stress imposed on the athlete (Foster et al., 1995) offers a practical tool in team TL assessment.

In the current study, attempts were made to compare session-RPE derived TL’s with other HR-based TL’s in a variety of different training modes in elite rugby union. Results showed significant and positive correlations between the session-RPE method and the other HR-based methods. The mean correlations between the HR-based TL methods and RPE are higher than those reported in recent studies in soccer (Alexiou and Coutts, 2008; Impellizzeri et al., 2005), rugby league (Coutts et al., 2007) and physically active men and women (Borresen and Lambert, 2008). As expected, training load values using the RPE-based method were
consistently higher than observed TL’s using Edwards SHRZ method. It has been previously suggested that HR is a comparatively poor method when evaluating very high intensity exercise such as weight training, high intensity interval training, and plyometric training (Foster et al., 2001). Furthermore, although both methods are similar in terms of TL calculation, both employ different intensity rating zones during calculation (HR = 1-5; RPE = 1-10) and as such, are not interchangeable because of scale variance.

Stronger correlations between the session-based RPE method and the HR-based methods were observed for general ‘conditioning’ work and rugby play. Poor correlations were observed during training sessions involving skills, phase play and technique. The reduced strength of these correlations may have been due to the low intensity nature of these activities. This is something which may not register as being stressful using the traditional HR-based methods, but instead given the duration, may have disproportionately increased TL values using the session-RPE method. Therefore care should be taken when interpreting data. Furthermore, there is a chance that player’s may over or under report loads depending on what they want or expect coaching staff to expect. With the above in mind, use of internal load reporting should be periodically validated against other objective method for external load quantification such as HR assessment and GPS monitoring. It has been previously suggested that muscularly strong individuals are comparatively poor at rating the intensity of aerobic exercise sessions, attending more to muscular tension than to sensations of dyspnea (Foster et al., 2001). Although comparative data to other athletic groups was not the focus of this investigation, it should be noted that small between player variations were observed for session-RPE TL estimation (range 0.85-0.94 for Bannisters TRIMP; 0.87-0.93 for Edwards SHRZ) TL methods. This within group consistency (rugby union players) is perhaps not surprising since the subjects were elite players and would be expected to be aware of acute changes in exercise intensity given their heavy and varied daily training schedule. Furthermore all players had been used to reporting training intensity via self-reporting in the lead up to the investigation. However such findings are made in light of small subject number employed in the current investigation which was an obvious limitation.

Use of RPE based TL load assessment may have many advantages in the assessment of physical state in team sports. This is because extrinsic factors such as varying levels of fitness, illness and/or psychological status could influence internal training loads; factors not always addressed or picked up by more objective HR-based methods. Indeed, it has been previously shown that the combination of HR and blood lactate predicts RPE more
accurately than either variable taken alone (Borg et al., 1985). Furthermore, subjective categorisation and weighting of drills employed by coaching staff may mask the training effort that formalised or planned exercise training has on the athlete. Therefore, monitoring and controlling of an athlete’s internal training load is very important to ensure each athlete receives adequate training stimulus (Impellizzeri et al., 2004). In rugby union, this may prove advantageous with respect to periodisation and planning of training loads, player welfare and overall player preparation. Also, use of this system may be valuable in identifying players who are overreached or at risk of overtraining since RPE for a given HR has been shown to increase during overreaching (Martin and Anderson, 2000). While session-RPE does seem to offer a practical non-expensive tool in training load calculation, limitations using this method and others should be acknowledged. Like Edward’s SHRZ TL method, the session-RPE method provides intensity weightings in a linear fashion. This is important since linear weighting of HR zones does not reflect the physiological responses above anaerobic threshold (Wasserman, 1987). Furthermore, Bannister’s TRIMP TL method utilizes mean exercise HR, something which may conceal important elevations in HR within stochastic sport (Stagno et al., 2007).

In summary, accurate calculation of TL in team athletes is not without its problems. Recent studies have pointed to the use of individual player training zone weighting from laboratory data (Stagno et al., 2007). While intriguing, these methods are nevertheless, time consuming when attempting to assess TL in large team squads over long durations. Given the moderate to strong correlations observed between the session-RPE TL method and the other HR-based methods, the present study suggests that the session-RPE method is a good practical method for quantifying internal TL in rugby union.
Case Study Three: Illness monitoring in team sports using a web-based training diary

SUMMARY
The use of web-based data recording systems has received little attention in sport. An ‘online’ training diary could provide a valuable alternative to pen-paper methods in the regular assessment of physical activity and illness occurrence in athletes. Given previous associations between heavy exercise and incidence of infection, the objective of this study was to design a user-friendly and efficient system to monitor illness occurrence in professional rugby union. Illness data was collected prospectively on a squad of 30 professional rugby union players (age, 26 years; height, 186 cm; mass, 101 kg; \( \text{VO}_{2\text{peak}} \), 52 ml.kg\(^{-1}\).min\(^{-1}\)) throughout a 48-week rugby season. Players were asked to register presence/absence of weekly illness symptoms with medical staff and also on a web based training diary. Submitted self-reported diary illness data was then compared to illness complaint data recorded by medical staff. Diary compliance in the reporting of weekly illness was 79% over the study period. A greater number of upper respiratory illnesses [URIs] were reported by players to the web diary (n = 118) than to medical staff (n = 23). Of the total number of URI episodes, 95.9% were registered on the web diary while 18.7% were recorded by medical staff. In conclusion, current findings suggest that professional rugby union players tend to under-report incidence of banal infections. Closer monitoring of self-reported illness using a similar system to the present study may provide a better alternative to previous methods in non-clinical illness assessment.

INTRODUCTION
Several case histories have been published demonstrating sudden and unexplained deterioration in athletic performance which can be traced to a recent upper respiratory illness (URI) or subclinical viral infection running a protracted course (Parker et al., 1996; Friman et al., 1998). In a recent longitudinal study on elite yachtsmen, incidence of URIs were found to account for 60% of days absent from sailing due to illness (Neville et al., 2006). Occurrence of banal infections has also been suggested as an early marker of overtraining (Foster et al., 1997; Lehmann et al., 1993) and overtrained athletes tend to report a greater number of recurring infections (Budgett, 1998). Furthermore, exercise training during the most virulent stage of an illness has been shown to exacerbate symptom severity in animals (Gross et al., 1998) and depending on viral cause, increase the risk of myocarditis (Roberts, 1986). Therefore, close monitoring of self-reported illness may be important in evaluation of the training process and assessment of athlete well-being.

Traditionally the prevalence and characterisation of upper respiratory illness in sport has been assessed through the use of health logbooks (Nieman et al., 1990; Klentrou et al., 2002; Novas et al., 2002; Fricker et al., 2005; Tiollier et al., 2005; Fahlman et al., 2005; Kostka et al., 2008), questionnaires/report forms (Neville et al., 2006; Peters and Bateman, 1983; Spence et al., 2007), or by subject recall (Nieman, 1994; Matthews et al., 2002). A few
studies have employed the use of physician/medical examinations (Tiollier et al., 2005; Gomez-Merino et al., 2005; Neville et al., 2008), or clinical verification of underlying illness (Spence et al., 2007; Cox et al., 2004; Gleeson et al., 2004). To the author’s knowledge, no studies have assessed illness incidence in elite athletes using a ‘web’ based data logging system. The use of web-based technology in the collection of data has so far received little attention in elite sport. This is despite rapid growth of this data collection tool in areas such as medical research and nursing (Ahern, 2005). A user may easily send data back to a designated server, which can process, code, filter, and save data electronically (Birnbaum, 2004). This has several advantages. In a recent study comparing ‘online’ and traditional ‘pen-paper’ questionnaires in athletes, Lonsdale et al. (2006) showed a better response rate in the ‘online’ group (57%) compared to the postal group (46%). Furthermore, online questionnaires were returned faster, contained fewer mistakes and missing entries. In longitudinal research studies, often involving multiple data sampling points; pen-paper methods can be off-putting and lack visual appeal. In addition to this, subjects may be more honest when reporting undesirable behaviour to a computer than on paper (Millstein, 1987; Skinner and Allen, 1983). Such shortcomings may be negated via use of web-based data technology. Data can be inputted directly and stored in electronic format, therefore reducing the time taken to transfer data from a questionnaire onto a computer for future analysis. Furthermore, the athlete may be ‘present’ or ‘away’ from training headquarters, therefore increasing data registration and overall diary compliance. At an elite level, this makes monitoring of illness in athletes possible when they are away during international competition or absent due to injury.

The aim of the current study was to investigate the potential use of ‘web’ based technology in the collection of illness data over a competitive season within elite team-sport athletes. It was hoped that the ‘online’ nature of the diary would provide an administrative friendly and effective alternative to traditional methods in the characterisation of illness.

METHODS

Subjects and Research design
Following university ethical permission, data was collected on a squad (n = 30) of professional rugby union players contracted to a top European club side participating in the Celtic league, Anglo-Welsh cup and European cup. Player characteristics are described in table 8.4. Before entry into the study, players were asked to complete a health-screen
questionnaire. This was to determine history of known allergies which may have effected interpretation of reported URIs. Each player provided written informed consent prior to study entry. Data was collected prospectively over the course of a competitive (2005-2006) season, starting from pre-season (July) to June of the following year (48-weeks in total). The current study formed part of a larger investigation examining the effects of training stress on markers of immune function and illness occurrence in rugby union. However, for purposes of clarity, and in line with study goals, details of exercise load and its possible interaction with illness will not be discussed in this paper.

