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List of Abbreviations

Abbreviation	*Explanation*
GAA	Gaelic Athletic Association
T	Testosterone
C	Cortisol
SAA	Serum Amyloid A
GH	Growth Hormone
IGF	Insulin-like Growth Factor
WADA	World Anti-Doping Association
$Nmol.L^{-1}$	Nanomole per litre
$Ng.mL^{-1}$	Nanogram per millilitre
SD	Standard Deviation
$Ng.dL^{-1}$	Nanogram per decilitre
Kg	Kilogram
Cm	Centimetre
ACTH	Adrenocorticotropic Hormone
CRH	Corticotropin Releasing Hormone
T:C ratio	Testosterone:Cortisol Ratio
REM-test	Rower Ergometer Test
SEM	Standard Error of the Mean
Yrs	Years
FCTR	Free Testosterone and Cortisol Ratio
M	Metre
H	Hour
$ml.kg^{-1}.min^{-1}$	Millilitre per kilogram per minute
$Kg.m^2$	Kilogram per metre squared
NT	Normal training
IT	Intensified training
FOR	Functional overreaching syndrome
NFOR	Non-functional overreaching syndrome
OTS	Overtraining Syndrome
OS	Overreaching Syndrome
RPE	Rating of Perceived Exertion
sRPE	Session Rating of Perceived Exertion
HR	Heart Rate
RPM	Revolutions per minute
W	Watts
$m.min^{-1}$	Metres per minute
HSR	High speed running
POMS	Profile of Mood State
AU	Arbitrary Units
GPS	Global Positioning System
$m.s^{-1}$	Metres per second
$m.s^2$	Metres per second per second
HREI	Heart Rate Exertion Index
Hz	Hertz
U20	Under 20
$Km.h^{-1}$	Kilometres per hour
$Sprint.min^{-1}$	Sprints per minute

TD	Total distance
Vmax	Maximum velocity
AFL	Australian football league
SJ	Squat Jump
CMJ	Countermovement Jump
1RM	One Repetition Max
Yo-Yo IR2	Yo-Yo Intermittent Recovery Test: Level 2
HSR%	High Speed Density
SHBG	Sex Hormone Binding Globulin
CK	Creatine Kinase
STAI-S	State Anxiety Inventory Questionnaire
$Pmol.L^{-1}$	Picomole per litre
CSAI-2	Competitive State Anxiety Inventroy-2
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Glutamate	Glu
Glutamine	Gln
Upper Respiratory Tract Infection	URTI
CRP	C-Reactive Protein
IL-6	Interleukin-6
IL-1	Interleukin-1
TNF	Tumour Necrosis Factor
NK cells	Natural Killer cells
NMF	Neuromuscular fatigue
PPO	Peak Power Output
ηp^2	Partial ETA squared
RCT	Randomised Control Trial
MSFT	Multistage Fitness Test
MAS	Peak aerobic running velocity
Gln:Glu Ratio	Glutamine to Glutamate Ratio
VO_2 Max	Maximal Oxygen Consumption
BAMS	Brief Assessment of Mood State
POMS	Profile of Mood States
LEG	Low Exposure Group
HEG	High Exposure Group
MBI	Magnitude Based Inferences
AOU	Amyloid of Unknown Origin
HDL	High Density Lipids
APR	Acute Phase Response
$\mu g.mL^{-1}$	Microgram per millilitre
$umol.L^{-1}$	Micromoles per litre
LC-MS-based tests	liquid chromatography–tandem mass spectrometry-based tests
NFL	National Football League
LFC	Leinster Football Championship
µl	Microlitre
Ml	Millilitre
L	Litre
°C	Degrees Celsius

$Rev.min^{-1}$	Revolutions per minute
nm	nanometre
ANOVA	Analysis of Variance
SEM	Standard error of the mean

The Effect of Competitive Gaelic Football Match Play on Player Immunoendocrine Status

Introduction

Research by Halson (2014) suggested the importance of monitoring both internal and external training load of athletes to optimise both recovery and subsequent performances. Global Positioning System (GPS) has become common place in quantifying external workload in team sport athletes. Salivary hormones, namely testosterone (T) and cortisol (C), have also been utilised to explore individual training load tolerance in team sports and have been reported to fluctuate in response to competitive rugby match-play (West et al., 2014), with temporary immunosuppression also reported (Cunniffe et al., 2010).