**Table 8.1:** Player (n = 30) characteristics as per positional group, forwards (n = 16) and backs (n = 14). $\dot{V}O_{peak}$, Peak oxygen uptake expressed per kilogram body mass; HRmax, maximum heart rate. Values expressed as mean ± SEM

<table>
<thead>
<tr>
<th></th>
<th>Forwards</th>
<th>Backs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>26.8 ± 0.9</td>
<td>25.9 ± 0.9</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>112 ± 2.6</td>
<td>91 ± 2.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>188.3 ± 1.7</td>
<td>182.6 ± 2.4</td>
</tr>
<tr>
<td>$\dot{V}O_{peak}$ (ml.kg$^{-1}$.min$^{-1}$)</td>
<td>50.4 ± 1.3</td>
<td>55.2 ± 1.4</td>
</tr>
<tr>
<td>HRmax (beats.min$^{-1}$)</td>
<td>184 ± 1.7</td>
<td>186 ± 2.5</td>
</tr>
</tbody>
</table>

**Description of Player-diary**

The diary was developed in HTML-format and PHP-scripting language (as internet web pages) for use on computers with operating system Windows ‘98 and higher. In addition to monitoring changes in illness incidence, the diary was designed to monitor player activity levels and training loads across the rugby year (not detailed in this paper). The web diary was set up 2-months prior to study commencement so that the majority of players were familiar with system requirements. At the start of investigation, all players were provided with an individual user-name and password and the software prevented entry of existing name files. Any new player entering the club was provided with user training on how to operate and submit data. Players were asked to enter presence/absence of weekly illness symptoms on a self-report illness log (Figure 8.2) contained within the web-based diary database. They were also given the opportunity to practice data entry during the initial testing week (week-1) and to resolve queries relating to data submission. The illness report form appeared every Tuesday and Wednesday of each week upon players logging into the system. Where
possible, all players were instructed to enter illness data on the Tuesday. In the event of a player failing to do this, a system built diary reminder was automatically sent to both the player and affiliated fitness/medical staff. The player then had further 12-hours to submit the data before it disappeared until the following week. In the event of no illness or physical complaints being present, players were instructed to enter the diary without submitting illness symptom data. This enabled the system to recognise player registration and ensure that no symptoms were present for that time. Players were also instructed to submit data while on international duty or absent from the team training-base due to competition, injury/illness or non-selection.

Data input was in the form of drop down menus. Aside from registration of illness data, players were presented with a series of accessory items which required submission of data upon diary log-in. These included fatigue ratings, current body-weight, mood-state, and training activity/loads. Illness data were saved in electronic form to the server and grouped by completion date. A file containing player name and time of entry was also stored on the system and a training summary for each player was available to fitness staff for each day/week/month of interest. Raw data was exportable to Microsoft excel (Microsoft Corporation, USA) for further analysis. No attempt was made by investigators to alter training loads or activity levels during the course of the investigation.

**Illness incidence and classification**

An upper respiratory illness (URI) was noted as having occurred when symptoms coding for a cold or flu were recorded on two or more consecutive days. If players failed to note any of these symptoms in the presence of non-illness related symptoms e.g. joints aches/pains, sneezing; no URI symptoms were noted. This was to exclude the possibility of over-reporting due to residual musculoskeletal complaints from training. An asymptomatic period of at least 7-days was required for a subsequent episode to be classified as a ‘new’ illness. In the event of a reported URI extending over this period, without the player reporting absence/cessation of original symptoms, a continuation/re-occurrence or complication of the original illness was noted. Duration of symptoms was recorded until symptom cessation or if players failed to report two or more symptoms over two consecutive days. Presence/absence of gastro-intestinal complaints was also noted and recorded within the ‘other illness’ section of the illness log. Presence of a GI illness was based on the player having reported at least one of the following symptoms: nausea, vomiting, and/or diarrhoea. Clinical verification of illness was not carried out in the current study.
Diary control

The diary was in a standard order format so that players could not neglect certain sections of the diary. Players were not able to view summary sheets and unable to view other teammates data on the diary. Registration (diary) data for each player was available to selected staff. In the event of a player failing to enter illness data, an ‘auto-alert’ system was employed. This alerted the player in question and fitness staff of data omission upon future diary log-in.

Treatment of data

Along with self-reporting of diary illness data, players were asked to inform medical staff of any illness presence across the study duration. Submitted diary data was subsequently compared to illness complaint data recorded by affiliated club medical staff. Given the nature of the study, the majority of data are of a descriptive nature only. Where warranted, data are expressed as mean values and standard error of the mean (SEM). Following assumption of normality, evaluation of between group (player position) differences in illness incidence was carried out using an independent student’s t test. Significant was set at \( P < 0.05 \). Diary compliance was calculated from the number of completed diary entries against the number of available/required entries over the season.

RESULTS

Illness data

There was 79% compliance in the reporting of weekly illness using the web diary across the 48-week period. A total of 141 URI reports were registered using both methods [web diary: \( n = 118 \); medical reporting: \( n = 23 \)]. When taking into account the number of duplicate URI entries, a total of 123 URI episodes were recorded. This resulted in a mean incidence of 4.1 [\( \text{min} = 0; \text{max} = 8 \)] URIs per player while 92% of players experienced at least one URI over the season of investigation. Recorded number of URI episodes appeared higher in backs than forwards [\( \text{mean} \pm \text{SEM}: 3.4 \pm 0.47 \) vs \( 4.3 \pm 0.51 \); forwards vs backs], although this finding was non significant (\( P = 0.08 \)). Average duration of reported URIs lasted 4.5 days (\( \text{min} = 2; \text{max} = 33 \)). A total of 23 URIs were registered by medical staff, which accounted for 18.7% of the total number of URI episodes. In turn, 95.9% (\( n = 118 \)) of all URI episodes were reported to the web-based diary (Table 8.5). Of those URIs reported to the web diary, 18 (15.3%) were also registered by medical staff. A total of 5 (21.7%) URIs registered by team
medics were not reported by players to the web based diary while 100 (84.7%) URIs recorded on the web diary were not reported to the team medical staff. Conversely, of those URIs reported to medical staff, 78.3% were also reported by players on the web diary.

Table 8.5: Breakdown of upper respiratory illness (URI) number registered using either of two methods; those reported to (a) web diary and (b) team medics across the season (n = 48 weeks). Percentage of respective URI totals is also displayed.

<table>
<thead>
<tr>
<th>Web based reporting</th>
<th>Reported</th>
<th>Not reported</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported</td>
<td>18</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Medical based reporting</td>
<td>100</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>5</td>
<td>123</td>
</tr>
</tbody>
</table>

* Percent of total URIs reported to medics
* Percent of total URIs collected on web diary
* Percent of total URI episodes

Table 8.6: Breakdown of gastrointestinal illness (GI) number registered using either of two methods; those reported to (a) web diary and (b) team medics across the season (n = 48 weeks). Percentage of respective GI totals is also displayed.

<table>
<thead>
<tr>
<th>Web based reporting</th>
<th>Reported</th>
<th>Not reported</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reported</td>
<td>13</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Medical based reporting</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>3</td>
<td>25</td>
</tr>
</tbody>
</table>

* Percent of total GIs reported to medics
* Percent of total GIs collected on web diary
* Percent of total GI episodes
A total of 38 gastrointestinal illnesses (GIs) were registered using both methods [web diary: $n = 22$; medical reporting: $n = 16$]. When taking into account the number of duplicate GI entries, a total of 25 GI episodes were recorded. This resulted in a mean incidence of 0.8 [min = 0; max = 3] GIs per player while 40% of players reported at least one GI complaint over the period of investigation. A total of 16 GIs were registered by medical staff which accounted for 64.0% of the total number of GI episodes. In turn, 88.0% ($n = 22$) of all GI episodes were reported by players to the web diary (Table 8.6). Of those GIs reported to the diary, 13 (59.1%) were also reported by players to medical staff. A total of 3 (18.8%) GIs reported to team medics were not reported to the web based diary while 9 (40.9%) GIs recorded on the web diary were not reported to medical staff. Conversely, of those GIs reported to medical staff, 13 (81.3%) were also reported by players to the web diary.
### Weekly Illness Form

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Present</th>
<th>Symptom severity:</th>
<th>No. days that symptoms persisted.</th>
<th>Normal Daily Activity:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Yes</td>
<td>Severe</td>
<td>1</td>
<td>Went to Bed</td>
</tr>
<tr>
<td>Persistent muscle soreness or tenderness (&gt;than 8h)</td>
<td>No</td>
<td>Light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sore, painful throat</td>
<td>No</td>
<td>Light</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Catarrh (runny or viscous fluid) in the throat</td>
<td>Yes</td>
<td>Moderate</td>
<td>3</td>
<td>Normal</td>
</tr>
<tr>
<td>Runny nose</td>
<td>Yes</td>
<td>Moderate</td>
<td>3</td>
<td>Normal</td>
</tr>
<tr>
<td>Cough</td>
<td>No</td>
<td>Light</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Repetitive sneezing</td>
<td>No</td>
<td>Light</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Joint aches and pains</td>
<td>Yes</td>
<td>Moderate</td>
<td>2</td>
<td>Reduced</td>
</tr>
<tr>
<td>Weakness/fatigue</td>
<td>No</td>
<td>Light</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>No</td>
<td>Light</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Loss of sleep</td>
<td>Yes</td>
<td>Moderate</td>
<td>2</td>
<td>Reduced</td>
</tr>
<tr>
<td>Headache</td>
<td>No</td>
<td>Light</td>
<td></td>
<td>Normal</td>
</tr>
</tbody>
</table>

If you have had any of the above symptoms please fill in the details below.

- **Have you taken any medication?**
  - Yes
  - If Yes please specify: paracetamol
- **Seen your doctor/GP?**
  - No
- **Any other illness not on the form please add here:**

**Figure 8.2:** Graphical readout of the ‘online diary’: presence/absence, duration and severity (function of impact on athletic performance) of upper respiratory illness (URI).
DISCUSSION

Weekly self-reporting of illness has been previously employed in the assessment of performance outcomes in elite international athletes (Pyne et al., 2005). To the author’s knowledge, this is the first study to use a web based diary as a tool in gathering illness data on a cohort of elite team-sport athletes (professional rugby union players).

Findings revealed that players reported an average of 4 URIs over the competitive season, although the impact of illness on performance was not considered in the present investigation. While over-reporting of illness may have been a factor, this is unlikely given the criteria involved in overall URI classification. Other studies have reported that up to four upper respiratory tract infections (URTI’s) would be considered normal (per given year) in clinical practice (Gleeson et al., 1999). Such data would suggest that the present athlete group do not suffer from a greater occurrence of upper respiratory illnesses than those individuals within the general population. It should be noted however that considerable variability existed in the number of URIs reported (range 0-8); ranges which were similar for each playing group (forwards and backs). Interestingly, the recorded number of URI episodes differed for player group with the ‘backs’ experiencing on average, one more URI (4.3 URI’s) during the season compared to their forward (3.4 URI’s) counterparts. It should be noted that the latter finding is preliminary and requires further investigation with larger subject numbers. Nevertheless these findings do suggest the possible existence of certain ‘at risk’ players/groups who were more prone to illness than others and confirm previous findings proposing that ‘practical strategies for limiting the risk of illness are needed on a case by case basis’ (Pyne et al., 2005).

Although clinical verification of illness was not performed in the current study, use of a similar web-based system should be considered in future studies despite the self-reported nature of data. The ability of individuals to provide subjective assessment of underlying disease pathology seems to be reliable. Research has shown that athletes, as well as the general population, are quite able to correctly diagnose symptoms of a URTI such as running nose, sore throat, and cough in combination with fatigue, headache and fever (Cohen et al., 1991). Also, following viral inoculation, research has shown the magnitude of impairments in muscle strength to be significantly correlated with the individuals own ratings of subjective symptoms (Friman et al., 1985). These studies provide foundation for the use of self-reporting in illness assessment and as such, this method may represent a practical tool in the medical
management of elite athletes. In the present study, 78.3% of URIs registered by medical staff were also reported by players to the web diary. However, considerably more URIs were reported to the computer diary (n = 118) than to medical staff (n = 23) with 84.7% of URIs recorded on the web diary not reported by players to the club medical staff. Data also revealed that the web diary accounted for 95.9% of all recorded URI episodes in comparison to 18.7% which were registered by medical staff. It is possible that these findings may have occurred due to a number of reasons.

Previous research has shown subjects to be more honest in reporting undesirable behaviour to a computer than using paper-pencil format (Millstein, 1987; Skinner and Allen, 1983). It is also feasible that players did not wish to disclose illnesses directly to fitness staff for reasons such as appearing fragile or because of perceived influence on team selection. Alternatively, players only felt the need to disclose the presence of a URI when severe enough to warrant medication and/or a decrease/absence from training. This is perhaps why more occurring GIs (64.0%) were reported to medical staff than URIs (18.7%). Typical URIs are self-limiting in nature (Fricker et al., 2005) and do not usually warrant medication in the absence of a complication, extended duration or known bacterial cause (Fahey et al., 1998). Furthermore, URIs are mild in symptom severity and have been suggested to have a minimal impact on physical performance (Fricker et al., 2005), something perhaps in contrast to the aetiology of a typical GI infection. Therefore, the recorded number of URIs reported by players to fitness staff in the present study may have represented those requiring an absence from training or further treatment. A combination of the above is the most probable scenario.

In the current study, 92% of players experienced at least one URI over the 48-week season of investigation; findings which are similar to those reported previously (Fricker et al., 2000). The players under-reported incidences of banal infections to medical staff suggesting that assessment criteria involving times when an athlete requires medication and/or missed a training session (Neville et al., 2008; 2006) may underestimate total incidence of URIs. This may also hide the possibility of ‘at risk’ individuals and partly explain why the incidence of illness in the current study (4.1 URIs) was higher than those recently reported in elite yachtmen’s (2.7 URIs) (Neville et al., 2008), despite similar study durations. Irrespective of illness severity, a more prudent protocol would be for athletes to disclose all illness presence to medical staff. Unresolved viral infections are not routinely assessed in elite athletes (Reid et al., 2004), and as observed in the current study, under-reported by athletes to medical staff. Exercise training during the most virulent stage of an illness has been shown exacerbate
symptom severity in horses (Gross et al., 1998) and depending on viral cause; increase the chance of myocarditis (Roberts, 1986). Furthermore, a sore throat, which is often a symptom of a URI warrants close attention as one of its causes, infectious mononucleosis, carries serious risks with exercise. One such risk is potential splenic rupture (Metz, 2003), and although very uncommon should precipitate prudent monitoring processes given the physical nature of rugby. Therefore, close observation of self-reported illness using a similar system in the present study may provide a better alternative to previous methods in non-clinical illness assessment. This may help fitness staff in identifying illness-prone individuals, those displaying symptoms below the neck, and those players with prolonged symptom duration. Appropriate remediation strategies may then be implemented and tailored to the individual athlete.

Regular monitoring of athletes within elite team sport environments provides many difficulties to both researchers and fitness staff alike. In rugby, absence from squads due to international representation, or injury can result in loss of players from the monitoring process. This limits study power and provides complications when making conclusions about the training. In the present study, the use of an online training diary proved to be a valuable tool in the collection of illness data. Although previous longitudinal studies have investigated the occurrence of illness in athletes using self-reported methods; (Klentrou et al., 2002; Fricker et al., 2005; Fahlman et al., 2005; Kostka et al., 2008) few have documented the response rates of athletes with diary completion. In an acute study, Nieman et al. (1990) reported that 46.9% of illness questionnaires mailed to finishers of the Los-Angles marathon were returned. In the present study, reporting of weekly illness data by players to the diary was 79%. Given the longitudinal nature of the present investigation (48 weeks), these diary compliance rates were deemed highly acceptable.

Practical Applications

While investigation of illness distribution or its association with training load was not the focus of this study, future studies should attempt to investigate such using a similar ‘web’ based system. As the current study was prospective and monitoring in nature, interventions were not considered so as to confound study goals. Future implementation of ‘diary illness alerts’ may notify medical personal of illness ‘prone’ athletes, those displaying signs of systemic illness as well as those showing a large number of missed training days. Furthermore, monitoring of other non-injury stressors such as mood disturbances and changes in performance may provide adjunctive tools in player management and help
practitioners in deciding if interventions are required. System development may subsequently aid tailoring of athlete training programs and help in medical evaluation. Given the relative success and appropriate levels of player compliance in the current study, future development of web-based data collection methods within elite sport represents a realistic goal.

Conclusions

The observed response rates and diary completion time (less than 3 minutes per day) seem to confirm the beneficial use of this system in the medical management of athletes within elite sport. Furthermore, the ability to incorporate a vast array of traditional pen-paper questionnaires on the same system is intriguing. Athletes tend to under-report incidence of illness to medical staff and this may hide certain ‘at risk’ players within the training process. Assessment of illness using an ‘online’ diary seems to provide greater resolution on prospective illness rates than data traditionally obtained using medical reports or pen-paper logbooks.
AN EVALUATION OF THE PHYSIOLOGICAL DEMANDS OF ELITE RUGBY UNION USING GLOBAL POSITIONING SYSTEM TRACKING SOFTWARE

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ABSTRACT

Cunniiffe, B., Proctor, W., Baker, J., and Davies, B. An evaluation of the physiological demands of elite rugby union using GPS tracking software. J Strength Cond Res 23(4): 600–600, 2009—The current case study attempted to document the contemporary demands of elite rugby union. Players (n = 2) were tracked continuously during a competitive team selection game using Global Positioning System (GPS) software. Data revealed that players covered on average 6,953 m during play (83 minutes). Of this distance, 37% (2,600 m) was spent standing and walking, 27% (1,900 m) jogging, 10% (700 m) cruising, 14% (950 m) striding, 5% (320 m) high-intensity running, and 6% (420 m) sprinting. Greater running distances were observed for both players (6.7% back; 10% forward) in the second half of the game. Positional data revealed that the back performed a greater number of sprints (> 20 km h⁻¹) than the forward (34 vs. 19) during the game. Conversely, the forward entered the lower speed zone (5–12 km h⁻¹) on a greater number of occasions than the back (515 vs. 226) but spent less time standing and walking (66.5 vs. 77.8%). Players were found to perform 87 moderate-intensity runs (>14 km h⁻¹) covering an average distance of 19.7 m (SD = 14.8). Average distances of 15.3 m (back) and 17.3 m (forward) were recorded for each sprint burst (>20 km h⁻¹), respectively. Players exercised at ~80 to 85% V0₂ max during the course of the game with a mean heart rate of 172 b min⁻¹ (~68% HRmax). This corresponded to an estimated energy expenditure of 6.9 and 8.2 MJ, back and forward, respectively. The current study provides insight into the intense and physical nature of elite rugby using "on the field" assessment of physical exertion. Future use of this technology may help practitioners in design and implementation of individual position-specific training programs with appropriate management of player exercise load.

KEY WORDS: demands, game analysis, GPS technology, rugby

INTRODUCTION

Rugby union is an intermittent high-intensity sport, in which activities that call for maximal strength and power are interspersed with periods of lower-intensity aerobic activity and rest (26). Until recently, it has not been possible to collect objective data on player work rates in situ other than via use of heart rate (HR) monitoring. Traditionally, the majority of studies have investigated game demands in rugby union through time-motion analysis systems incorporating the use of game video recordings (24,9,10,14,30). Problems using video recordings may occur however as a result of errors involved in categorization of locomotor activity. This is important because rugby union is a dynamic intermittent sport with many gain changes during game phases. Furthermore, notational analysis systems are largely dependent on trained users, and considerable subjectivity may exist when interpreting data. Therefore, accurate performance assessment may be technically difficult given the complex interactions of players and the varied nature of game play.

With the development of Global Positioning System (GPS) technology for use in sport, investigators can now evaluate training loads and activity profiles of players on the field. This is achieved via use of portable tracking devices, which permit quantitative measurement of activity profiles through traditional GPS triangulation methods and accelerometer software. Positional data are normally achieved by comparing the signal travel time of radio frequency signals sent from the orbiting satellite and the GPS receiver worn by the player/athlete. The distance to the satellite is then calculated by multiplying the signal travel time with the speed of light. By calculating the distance to at least 4 satellites, the exact position can be trigonometrically determined (19). Changes in speed (velocity) are usually determined via the
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Supporting Case Studies

Doppler shift method, that is, measurements of the changes in signal frequency due to movement of the receiver. With this, an opportunity exists to gain valuable data on game demands in team sports like rugby union through objective distance and speed calculations not previously available using the same recording system. Through investigation of game demands, training methods can be targeted to mimic the positional requirements and physiological loads, thus optimizing player conditioning to the energy demands of the sport.

"On the field" GPS technology has been used previously in Australian Football League to profile positional demands and enhance knowledge on injury potential. To the author’s knowledge, these data have not been published within scientific literature. Limited data using this type of technology exist in rugby union despite its initial use within domestic southern hemisphere rugby. Furthermore, evaluation of game demands at the elite level is currently difficult as such communication devices are not allowed during competitive league and cup games.

With this in mind, the aim of this pilot study was to gather information on rugby union forward and back play at the elite level and demonstrate the potential use of GPS technology in the assessment of the games’ physiological demands. It should be noted that this study focuses on a small subject number, the goal being to provide some insight into the contemporary demands of rugby union with an additional focus on future research. An improved understanding of the game and training leads may help facilitate best practice advice for player management issues and appropriate training periodization.