Methodology

Saliva and blood samples were collected 46- and 2h pre-game, as well as immediately, 48- and 72h post-game. GPS data was collected during trainings and games. Saliva samples were pooled (n = 17) to explore a larger sample size, before individual game analysis (n=7) was conducted to explore the largest sample size in a single game. Pooled serum samples (n = 8) and individual samples (n = 4) were also analysed.

Results

A significant effect for time was reported for salivary C concentrations in pooled samples (P=0.001). While a significant effect for time was reported for SAA in individual game samples (P=0.026). Analysis of the correlations between Δ immunoendocrine concentrations and GPS metrics for the individual game samples, reported only three significant correlations. The first significant correlation was between TΔ0-48 and HSR% (r = 0.83, P=0.020). The other two significant strong negative correlations were described between SAAΔ2-0 and both TD (r = -0.97; P=0.035) and relative TD (r = -0.97; P=0.035).

Discussion

Salivary hormones returned to baseline concentrations at 48h post-game in line with previous research (Cunniffe et al., 2011). Correlation analysis suggested that players who endure greater HSR% during the game, will experience a greater decrease in salivary T concentrations from 0- to 48h post-match in elite Gaelic footballers.

Conclusion

Quantifying individual recovery timelines, using objective measures, provides coaches with the ability to appropriately monitor their athletes and optimise subsequent performances. Despite the fact that internal load does not appear to dictate the magnitude of change in immunoendocrine markers, the benefits of load monitoring are still evident.

Chapter 1

1.0 Introduction

1.0 Introduction

Intercounty Gaelic Football is an amateur sport played in Ireland where the season runs from December to September if a team is successful in making it to the All-Ireland Final. Players usually train 3-5 days per week with a combination of gym and pitch-based sessions with games taking place on the weekends. Intercounty matches last for 70 minutes; 35 minutes per half. Depending on the time of year, competitive match demands vary with most games taking place between January and May. In addition to their training demands, players usually work full-time, 9am-5pm, jobs to finance their lives outside of sport as players are not paid while playing at the elite level. Depending on the training demands of the team, players may be required to train at 7am before work and are mostly required to train after work.

There are significant psychological demands associated with training at this level, which include competitive stressors, organisational stressors and personal stressors, as defined by Sarkar and Fletcher (2014). The competitive stressors have been further identified as the stress associated with preparation, injuries, pressure, underperformance, expectations, self-presentation and rivalry. Organisational stressors on the other hand are related to the environment in which the athletes train and compete. Stressors classed in this subcategory include relationships with coaches or team-mates, leadership, cultural and team issues, as well as logistical and environmental issues. Finally, personal issues relate to events that occur outside of the athlete's sporting life. They include, work-life interface, which is particularly pertinent to players who are members of the Gaelic Athletic Association (GAA) as they are amateur athletes that require full-time jobs to finance their lives. Moreover, family and other relationships outside sport play a role in the personal issue subcategory. With the demands placed on GAA players, many of which are encompassed in these categories of stressors, it is of paramount importance that players are monitored and managed throughout the training process, in a manner beyond education and self-awareness initiatives. The coach and support staff need to be aware of the internal and external load the players experiences and manage the player accordingly in order to bring about sustained optimal performance throughout the competitive season calendar. Developing an understanding of the timeline of recovery following competitive match-play and its' accompanying load is, therefore, of significance to firstly, define a recovery window and, secondly, determine the time during which a subsequent training load will be effective rather than detrimental to the athlete.

Training load is defined as the input variable manipulated by practitioners to elicit change in athletes' performance (Borresen and Lambert, 2009). Halson (2014) discussed the benefits of using a combination of internal and external load monitoring protocols to effectively monitor athletes training

load during competition. Impellizzeri *et al.* (2019) described external load as the work completed by an athlete during a given training session, while the internal load is the athletes' psychophysiological response to the external workload. No single measure was identified; however, it was recommended that utilising effective load monitoring strategies coupled with efficient recovery protocols can reduce the risk of maladaptation associated with intensified training periods (Halson, 2014). Disturbances in both internal and external load monitoring measures have been reported in rugby union players from pre- to post-match, before returning to baseline measures between 38- and 60h post-match (West *et al.,* 2014). Effective monitoring of athletes in this recovery window is imperative to ensure players can recover prior to the next training stimulus, subsequently minimising the risk of experiencing symptoms of overreaching syndrome (Halson and Jeukendrup, 2004).

To date, no research has been conducted into the hormonal responses of elite Gaelic football players to competitive match-play. The purpose of this study is to identify the recovery timelines of internal load monitoring markers, namely testosterone (T) and cortisol (C) concentrations, as well as serum immune markers, in order to better educate practitioners on player management around competitions. The physiological relevance of these immunoendocrine markers will be discussed extensively in the coming sections.