**Accuracy of Global Positioning System Technology**

Limited data exist on the accuracy and reliability of current GPS software estimation of true distance and velocity. In a study using a generic GPS logger, it has been stated that errors in data logging may increase during activity over circular paths most likely because of underestimations in speed (35). A recent study comparing current GPS software with timing gates in soccer demonstrated that both methods produced comparable speed and distance data in a linear running protocol (28). In the same study, it was concluded that GPS data recording at 1 Hz seemed appropriate for calculating distance at lower velocities but that greater error in estimation may occur at higher velocities. The accuracy of this system in calculation of distances traversed has been previously shown to be within 4.8 (15) and c<1% (22) in estimation of true distance measured using a treadmill. In the latter study, GPS technology was shown to be accurate in the assessment of speed (within 0.01 m s<sup>-1</sup> of true value).

Limited data exist on accuracy of GPS in calculation of distance and speed in field activities requiring repeated changes in running intensity.

**METHODS**

**Experimental Approach to the Problem:**

To examine game play at the elite level, data in this study were taken from players during an out-of-season competitive 89-minute game. This involved 2 teams normally participating in the Celtic (Magpies) League and Guiness Premiership, respectively. Both leagues represent the highest standard of club play among the Celtic nations (Ireland, Scotland, and Wales) and England.

**Subjects**

Data were obtained from 3 home team players (age: 25.0 ± 3.6 years; weight: 104.6 ± 10.4 kg; height: 193.3 ± 9.4 cm; V<sub>0</sub>max: 53.3 ± 21.6 ml.min<sup>-1</sup>kg<sup>-1</sup>; mean ± SD). These included 1 back (out-half) and 2 forwards (lock, prop, and hook). However, as one of the players (lock forward) only participated in a quarter of the game, discussion will concentrate on the 2 players with full data sets. Forward and back players were selected in an attempt to investigate game play differences for different playing positions. Before participation in the study, players provided informed consent and were made aware of their ability to withdraw from testing at any time. Ethical procedures for the study were obtained from the University of Glamorgan Ethics Committee. All players were fully habituated and familiarized with the data collection systems. This was done on several occasions during training sessions before the actual game itself.

**Procedures**

Players were asked to wear an individual GPS unit (mass: 89g; dimensions: 91 x 45 x 21 mm) excised within a protective harness between the player’s shoulder blades in the upper thoracic spine region (estimated). Players also wore a HR transmitter belt (Polar Electro, Kempele, Finland) to incorporate HR data. This was recorded synchronously (1-second interval) with the GPS tracking device (SPL Elite; GPSports Systems, Canberra, Australian Capital Territory, Australia). Devices were switched off 5 minutes before the start of the game and turned off immediately after the game had ended. Data stored included time, velocity (calculated via Doppler shift), distance, position, direction, HR, and number and intensity of player impacts as measured in ‘g’ force. GPS data were recorded at 1 Hz and accelerometry (triaxis) data at 100 Hz, respectively. After collection, data were downloaded to a personal computer where further analysis was carried out via use of the system software provided by the manufacturer (Team AMS; GPSports, V1.2).

**Measures**

**Heart Rate and Locomotor Activity:** Recorded game HRRs were categorized into 6 HR zones based on each player’s known maximum HR (HRmax) monitored using an incremental treadmill running test in the laboratory. HR zones were as follows: (a) 0 to 60% HRmax, (b) 60 to 70% HRmax, (c) 70 to 80% HRmax, (d) 80 to 90% HRmax, (e) 90 to 98% HRmax, and (f) 95 to 100% HRmax. Total values for HR exertion were achieved using a similar weighting system carried out by Edwards (16).

Frequency and duration of locomotor efforts were evaluated from the time spent in 6 player speed zones. Allocations
of speed zones were those thought typical of varying locomotor categories during intermittent team sport. These were as follows: (a) standing and walking (0.6 km h⁻¹), (b) jogging (6-12 km h⁻¹), (c) cruising (12-14 km h⁻¹), (d) striding (14-18 km h⁻¹), (e) high-intensity running (18-29 km h⁻¹), and (f) sprinting (>29 km h⁻¹). The above categories were later divided into 2 further locomotor categories to provide a crude estimate of player work to rest ratios: (a) low-intensity activity (0-8 km h⁻¹) and (b) moderate- and high-intensity activity (>8 km h⁻¹). This categorization was based on data obtained from a similar previous study in Australian rules football using GPS software (34).

Body Load and Game Impacts. Player impact data (intensity, number, and distribution) were gathered from accelerometer data provided in “g” force. Intensity of impacts was graded according to the following scaling system provided by system manufacturers: 5-6g: light impact, hard acceleration/deceleration/change of direction; 6-6.5g: light to moderate impact (player collision, contact with ground); 6.5-7g: moderate to heavy impact (tackle); 7-7.5g: heavy impact (tackle); 8-10g: very heavy impact (serious engagement, tackle); and 10g: severe impact/tackle/collision.

Computation of player body load during exercise also involved use of the above acceleration zone forces. Body load was calculated automatically using the system software provided by the manufacturers.

Estimation of Energy Expenditure. Information regarding estimation of energy expenditure (EE) was obtained from continuous measurement of HR during the game. In turn, corresponding values for VO₂ were estimated from player’s individual HR-VO₂ relationships (2), which were obtained during a standard incremental running protocol (VO₂max test) collected on the players in our laboratory. Rates of energy expenditure were subsequently calculated using methods similar to those previously shown in soccer (3,17) and rugby league (8). Non-playing periods (halftime, warm-up, and cooldown) were omitted from all analyzed GPS and energy expenditure data.

Statistical Analyses. Given the nature of the present investigation and small subject number, data presented below are of a descriptive nature only. Where appropriate, the duration for each activity is presented as the mean and SD.

RESULTS

Heart Rate. Players recorded mean and peak HRs of 172 and 209 b min⁻¹, respectively, during the game (Figure 1). Both players reached prestablished maximum heart rate (HRMAX) during the game. The back spent more time at 80 to 95% HRMAX (42% of heart rate) (27.7%), whereas the forward spent more time at heart rate HRMAX (15.4%) of the back (47.2%) (Table 4). Mean HR was higher in the first half than the second half (173 vs. 169 b min⁻¹; Table 1).

Motion Analysis. During the game time of 83 minutes, 72% was spent standing and walking, 18.6% jogging, 3.3% cruising, 3.8% striding, 1% high-intensity running, and 1.2% sprinting (mean data for both players). These values represent a work to rest ratio of 1.5:7. Players covered on average 6,953 m during the game (Table 1). Of this distance, 37% (~2,800 m) was spent standing and walking, 27% (~1,900 m) jogging, 10% (~700 m) striding 1% (~500 m) striding, 5% (~320 m) high-intensity running, and 6% (~420 m) sprinting (Table 6). The majority of moderate to intense accelerations occurred over running intervals of 4 to 6 seconds with little difference between player positions.

Acceleration data (Table 2) refer to the number of times the players changed velocity in defined categories over 1-second time intervals. Changes in velocity over 1.5, 2.0, 2.5, and 2.75 m·s⁻¹ correspond to changes in running speed of 5.4, 7.2, 9, and 10 km h⁻¹ in 1-second intervals, respectively.

During the game, the players encompassed 712 changes in tempo, occurring approximately every 3 to 4 seconds. The back entered the high-speed zone (>20 km h⁻¹) on a greater number of occasions (34 vs. 19) than the forward (Table 7). In turn, the forward entered the lower speed zone (6-12 km h⁻¹)
on a greater number of occasions than the back (315 vs. 229) but spent less time standing and walking than the back (66.5 vs. 75.0%).

Players reached maximum speeds of 28.7 km·h⁻¹ (back) and 26.3 km·h⁻¹ (forward), respectively. Peak speeds for both players occurred during the second half (Table 2). Both players' work to rest ratios (average, 1.57) were also lower during the second half (Table 1), indicating less recovery time (i.e., time spent below 8 km·h⁻¹) between play periods. Average player running speed over the game was 4.2 km·h⁻¹, values greater during the second half for both players (Table 2). Within half comparisons revealed that values for maximum speed, average speed, total distance covered, and peak HR were higher at the start of the each playing (20 minutes) quarter (Q) such that values for Q1 > Q2 and Q3 > Q4 (both players). Cross-quarter comparisons revealed that values for the above variables were highest during the third quarter of the game, that is, first 20 minutes after halftime.

### Body Load and Game Impacts

Both players received a large number of impacts during the game with positional differences observed between the number of impacts received by the back and forward (798 vs. 1,274). Grouping of game impacts within the latter 3 categories (heavy + very heavy + severe) revealed that the forward was involved in 69% more high-level impacts than the back (Table 3). Furthermore, 66% of the high-level impacts received by the forward occurred during the second half. This resulted in greater overall body load and body load per minute for the forward player (Table 3).

### Discussion

To the author's knowledge, this is the first study to evaluate player demands during a competitive game of rugby union using objective "on the field" software. During the game, players covered an average distance of 6,953 m (83.7 m·min⁻¹) (Table 1). These values are less than distances...
Table 3. Game impact and body load data per position and half.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Back</th>
<th>Forward</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light impact (5-8g)</td>
<td>349 (168; 169)</td>
<td>563 (201; 317)</td>
</tr>
<tr>
<td>Light/moderate (6-6.5g)</td>
<td>328 (152; 171)</td>
<td>346 (174; 188)</td>
</tr>
<tr>
<td>Moderate-heavy (6.5-7g)</td>
<td>55 (25; 30)</td>
<td>143 (37; 97)</td>
</tr>
<tr>
<td>Heavy (7-8g)</td>
<td>38 (15; 23)</td>
<td>101 (24; 71)</td>
</tr>
<tr>
<td>Very heavy (8-10g)</td>
<td>24 (15; 9)</td>
<td>56 (19; 35)</td>
</tr>
<tr>
<td>Severe (10+g)</td>
<td>4 (2; 2)</td>
<td>15 (8; 7)</td>
</tr>
<tr>
<td>Total no. of impacts</td>
<td>798 (377; 401)</td>
<td>1,274 (461; 715)</td>
</tr>
<tr>
<td>Total body load (AU)</td>
<td>31,402 (16,483; 14,372)</td>
<td>119,103 (35,477; 78,058)</td>
</tr>
<tr>
<td>Body load/min</td>
<td>375 (392; 344)</td>
<td>1,426 (845; 1,883)</td>
</tr>
</tbody>
</table>

*AU = arbitrary units.
**Values inside parentheses are those for first and second halves, respectively.

*reported in professional soccer players (118 ± 7.5 m-min⁻¹) using similar GPS technology (1) but greater than previous estimations of running distance in rugby union (10,30). Similar to the study by Roberts et al., the present data suggest that backs travel greater total distances during a game than their forward counterparts (7.6% further in the current study). Interestingly, both players recorded greater running distances in the second half of the game (6.7% back, 10% forward), indicating that deterioration in running ability or, perhaps, depletion of energy reserves was not an issue in this player group. During game activity, players performed 87 moderate-intensity runs (>14 km h⁻¹) (18) over an average distance of 19.7 ± 14.6 m. Along with running a greater total distance, the back entered the high-speed zone (>20 km h⁻¹) on a greater number of occasions (34 vs. 19; Table 7) than the forward. The back also covered a greater total distance sprinting (>20 km h⁻¹, 524 vs 315 m) when compared with the forward. Not surprisingly, values for total sprinting distance observed in the current study (elite senior players) are substantially greater than those reported previously in elite U19 rugby for backs (253 ± 45 m) and forwards (94 ± 27 m) (30). Overall, the data would suggest that backs participate in a greater amount of higher intensity locomotor work when compared with forwards, although the forward was found to cover a greater average distance per sprint burst activity (15.3 m back, 12.3 m forward) in the present investigation.

Several studies have demonstrated that estimated total work performed (quantified by HR and movement patterns) is lower for backs than forwards (24,10,11). This is thought to occur despite the fact that forwards spend more time in the lower speed zones. Data in the present study revealed that the forward entered the lower speed zone (6-12 km h⁻¹) on a greater number of occasions than the back (315 vs. 229; Table 4).

Table 4. Percent (%) time spent in each HR zone.

<table>
<thead>
<tr>
<th>Percent time per HR zone</th>
<th>Back</th>
<th>Forward</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1: 0–60% HRmax</td>
<td>0 (0; 0)</td>
<td>1.8 (3.4; 0.1)</td>
</tr>
<tr>
<td>Zone 2: 60–70% HRmax</td>
<td>2.5 (2.0; 5.9)</td>
<td>3.7 (3.2; 4.8)</td>
</tr>
<tr>
<td>Zone 3: 70–90% HRmax</td>
<td>13.9 (5.3; 25.2)</td>
<td>15.7 (18.2; 18.5)</td>
</tr>
<tr>
<td>Zone 4: 80–90% HRmax</td>
<td>42.2 (45.6; 50.1)</td>
<td>27.7 (33.7; 30.6)</td>
</tr>
<tr>
<td>Zone 5: 90–95% HRmax</td>
<td>36.7 (38.4; 19.4)</td>
<td>35.7 (27.2; 26.0)</td>
</tr>
<tr>
<td>Zone 6: 95–100% HRmax</td>
<td>4.7 (8.7; 2.3)</td>
<td>16.4 (18.4; 18.1)</td>
</tr>
</tbody>
</table>

*HR = heart rate.
**Values inside parentheses are values representative for first and second halves, respectively.
Table 5. Percent (%), time spent by players in each speed zone.∗

<table>
<thead>
<tr>
<th>Percent time within speed zone</th>
<th>Player position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Back</td>
</tr>
<tr>
<td>Standing and walking: 0–6 km h⁻¹</td>
<td>77.8 (76; 72.8)</td>
</tr>
<tr>
<td>Jogging: 6–12 km h⁻¹</td>
<td>16 (12.8; 18.8)</td>
</tr>
<tr>
<td>Cruising: 12–14 km h⁻¹</td>
<td>3.1 (3.6; 3.7)</td>
</tr>
<tr>
<td>Striding: 14–18 km h⁻¹</td>
<td>3.7 (4.8; 4.0)</td>
</tr>
<tr>
<td>High-intensity running: 18–20 km h⁻¹</td>
<td>0.9 (1.3; 0.6)</td>
</tr>
<tr>
<td>Sprinting: &gt;20 km h⁻¹</td>
<td>1.4 (1.8; 1.6)</td>
</tr>
</tbody>
</table>

∗Values inside parentheses are those for first and second halves, respectively.

Table 7) but spent less time standing and walking than the back (66.5 vs. 77.8% of total time; Table 5). It should however be noted that the percentage of time or effort exerted by the forwards in static activity and tackling was not measured in the current study and such activity would be anticipated to significantly contribute to game workload in this player group (30). Overall, the above findings demonstrate that the back participated in more anaerobic high intensity activity interspersed with longer recovery periods in the lowest speed zones, whereas the forwards spent more time in the moderate speed zones as recovery time between high-intensity activities. This may have implications for positional specific training requirements.

The typical sprint distances of 15 to 20 m in the current study and number of intense accelerations (Table 2) imply that the ability to accelerate quickly is highly important within professional rugby union. Of interest, the majority of intense accelerations did not occur from standing starts (0 km·h⁻¹), implying that quick changes in player running gait are of essence in game performance. During the game, approximately 10% of game time was spent performing intense locomotor activity. This corroborates previous studies using less objective methods of analysis (9,10). Because the current study did not include time spent performing intense game-specific efforts and utility movements, it is likely that this figure is greater in modern day rugby union. Nevertheless, the longest continuous time recorded above speeds of 29 km·h⁻¹ was just 7 seconds (46.6 m), with the majority of high-intensity work periods below 6 to 7 seconds in duration. These data support the previous findings where typical sprint distances of just 10 to 20 m have been shown (10,11). Such findings point to the contribution of the anaerobic energy system, in particular the phosphocreatine system during high-intensity activity of which is interspersed with long periods of lower intensity activity, primarily aerobic in nature. Interestingly, there was a general trend for both players’ speed and distance to decrease after intense 5-minute play periods. It is not known what effect, if any, interval or aerobic type training may have on repetitiveness of intense efforts during rugby play as measured through GPS technology. This is important because improved aerobic fitness has been implicated in sprint and

Table 6. Distance (meters) covered by each player in designated speed zones.∗

<table>
<thead>
<tr>
<th>Distance (m) within speed zone</th>
<th>Player position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Back</td>
</tr>
<tr>
<td>Standing and walking: 0–6 km h⁻¹</td>
<td>2,802 (1,247; 1,314)</td>
</tr>
<tr>
<td>Jogging: 6–12 km h⁻¹</td>
<td>1,956 (794; 1,054)</td>
</tr>
<tr>
<td>Cruising: 12–14 km h⁻¹</td>
<td>978 (392; 392)</td>
</tr>
<tr>
<td>Striding: 14–18 km h⁻¹</td>
<td>978 (332; 439)</td>
</tr>
<tr>
<td>High-intensity running: 18–20 km h⁻¹</td>
<td>202 (172; 120)</td>
</tr>
<tr>
<td>Sprinting: &gt;20 km h⁻¹</td>
<td>524 (241; 283)</td>
</tr>
</tbody>
</table>

∗Values inside parentheses are those for first and second halves, respectively.
Table 7. Number of entries/surges by players into each speed zone.*

<table>
<thead>
<tr>
<th>Speed Zone</th>
<th>No. of surges</th>
<th>Player position</th>
<th>Back</th>
<th>Forward</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surge below 8 km h⁻¹</td>
<td>207 (88, 111)</td>
<td></td>
<td>229 (133, 159)</td>
<td>315 (132, 159)</td>
</tr>
<tr>
<td>Surge between 6 and 12 km h⁻¹</td>
<td>123 (64, 66)</td>
<td></td>
<td>179 (83, 61)</td>
<td>105 (41, 61)</td>
</tr>
<tr>
<td>Surge between 12 and 14 km h⁻¹</td>
<td>116 (63, 51)</td>
<td></td>
<td>179 (83, 61)</td>
<td>105 (41, 61)</td>
</tr>
<tr>
<td>Surge between 14 and 16 km h⁻¹</td>
<td>43 (26, 17)</td>
<td></td>
<td>48 (20, 24)</td>
<td>19 (10, 9)</td>
</tr>
<tr>
<td>Surge above 20 km h⁻¹</td>
<td>34 (20, 14)</td>
<td></td>
<td>19 (10, 9)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers of surges per speed zone are not exclusive locomotor efforts but rather the number of times players reached speeds above designated speed interval.

recovery and fatigue resistance (6,25,32,33). Future investigation using a larger data set on typical work rates in elite rugby union may be of interest. With this, it may be possible to determine minimal requirements in aerobic fitness and role of VO₂ kinetics in recovery from short-term anaerobic efforts.

Previous research has shown that despite a disparity in distances (assessed through 10-minute intervals) covered between game halves, a rugby player’s ability to perform high-intensity activity with increasing game duration is not limited (20). In the present study, total running distance, peak running speed, number of peak accelerations, average speed, and peak HR were all greater during the second 40-minute play period. Cross-quarter (~20 minutes) comparisons revealed the third playing quarter (after halftime) to be most intense. This was reflected by lower work to rest ratios and high-intensity (>18 km h⁻¹) running distance per minute. Interestingly, these results compare favorably with previous work, which has shown that most injuries occur in the third quarter of the game (5). These data suggest that player fatigue was not a factor between halves. Indeed, of the high-level impacts undertaken by the forward, 60% of them occurred during the second half of play. Furthermore, players reached maximum speeds of 287 km h⁻¹ (back) and 263 km h⁻¹ (forward), both of which occurred during the second half. Expressing these values as a percentage of individual peak running speed (measured using GPS 2 weeks before the study) revealed that both players were capable of reaching 92% of their peak running speed. This shows that players do reach maximum levels of locomotor activity during rugby play despite previous game activity.

In this study, the average work to rest ratio during the game was 1.5:7, indicating that for every 1 minute of running, there was almost 6 minutes of lower intensity activity. Figures drop for both players during the second half, indicating that play periods were more frequent with less recovery (Table 1). Although work to rest ratios provide important information on demands of the sport, in the case of rugby union, player work to rest ratios calculated from player locomotor activity may underestimate actual work time. Considerable time spent in specific game-related phases such as pushing/pulling in rucks/mauls/scrums may register as low-intensity activity using current GPS technology despite intense static player efforts. Although the above ratio provides information on the intermittent nature of elite rugby union, it may not provide a true reflection of player work rates, in particular for forwards. Combining objective GPS data with qualitative analysis of time spent in non-running exertion and utility movements may help in establishing more defined work to rest ratios and setting of fitness goals.