While a combination of internal and external load monitoring tools has previously been identified by Halson (2014), there is limited research on the effects that external workload has on the immunoendocrine markers which represent the internal workload of athletes. This study aims to determine the immunoendocrine response to Gaelic games match-play, and explore the effects commonly collected using Global Positioning System (GPS) metrics have on the fluctuations and subsequent recovery timelines of these immunoendocrine markers. A better understanding of the relationships between these internal and external load monitoring measures will assist practitioners in quantifying the extent of recovery required by elite Gaelic footballers post-match.

Chapter 2

2.0 Literature Review

2.0 Literature Review

2.1 Introduction

The purpose of this literature review and subsequent research is to outline the effects that competitive match-play and intense training blocks have on athletes. Furthermore, this research aims to provide coaches with guidelines and considerations for the proper management of players based on their internal responses to competitive match-play. Ensuring optimal recovery for players allows coaches to maximise the training stimulus players are exposed to while attempting to minimise the risk of overtraining and the detrimental effects this can have on performance.

It has been recommended that a combination of internal and external load monitoring tools are employed to best monitor athletes in the post-match recovery period (Halson, 2014). The effects that T and C have on athletes have been previously outlined (Crewther *et al.,* 2011), in addition to the research identifying methodological considerations that researchers should consider (Hayes *et al.,* 2016). Despite this, no research has been conducted on elite Gaelic football players to determine their immunoendocrine responses to competitive match-play.

This research study aims to be the first study to outline the immunoendocrine responses of elite Gaelic football players in addition to exploring what contributes to the magnitude of change that is described. While there is limited research exploring the effects that external workload has on the magnitude of change in commonly collected immunoendocrine responses, this study intends to explain the relationships between external workload as quantified by GPS metrics and immunoendocrine markers in elite Gaelic football players.

2.2 Physiology of Training

Team sport athletes are exposed to multiple training modalities during a competitive season, however there are two forms of training that players are most commonly exposed to; on field training, specifically aerobic and anaerobic training, and resistance training in the gym. The latter can be differentiated by the training modality employed by coaches during the session in order to improve an element of the force-velocity curve, ranging from maximal strength to speed work. Aerobic training is an alternative method of training most commonly implemented during training sessions conducted on the pitch. These aerobic sessions are most often completed using a range of sport-specific drills to replicate the varying volumes and intensities relative to match-demands. A range of hormones can

control the responses of athletes to these sessions, however for the purpose of this research, two hormones that will be discussed are T and C, in addition to the immune marker Serum Amyloid A (SAA).

2.2.1 Hormonal Influence on Training Adaptation

2.2.1.1 Testosterone

T is one of the primary physiological markers of anabolic status. It is produced by the interstitial cells of the testes in males. Aside from having a direct effect on muscle protein synthesis, it also has several secondary benefits. T indirectly affects a muscle fibre's protein content by promoting the release of growth hormone (GH). The release of GH has a cascading effect on the synthesis and release of Insulin-like Growth Factors (IGF) from the liver. Research demonstrated that the relationships between circulating T concentrations and changes in fat-free mass and muscle size, conform to a log-linear dose-response curve (Bhasin *et al.,* 2001). While this research included exogenous T supplementation, which is commonly known as 'anabolic steroids' and prohibited by the World Anti-Doping Association (WADA), the positive effects associated with T supplementation outlined by Bhasin *et al.* (2001), would be beneficial to athletes who are required to be in peak physical condition for competition. T also affects the force-production capabilities of muscles by interacting with neural receptors. T causes neurotransmitters to be released, initiating change in structural proteins that alter the size of the muscle's neuromuscular junction. T binds to the protein transporter, sex-hormone binding globulin (SHBG), and is transported to the target tissue. Once at the target tissue, it associates with a membrane-bound or cytosolic receptor and subsequently migrates to the target cell where it interacts with nuclear receptors and protein synthesis is initiated. Research conducted in animals have provided the most detailed insight into how exactly T influences neuromuscular function through various rapid and short-term mechanisms. In adult male rats, T can cause an increase in firing rates within 2- and 30s when administered directly to individual neurons in the anterior hypothalamus (Yamada, 1979). This research shed light on the rapid androgen activity and its actions in the brain. Such data have since been supported by research in athletic populations reporting the importance of T as a key steroid-hormone, especially in a competitive population like athletes (Crewther *et al.,* 2011).