Although the game in the present investigation was outside normal competition, it was played between 2 of the top sides normally participating in the Celtic League and Guinness Premiership. The game occurred at the end of pre-season training before commencement of the regular club season. Both teams contained a large number of first-team regulars, were evenly matched, and the game served as an important element in seasonal preparation. Therefore, data, although limited by subject number, do provide some insight into game demands at the top level in European rugby. Our results suggest that players exercise at ~80 to 85% VO₂max (Table 8) during the course of the game. This is similar to those reported in rugby league (~80% VO₂max) (6) and higher than values observed in Gaelic footballers (~75% VO₂max) (29) during competitive matches. It is possible that elevations in HR may have overpredicted aerobic demand because it has been suggested that changes in HR may not accurately reflect changes in energy cost occurring over short-term high-intensity activities (20). Factors other than oxygen uptake such as environmental temperature, emotions, continuity of exercise, and perhaps more importantly muscle actions and body position can influence HR response to exercise. With particular reference to rugby, players are required to exert forces dynamically and statically during various game activities. These activities often demand both upper- and lower-body muscle action, for example, during scrummaging, rucking, and mauling. In such cases, elevations...
Table 8. Estimated values for energy expenditure, oxygen consumption, and percentage of maximal oxygen consumption ($V_o_{2\text{max}}$) between position and half.*

<table>
<thead>
<tr>
<th></th>
<th>Estimated oxygen consumption (ml·kg$^{-1}$·min$^{-1}$)</th>
<th>$V_o_{2\text{max}}$</th>
<th>EE (MJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Back</td>
<td>Forward</td>
<td>Back</td>
</tr>
<tr>
<td>First half</td>
<td>47</td>
<td>46</td>
<td>85</td>
</tr>
<tr>
<td>Second half</td>
<td>43</td>
<td>46</td>
<td>78</td>
</tr>
<tr>
<td>Total game</td>
<td>45</td>
<td>46</td>
<td>82</td>
</tr>
</tbody>
</table>

*EE = estimation of energy expenditure.

in HR may not accurately predict actual oxygen uptake. Indeed, it has been shown that when muscles act statically in training-type exercise, HRs are consistently higher compared with dynamic leg exercise only at a particular oxygen uptake (21,27). This should be taken into account when prescribing training drills based on HR. Nevertheless, measurement of game HR does provide a useful index of overall physiological strain. Mean game HR was 172 b·min$^{-1}$ (88% $H_R\text{max}$; Figure 1), higher than values of 166 ± 10 b·min$^{-1}$ recorded within semi-professional rugby league (8).

Extrapolation of laboratory-based HR-$V_o_{2}$ relationships in the EE during intermittent activity has been previously shown to reflect metabolic expenditure during soccer activity (17). Furthermore, the HR-$V_o_{2}$ regression has been shown to be a good predictor of aggregate responses to irregular exercise including vigorous anaerobic activity (7). Using this method, data above (Table 8) show that estimated values for EE were 6.9 and 8.2 MJ for the back and forward, respectively. These values correspond to 12 metabolic equivalents and are similar to those reported in semi-professional rugby league (7.9 MJ) but 25% greater than those reported in professional soccer players (3.13). It is perhaps not surprising that EE is so high in professional rugby union given the nature and intensity of the game, involvement of total body musculature, and, most importantly, player size. This is significant because energy cost of locomotion increases directly with increasing body mass (23). In this study, players weighed 92 kg (back) and 107 kg (forward), respectively. Nevertheless, potential errors in estimation of EE using this method may occur and have been the subject of recent attention (12). This "averaging out" approach may be criticized on the basis that the regression line is based on steady rate responses, conditions not found in intermittent sport (12). Consequently, the current EE data should be regarded as a crude estimation only and does not take into account resting energy expenditure. Nonetheless, the data suggest that replenishment of energy after a game is of great importance. This has obvious implications for maintenance of muscle mass during a season and for replenishment of energy reserves between games. Previous research has shown that there is a 2-day delay in muscle glycogen replenishment after a game of soccer despite administration of a high carbohydrate diet (4). Therefore, repeated exposure to heavy exercise should be monitored closely so as to avoid any adverse effects on player well-being.

**Practical Applications**

The data presented in this case study are of a descriptive nature only, are limited by subject number, and do not reflect variations in game activity/player demands, which may occur within and between participation levels. Further data on players from different playing levels, positions, and teams will help in defining physiological demands and evolutionary trends. Nevertheless, the current report does provide insight into the intense intermittent nature of elite rugby union. These findings seem to confirm that the contemporary rugby union player runs longer and harder than previously thought, data of which have not been described previously using this technology. Such data may have important applications in terms of training replication of game demands, conditioning of player groups, and in the evaluation of overall game stress.

Use of GPS-accelerometry technology offers a valuable insight into physiological demands during match play, not previously available through HR-based collection methods and video analysis. Further work using this technology, in particular detailed analysis of accelerometer and player impact data, may help fitness experts in evaluating player work rates outside that of traditional locomotor activity. Combination of GPS software with game recordings may provide more insight into categorization of forces/accelerations received/exerted during the many contact elements within the game. Appropriate classification of these contact loads may help in devising individual recovery programs specific to the player in question.

**Acknowledgments**

The author wishes to thank the players and staff of Llanelly Scarlets RFC, Wales, for participation and facilitation of this
study. No grant aid was received in conjunction with this work, and no conflicts of interest are declared.

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REFERENCES


References


References


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References


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APPENDIX A

INFORMED CONSENT FORMS
UNIVERSITY OF GLAMORGAN
SCHOOL OF APPLIED SCIENCES

INFORMED CONSENT

EXERCISE STRESS AND IMMUNOSUPPRESSION IN PROFESSIONAL RUGBY

NAME OF VOLUNTEER: ..............................................................................

PRINCIPAL INVESTIGATOR(S): Mr Brian Cunniffe\textsuperscript{1,2}
Prof. Bruce Davies\textsuperscript{1}
Prof. Julien Baker\textsuperscript{1}
Mr. Andrew Hore\textsuperscript{2}
\textsuperscript{1}Welsh Rugby Union & \textsuperscript{2}University of Glamorgan

I have read the player information sheet on the above study and have had the opportunity to discuss the details and ask questions. The investigator has explained to me the nature and purpose of the tests to be undertaken. I understand fully what is proposed to be done.

I have agreed to take part in the study as it has been outlined to me, but I understand that I am completely free to withdraw from the study at any time I wish.

I understand that these trials are part of a research project designed to promote scientific knowledge, which has been approved by the SAPS Ethics Committee, and may be of no benefit to me personally.

SIGNATURE OF VOLUNTEER: ........................................................................

Subject confidentiality will be maintained at all times. All personal information will be encoded or anonymised as far as is possible and consistent with the needs of the study.

In the event of abnormal results you have three options. Please tick \checkmark which option as appropriate.

(1) Please contact me directly so I can withhold/disclose this information as I wish to do so.

(2) Please contact my local GP.

(3) Please contact the affiliated WRU Medical officer.

Should you decide to pick option 1, you will be made aware as soon as possible and advised to seek a follow up blood test (if required) by your local/affiliated GP. Option 2 requires you to provide a name and contact number of your local GP.

I hereby fully and freely consent to participate in the study which has been fully explained to me.

SIGNATURE OF VOLUNTEER: ..........................................................

I confirm that I have explained to the volunteer named above, the nature and purpose of the tests to be undertaken.

SIGNATURE OF INVESTIGATOR: ............................................ Date........................
APPENDIX B
STUDY INFORMATION SHEETS
Dear Player,

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Introduction

The aim of this study will be to investigate the effects of player work rates and game play on stress responses within the professional rugby player. This is important since brief intense exercise has been shown to temporarily suppress the body’s immune system. This temporary suppression of the body’s immune system following heavy exercise may predispose an athlete to an enhanced risk of post-exercise infection, injury and even overtraining. Eliminating and/or minimizing these problems may be achieved by quantification of individual player work rates during game play.

While rugby is considered an intense game of physical nature, little research has documented its role as an exercise stress on the immune system. Investigation into this may help fitness staff with training periodisation and in turn, exercise recovery. Players with adequate recovery between heavy exercise bouts have a greater chance to avoiding the risk of fatigue and overtraining. This is essential team sports like rugby where players are required to train almost all year round.

What will I do if I take part?

You will assigned to a given group within which specific criteria will apply. This will be further explained to you in detail upon study commencement. In all cases, you will be required to provide blood, saliva and/or urine samples for determination of hydration status and your immune/hormone response prior to exercise. This exercise will be in the form of a normal competitive rugby game. These measurements will take place before and after exercise, more specifically on the morning of the game and 1 h after the rugby game. Additional bloods will be taken at specific time periods during recovery to investigate the time period required for immune/stress markers to return to normal resting values. Activity levels during the game will be quantified by use of video and statistical analysis. All measurements will take place during home game and/or at your normal training base. Prior to collection of bloods, you will be required to enter a warm room. During this time you will be required to sit quietly for 10 min before a resting blood sample will be taken from your arm. This will not result in any undue discomfort expect a dull ache lasting only a few seconds. For blood collection, a small needle will be placed in the
back of the arm. Blood collection should last no longer than 15 sec and the needle will be removed immediately. The volume of blood collected will be small, approximately 20 ml or 3 tablespoons! You will also be required to provide a saliva sample for determination of hydration status and hormonal analysis. Following this will be free to leave the room to and undergo normal pre-game activities. Pre-game bloods samples will take place in the vale of Glamorgan hotel between 7-9 am before you eat breakfast. After the game this procedure will be repeated and you will be asked to sit for 15 minutes in a comfortable environment to allow post-exercise measurements to be taken (a further blood & urine sample) in the Millennium stadium. Body mass will then be re-measured, to allow us to estimate fluid losses during exercise, after which you will be free to undergo normal post-game activity.

Additional blood samples will be taken the following two mornings (12 h & 24 h) to determine the time frame required for stress responses to return to resting levels. A blood sample will also be taken when you enter training camp. This sample will serve as a reference sampling point when you are 48 h rested from previous exercise. These will also take place at the vale of Glamorgan hotel before breakfast. Normal dietary practices will resume after this period. It is important to ensure that conditions are kept similar for all trials. We will therefore ask you not to consume any alcohol during the day before or after the rugby game. This will be made aware to you in the days leading up to the game.

If I decide to take part, can I later change my mind?
You are of course entirely free to withdraw from the study at any time should you wish to do so, without giving any reason for doing so. Additionally, all information which is collected about you during the course of the research will be kept strictly confidential

Other information
This study is part of an ongoing series of investigations approved by the local Ethics Committee. Thank you for considering taking part in this research. Please discuss this information with your family, friends or GP if you wish.

Should you have any further questions regarding this study we will be pleased to try to answer these and can be contacted on:

Mr Brian Cunniffe 07709 566375 or via email (bcunniffi@glam.ac.uk)
Prof Bruce Davies 01443482577 or via email (bdavies1@glam.ac.uk)
PLAYE R INFORMATION SHEET

STRESS AND IMMUNE RESPONSES IN THE SEASONAL PREPARATION OF PROFESSIONAL RUGBY PLAYERS: A MONITORING STUDY

Dear Player

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Introduction

The aim of this study will be to investigate the effects of training load and game play on stress and immune responses within the professional rugby player. This is important since exercise stress has been shown to affect the body’s immune system. While light to moderate exercise appears to have a beneficial effect on the immune system, it is thought that heavy training bouts cause a temporary impairment in immune function. Repeated stimulation of the immune system through exercise stress may result in chronic suppression if training load is not reduced and/or during intensified training periods. This is important since increased exercise stress may predispose an athlete to an enhanced risk of post-exercise infection, injury and even overtraining. This study will focus on seasonal variations in stress responses and more specifically, during periods of intensified training periods and recovery. The influence of seasonal progression, contact and non contact training phases as well as minutes of exercise exposure will be documented throughout the season. It is anticipated that this longitudinal assessment will identify periods when YOU the player is at increased risk of immune suppression and illness. This will help to determine time periods when you are at most risk of immune suppression and illness.

What will I do if I take part?

As this study is designed to monitoring your immune function across a season, collection of body fluids will be required to determine this. Hence you will be required to provide blood and saliva samples at specific dates during the year. These dates will be set out in conjunction with your club fitness staff and will include a maximum of 8 sampling points spread over the seasonal structure. This first of these dates will take place next Tuesday 14th at 9am and you will be briefed on this in the near future.
During each sampling point you will be required to enter a warm room located at your club base at 9 am following an OVERNIGHT FAST the night before. Additionally you will be required to drink 500 ml or 1 pint glass of water on waking. Once you reach Stradey Park you will be asked to sit quietly for 10-15 min so that a resting blood sample can be taken from your arm. This will not result in any undue discomfort and you will experience a dull ache lasting only a few seconds. For blood collection, a small needle will be placed in the back of the arm. Blood collection should last no longer than 10 sec and the needle will be removed immediately. The volume of blood collected will be small, approximately 20 ml or 3 tablespoons! During saliva collection, you will be required to lightly spit/dribble into a small plastic tube for a 2 min collection period. This will be similar to the saliva sampling already undertaken at your club. Following this you will be asked leave the room for a light breakfast (prescribed) before undergoing your normal daily activities. Each week you will be required to fill out a weekly illness log stating any infections/illness/fatigue you encountered over the previous 7 days. Additionally, you will be required to fill out a periodic questionnaire designed to measure your mood state.

It is important to ensure that conditions are kept similar for all trials. We will therefore ask you not to perform any strenuous exercise or consume any alcohol and caffeine during the day before each experimental trial. We will also require you to keep a RECORD OF ALL FOOD AND FLUID INTAKE during the 24 hours before the first sampling point. Each sampling point will take place at the same time of day and day of week so as to control sampling conditions as much as possible.

If I decide to take part, can I later change my mind?
You are of course entirely free to withdraw from the study at any time should you wish to do so. All information which is collected about you during the course of the research will be kept strictly confidential.

Other information
This study is part of an ongoing series of investigations approved by the local Ethics Committee. Thank you for considering taking part in this research. Please discuss this information with your family, friends or GP if you wish.

Should you have any further questions regarding this study we will be pleased to try to answer these and can be contacted on Mr Brian Cunniffe 07709 566375 or via email (bcunniff@glam.ac.uk)

Yours in Sport,

Brian Cunniffe, Andrew Hore, Wayne Proctor, Bruce Davies
APPENDIX C

HEALTH SCREEN QUESTIONNAIRES
MUCOSAL IMMUNITY AND INFECTIOUS INCIDENCE IN RUGBY

HEALTH SCREEN FOR STUDY VOLUNTEERS  Name or Number  .........................

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

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present**, do you have any health problem for which you are:
   
   (a) on medication, prescribed or otherwise  Yes  No
   
   (b) attending your general practitioner  Yes  No
   
   (c) on a hospital waiting list  Yes  No

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

   (a) Convulsions/epilepsy  Yes  No

   (b) Asthma  Yes  No

   If ‘yes’ are you on any medication relating to such?  Yes  No

   (c) Known exercise induced bronchoconstriction  Yes  No

   Does your chest ‘tighten up’ during/after exercise?  Yes  No

   (d) Diabetes  Yes  No

   (e) A blood disorder  Yes  No

   (f) Arthritis  Yes  No

   (g) Digestive problems  Yes  No

   (h) Heart problems  Yes  No

   (i) Thyroid problems  Yes  No

   (j) Post viral fatigue  Yes  No

   If so, for how long?  

   (k) Flu in last 3 months  Yes  No

   If so, were you on any medication relating to such?  Yes  No

   (l) Cold ‘cough/runny nose’ in last 4 weeks?  Yes  No

   If so, were you on medication relating to such?  Yes  No

   (m) Kidney or liver problems  Yes  No

   (n) History of blood clots/poor circulation  Yes  No

   (o) Known allergies  Yes  No

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

4. Are you presently taking any ‘antibiotics’?  Yes  No

If so, for what and for how long…………………………………………………………………………

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

Thank you for your cooperation!
EXERCISE STRESS AND IMMUNE FUNCTION IN PROFESSIONAL RUGBY

**HEALTH SCREEN FOR STUDY VOLUNTEERS**

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

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present**, do you have any health problem for which you are:
   - (a) on medication, prescribed or otherwise............................ Yes ☐ No ☐
   - (b) attending your general practitioner................................... Yes ☐ No ☐
   - (c) on a hospital waiting list................................................... Yes ☐ No ☐

2. **In the past two years**, have you had any illness which require you to:
   - (a) consult your GP................................................................ Yes ☐ No ☐
   - (b) attend a hospital outpatient department ............................ Yes ☐ No ☐
   - (c) be admitted to hospital ..................................................... Yes ☐ No ☐

3. **Have you ever** had any of the following:
   - (a) Convulsions/epilepsy ........................................................  Yes ☐ No ☐
   - (b) Asthma .............................................................................  Yes ☐ No ☐
   - (c) Eczema .............................................................................  Yes ☐ No ☐
   - (d) Diabetes ............................................................................  Yes ☐ No ☐
   - (e) A blood disorder ...............................................................  Yes ☐ No ☐
   - (f) Head injury .......................................................................  Yes ☐ No ☐
   - (g) Digestive problems ...........................................................  Yes ☐ No ☐
   - (h) Heart problems .................................................................  Yes ☐ No ☐
   - (i) Problems with bones or joints ............................ Yes ☐ No ☐
   - (j) Disturbance of balance/coordination .................................  Yes ☐ No ☐
   - (k) Numbness in hands or feet ...............................................  Yes ☐ No ☐
   - (l) Disturbance of vision ........................................................  Yes ☐ No ☐
   - (m) Ear / hearing problems ......................................................  Yes ☐ No ☐
   - (n) Thyroid problems .............................................................  Yes ☐ No ☐
   - (o) Kidney or liver problems ..................................................  Yes ☐ No ☐
   - (p) Allergy to nuts ..................................................................  Yes ☐ No ☐
   - (q) Post viral Fatigue .............................................................  Yes ☐ No ☐
   - (r) Cold/Flu in last 3 months............................... Yes ☐ No ☐
   - (s) History of blood clots/poor circulation

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

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

............................................................................................................

Thank you for your cooperation!
APPENDIX D

FOOD DIARES [4 day and 24 h]
<table>
<thead>
<tr>
<th>TIME</th>
<th>DESCRIPTION OF FOOD CONSUMED</th>
<th>AMOUNT</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>9am</td>
<td>Water</td>
<td>1 pint</td>
<td></td>
</tr>
<tr>
<td>9am</td>
<td>Cornflakes</td>
<td>Lrg bowl</td>
<td></td>
</tr>
<tr>
<td>9am</td>
<td>Semi skimmed milk</td>
<td>Half litre</td>
<td></td>
</tr>
<tr>
<td>9am</td>
<td>Toasted brown bread</td>
<td>2 slices</td>
<td>with strawberry jam</td>
</tr>
</tbody>
</table>

Thank you for your cooperation!!
Four day food diary

The four day food diary has been designed to allow staff to understand how you are currently eating during a typical week, including match day. This diary will give us important information about the type of food you are eating, how much, when and under what circumstances. Please include ALL information relating to your daily food consumption and dietary habits.

Please complete the diary on 2 weekdays and one match day and the recovery day after the match.

Overleaf is an example of how your food diary will look when it is filled in (you do not need to type it). If you would like to make further comments, write on a spare sheet of paper and enclose it with the questionnaire.
Measurement and amount

To help us to understand and analyse your dietary intake, it is important that you describe the AMOUNT of food you have eaten. The following measurements should be used to describe different foods. All measurements are household items making it easy to understand and relate to.

A cup

The cup should be a normal tea-cup and not a coffee cup or mug. A cup measurement can be used to describe milk and beverages, vegetables and some fruits, cereals and porridge. E.g. 1 cup of oats porridge with ½ cup skim milk and ½ cup of strawberries

Whole

Fruit and some vegetables can be described as a whole fruit or half or a quarter. Fruit and vegetables can also be described as small, medium or large. E.g. ½ large orange and 2 small prunes or 2 medium baked potatoes.

Slices

Bread is described in slices, but can also be better qualified as a thick or thin slice. Bread can also be described as bread rolls. The size of the roll should be given and the type. E.g. a thick slice of whole-wheat bread and a small white crispy roll (6cm x 4 cm).

Size

When a cracker, pancake or pastry is eaten, the size should be described when it is not an accepted brand name. E.g. 2 pancakes (5cm x 5 cm) and 6 Ritz crackers or 2 digestive biscuits. Sausages, salami, etc. should be described as a number and a size.

Match boxes

Meat, chicken, fish and cheese can be best described in ounces or grams. 1 match box = 30g = 1 ounce
E.g. 1 slice of beef fillet = 3 match boxes = 90 grams = 3 ounces

Meat and fish can also be described as a ‘fist size’ = approximately 100g or
A flat hand size of fish = approximately 150 g

Cheese is easily measured in match box sizes, except grated, ricotta or cream cheese which can be measured in cups and tablespoons.
24 hour recall data sheet

Here is an example of how to fill in your food diary (you do not need to type it). If you need more room, continue to write on a spare sheet of paper or photocopy this or the next page before you start.