Research suggested that the short-term expression of neuromuscular performance may contribute to the long-term adaptation to training (Crewther *et al.,* 2011). In addition to the chronic effects of T activity, Kraemer *et al.* (1990) reported the acute T responses to two resistance training sessions; one strength session and one hypertrophy session. The authors reported increased serum T

concentrations after both strength, and hypertrophy resistance training workouts in nine healthy males. Notably, all protocols elicited different magnitudes and duration of serum T increases with an increased rest in the hypertrophy workout and a decreased load in the strength workout, both reducing serum T concentrations. Decreases in total serum T concentrations have also been demonstrated in aging American males with an average decrease of 0.11nmol.L^{-1} per year (Harman *et al.,* 2001). Low serum free T concentrations (<11.5ng.mL^{-1}) have been shown to have several negative effects including, loss of muscle mass, increased fat mass, reduced aerobic capacity, and increased cardiovascular disease risk (Szulc *et al.,* 2003; Herbst and Bhasin, 2004; Sellami *et al.,* 2018). Research by Baumgartner *et al.* (1999) concluded that a reduction in both serum free and total T concentrations was related to a reduction in muscle mass in men with a mean ± SD BMI of 25.5 ± 3.1kg.m^2. The elderly men (*n* = 121) in this study displayed a mean ± SD serum free T-index of 10.8 ± 5.8ng.dL^{-1} and total serum T concentrations of 362.8 ± 151.4ng.dL^{-1}. The same sample of males possessed a mean ± SD, 21.6 ± 3.0kg of muscle mass and a mean ± SD grip strength of 34.0 ± 8.4kg. T can minimise these negative effects by increasing protein synthesis and decreasing protein degradation, while C works in reverse. Ensuring C does not outweigh the effects of T to ensure a positive net balance, will result in an increase or maintenance of muscle fibre and whole muscle size (Herbst and Bhasin, 2004), which is inherently important for athletes who must operate at peak performance during a season. Unlike C, however, higher or lower T concentrations do not affect substrate utilization, specifically glucose, glycogen and lipid utilization, during 90min of submaximal exercise in moderately-active young males (Braun *et al.,* 2005). This is one of the main contrasting effects of both C and T and plays an important role in their uses in athletic research. The role of T in positively impacting force production and the individualised anticipatory response has been reported in judo competitors prior to competition (Salvador *et al.,* 2003). This anticipatory effect has been associated with higher motivation to win alongside higher C concentrations before the game. Similar research suggested that competitive canoeists who experienced these elevated C responses seen in parallel to the elevated anticipatory T responses, as seen by Salvador and colleagues (2003), appraised their somatic anxiety as enhancing, rather than worsening, their subsequent performance (Eubank *et al.,* 1997).

2.2.1.2 Cortisol

C is released in response to both physical and psychological stress and acts to mobilize substrates as well as modulate the immune system (Russell *et al.,* 2012; Gatti and De Palo, 2011). It is the major glucocorticoid produced by the adrenal cortex and targets an array of both peripheral and central processes, while also responding to the circadian rhythm. C concentrations are suppressed during

nocturnal sleep while the body is in a fasted state, and spike to peak concentrations in the morning (Lange *et al.,* 2010). Lange *et al.* (2010) also report that the fasted state that occurs in the latter stages of sleep, as the body's glucose availability is limited, results in an increase in C concentrations, before peaking in the morning, to promote gluconeogenesis and glycogenolysis via glycogen, protein and lipid metabolism resulting in increased glucose circulation. Furthermore, C can also affect human sympathetic nerve activity which can have a subsequent effect on metabolic and cardiovascular function (Crewther *et al.,* 2011). When C is triggered outside of circadian dependencies, its release is specific to stress (Herman *et al.,* 2005). When the body perceives acute stress, cells within the paraventricular nucleus of the hypothalamus release corticotropin releasing hormone (CRH), which travels through the infundibulum to the pituitary gland. Within the pituitary gland, the CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. When the ACTH eventually reaches the adrenal cortex, it binds to receptors that stimulate the release of C into the bloodstream. Due to the fact that the majority of human cells have receptors for C (Dedovic *et al.,* 2009), it has a myriad of effects throughout the system including metabolic, cardiovascular and immune responses (Buckingham, 2006). Furthermore, C affects macronutrient metabolism in several ways and some of these can have a negative effect on exercise if there is repeated exposure that goes unnoticed. C promotes the breakdown of protein into amino acids to produce glucose via gluconeogenesis with this process occurring in all cells except in the liver. C also acts as an insulin antagonist by inhibiting several metabolic reactions including glucose uptake and oxidation by cells, triglyceride breakdown to fatty acids and glycerol, immunosuppression and a negative calcium balance. These effects can create unfavourable conditions for athletes and maladaptation could ensue if sufficient recovery periods are not administered (Crewther *et al.,* 2011).