Please specify all food and fluid intake from time of awakening until the next morning for the two weekdays and one match day and the recovery day after the match.

<table>
<thead>
<tr>
<th>Time</th>
<th>Name</th>
<th>Type &amp; description</th>
<th>Amount</th>
<th>Comments e.g. appetite, place, feelings etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9am</td>
<td>Cottage cheese</td>
<td>Low fat 2%</td>
<td>½ cup</td>
<td>I was really hungry, but in a hurry</td>
</tr>
<tr>
<td></td>
<td>Bread</td>
<td>Whole wheat, thin</td>
<td>2 slices</td>
<td>Ate my cottage cheese in the car on the way to work</td>
</tr>
<tr>
<td></td>
<td>Margarine</td>
<td>25% less fat, spread</td>
<td>2 tsp.</td>
<td>I was still hungry after my sandwich</td>
</tr>
</tbody>
</table>

DAY:    DATE:
APPENDIX E
NUTRITIONAL SUPPLEMENT QUESTIONNAIRE
NUTRITION SUPPLEMENT QUESTIONNAIRE

This supplement Questionnaire is designed to provide researchers with appropriate information relating to dietary habits and player welfare. Please note: this information is anonymised and player confidentiality will be maintained at all times. There is no need to note player name on this form.

Additional information: Contact Brian Cuninffs- bcunniff@glam.ac.uk

SECTION 1 PLAYER INFORMATION

<table>
<thead>
<tr>
<th>PLAYER AGE</th>
<th>PLAYER POSITION</th>
<th>(Optional)</th>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>PLAYING EXPERIENCE</th>
<th>REGIONAL</th>
<th>SEMI PRO</th>
<th>REGIONAL</th>
<th>INT ‘U19’</th>
<th>INT ‘U21’</th>
<th>INT ‘A’</th>
<th>INTERNATIONAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Please Tick)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SECTION 2 – SUPPLEMENT TYPE

PLEASE TICK APPROPRIATE BOX(S) BELOW FOR SUPPLEMENT USE WITHIN THE LAST 12 MONTHS. IF YOU ARE UNSURE ABOUT SUPPL’S CONSUMED, NOTE PRODUCT NAME IN BOX BELOW E.G. MAXIMUMUSCLE; CYCLONE PROTEIN FORMULA ETC.

SUPPLEMENT TYPE

1. CREATINE
2. PROTEIN (Casein whey)
3. CALCIUM
4. IRON
5. PROBIOTICS
6. VITAMIN & MINERAL COMPLEX
7. ANTIOXIDANTS (Vita C & E)
8. GLUCOSAMINE /CHONDROITIN
9. GLUTAMINE
10. COLOSTRUM
11. CAFFEINE SUPPLEMENT
12. HMB (Hydroxy-beta-methylbutyrate)
13. HMB-CREATINE COMBO
14. AMINO ACID COMPLEX
15. HERBAL SUPPLEMENT (Specify here)
16. PROTEIN-CARB COMBO
17. OTHER (NOT LISTED ABOVE OR PRODUCT NAME)

SECTION 4 – FREQUENCY OF USAGE

PLEASE TICK APPROPRIATE BOX(S) BELOW INDICATING HOW FREQUENT YOU USE(D) THE ABOVE LISTED SUPPLEMENT

<table>
<thead>
<tr>
<th>SUPPLEMENT TYPE (S)</th>
<th>OCCASIONAL</th>
<th>DAILY</th>
<th>WEEKLY</th>
<th>PAST 3 MTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SECTION 5 – REASON(S) FOR CONSUMPTION

PLEASE TICK APPROPRIATE BOX(S) BELOW INDICATING REASON FOR USAGE. NOTE ALSO ASSIGNED SUPPLEMENT NO. Beside Ticked Box(S)

<table>
<thead>
<tr>
<th>No.</th>
<th>IMPROVE PERFORMANCE</th>
<th>DOCTORS RECOMMENDATION</th>
<th>INCREASE MUSCLE MASS</th>
<th>TISSUE MAINTENANCE</th>
<th>REDUCE BODILY FAT</th>
<th>OTHER (Please specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Yes | No

Are you most likely to use supplements during phases of high training loads?

Yes | No

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APPENDIX F

SAMPLE TRAINING LOAD CALCULATION
<table>
<thead>
<tr>
<th>Day</th>
<th>Training activity</th>
<th>Activity RPE</th>
<th>Duration (min)</th>
<th>Load [AU]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunday</td>
<td>Rest</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Monday</td>
<td>Recovery</td>
<td>2</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Tuesday</td>
<td>(1) Rugby</td>
<td>7</td>
<td>60</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>(2) Weights</td>
<td>6</td>
<td>50</td>
<td>300</td>
</tr>
<tr>
<td>Wednesday</td>
<td>Speed</td>
<td>10</td>
<td>25</td>
<td>250</td>
</tr>
<tr>
<td>Thursday</td>
<td>(1) Conditioning</td>
<td>8</td>
<td>60</td>
<td>480</td>
</tr>
<tr>
<td></td>
<td>(2) Weights</td>
<td>7</td>
<td>60</td>
<td>420</td>
</tr>
<tr>
<td>Friday</td>
<td>Rugby</td>
<td>4</td>
<td>65</td>
<td>260</td>
</tr>
<tr>
<td>Saturday</td>
<td>Game</td>
<td>10</td>
<td>70*</td>
<td>700</td>
</tr>
</tbody>
</table>

Daily Mean Load 482
Daily Standard Deviation of load 335
Monotony (Daily mean/std dev) 1.44
Total Weekly Load 2890
Strain (Weekly Load * Monotony) 4159

Schematic evaluation of training load, monotony, and strain calculations. Data representative of a typical weekly program on an elite rugby player. Days with more than one training session are summated yielding a total daily TL value.* Player substituted at 70 min.
### In the past 30 days/night(s)

<table>
<thead>
<tr>
<th>Statement</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
<th>Score 5</th>
<th>Score 6</th>
<th>Score 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>I watched TV</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I laughed</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I was in a bad mood</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I felt physically relaxed</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I was in a good mood</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I had difficulties in concentrating</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I felt so bad that I cried</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I was very good at making decisions</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I felt as if I could get everything done</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I was upset</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I felt successful in what I did</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
<tr>
<td>I felt accountable</td>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
</tbody>
</table>

This questionnaire contains a series of statements. These statements possibly describe your physical or mental health during the past few days. Please select the answer that most accurately reflects your thoughts and feelings. Indicate how often each statement was true in your case in the past days. For each statement, there are seven possible answers. Please make your selection by marking the number corresponding to the appropriate answer.

### Example

**In the past 30 days/night(s)**

- I read a newspaper

<table>
<thead>
<tr>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
<th>Score 5</th>
<th>Score 6</th>
<th>Score 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>never</td>
<td>seldom</td>
<td>sometimes</td>
<td>often</td>
<td>more often</td>
<td>very often</td>
<td>always</td>
<td></td>
</tr>
</tbody>
</table>

In this example, the number 3 is marked. This means that you read a newspaper very often in the past three days.

Please do not leave any statements blank.

If you are unsure which answer to choose, select the one that most closely applies to you.

Please turn to the page and respond to the statements in order without interruption.
In the past (3) days/night(s):

37: I am in a good condition physically
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
38: I trained myself during performance
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
39: I felt emotionally tired during performance
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
40: I had muscle pain after performance
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
41: I was convinced that I performed well
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
42: Too much was demanded of me during the breaks
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
43: I psyched myself up before performance
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
44: I felt that I wanted to quit my sport
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
45: I felt very energetic
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
46: I easily understood how my teammates felt about things
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
47: I was convinced that I had trained well
never seldom sometimes often more often very often always
0 1 2 3 4 5 6
48: The breaks were not at the right times
never seldom sometimes often more often very often always
0 1 2 3 4 5 6

Thank you very much!
Scales and Items of the RESTQ-52 Sport

Scale 1: General Stress
14) I felt down.
15) I was in a bad mood.

Scale 2: Emotional Stress
9) I was anxious or worried.

Scale 3: Social Stress
19) I was annoyed by others.
22) I was upset.

Scale 4: Conflicts/Pressure
7) I worried about unresolved problems.
23) I felt under pressure.

Scale 5: Fatigue
10) I felt dead tired after work.
29) I was overtrained.

Scale 6: Lack of Energy
6) I had difficulties in concentrating.
22) I got off making decisions.

Scale 7: Somatic Complaints
5) I had a headache.
12) I felt uncomfortable.

Scale 8: Success
10) I was successful in what I did.
24) I made important decisions.

Scale 9: Social Relaxation
2) I laughed.
9) I had a good time with my friends.

Scale 10: Somatic Relaxation
4) I felt physically relaxed.
21) I felt as if I could get everything done.

Scale 11: General Well-being
5) I was in good spirits.
27) I was in a good mood.

Scale 12: Sleep Quality
15) I had a satisfying sleep.
30) I slept restlessly.

Scale 13: Disturbed Breaks
27) I could not get rest during the breaks.
34) I had the impression there were too few breaks.
42) Too much was demanded of me during the breaks.
46) The breaks were not at the right times.

Scale 14: Burnout/Emotional Exhaustion
30) I felt burned out by my sport.
39) I felt emotionally drained from performance.
40) I felt that I wanted to quit my sport.
52) I felt frustrated by my sport.

Scale 15: Fitness/Injury
26) Parts of my body were aching.
33) My muscles felt stiff or tense during performance.
40) I had muscle pain after performance.
49) I felt vulnerable to injuries.

Scale 16: Fitness/Being in Shape
29) I recovered well physically.
37) I was in a good condition physically.
45) I felt very energetic.
51) My body felt strong.

Scale 17: Burnout/Personal Accomplishment
31) I accomplished many worthwhile things in my sport.
36) I dealt very effectively with my teammates’ problems.
40) I easily understood how my teammates felt about things.
53) I dealt with emotional problems in my sport very calmly.

Scale 18: Self-Efficacy
26) I was convinced I could achieve my set goals during performance.
35) I was convinced that I could achieve my performance at any time.
41) I was convinced that I performed well.
47) I was convinced that I had trained well.

Scale 19: Self-Regulation
32) I prepared myself mentally for performance.
33) I pushed myself during performance.
45) I psyched myself up before performance.
50) I set definite goals for myself during performance.

Note: The item 39 of Sleep Quality has to be inverted for analysis.