Much like T, C is an unbound steroid hormone in saliva, and as a result, can pass freely through the salivary gland (Gatti and De Palo, 2011). Furthermore, both salivary T and C show strong correlations with their 'free' serum concentrations. It is important to note from a research perspective, however, that the concentrations of these hormones differ, with salivary C concentrations being only 50-60% of the corresponding serum values (Wood 2009). Salivary C concentrations are significantly lower than plasma C concentrations; 3-30nmol.L^{-1} *vs.* 200-800nmol.L^{-1}, respectively (Schwartz and Granger, 2004). As a result, the medium through which samples are collected is key when analysing and cross-referencing research. For C, studies comparing salivary and serum total free steroid concentrations revealed strong correlation coefficients across studies (r = 0.80; Wood, 2009). Individual studies referenced in the review by Wood (2009), reported a distinct change in the slope when serum C concentrations passed 500nmol.L^{-1}, yet strong correlations were reported both above and below the threshold at 500nmol.L^{-1} (r = 0.84 and r=0.92, respectively). Similarly, a strong correlation coefficient

was reported by Wang *et al.* (1981) between serum free T concentrations and salivary T concentrations (r=0.94; n = 65). Mean ± SD serum total T concentrations in normal adult males were described as 6.09 ± 1.8 ng.mL^{-1}, while mean ± SD salivary T concentrations were reported as 84 ± 35 pg.mL^{-1}. It was subsequently reported that free T in saliva represented mean ± SD 1.38 ± 0.44% of the total T concentrations (Wang *et al.,* 1981). These findings suggest that salivary steroid concentrations are appropriate alternatives to serum total steroid concentrations. In addition to the research that has identified salivary T (Wang *et al.,* 1981) and salivary C concentrations (Wood, 2009) as being strongly correlated to their respective serum total concentrations, research conducted by Lippi *et al.* (2016) reported that 100% of the athletes included in the study, felt more comfortable providing a saliva sample rather than a blood sample. These findings by Lippi and colleagues (2016) provide further evidence that serial saliva sampling may be more practical in team sports as they are perceived by athletes as being less invasive than blood sampling.

2.2.1.3 Testosterone:Cortisol Ratio

When T:C ratios decrease due to deviations from homeostasis as a result of acute stress from training and matches, T decreases (Zilioli and Watson, 2013) and C increases (West *et al.,* 2014) which results in a significantly lower T:C ratio than would be reported prior to the game (Cunniffe *et al.,* 2010). Alternatively, as T concentrations increase to baseline concentrations during the recovery period, C concentrations subsequently decrease which results in a greater T:C ratio (Cunniffe *et al.,* 2011). A blunted response in either of these hormones, as a result of the inability for the body to effectively return to homeostasis, would be reflected in the T:C ratio response (Hayes *et al.,* 2010). It is for this reason that the combination of T and C concentrations as well as their ratio value, have been suggested as modes of monitoring overreaching and the subsequent overtraining of athletes as a result of intensified periods of training and match-play (Crewther *et al.,* 2011).

The ratio between T and C, has also been suggested as a means of monitoring the progression of overreaching syndrome in athletes. This ratio is more commonly known as the testosterone:cortisol ratio (T:C ratio) and was originally addressed by Adlercreutz *et al.* (1986) and was suggested to be associated with tiredness. Vervoorn *et al.* (1991) identified the ratio as a way of monitoring "hormonal" overstrain as the research sought a tool to aid in the diagnosis and prevention of overreaching in six elite male Dutch rowers, with a mean age of 23.2yrs (range 20-26yrs) and a mean weight of 91.2kg (range 85-103kg). Blood samples were collected between 8am and 10am to determine serum free T and C concentrations, subsequently calculating the T:C ratio. Participants were asked to refrain from intensive exercise before sample collection. Ten samples were collected at five-

week intervals, except during a two-week training camp where samples were collected every four days (test 3, 4, and 5). On sample collection days, the rowers also completed a rower ergometer test (REM-test) which consisted of three blocks, including a three-minute warm-up and a five-minute loading block which correlated to the anaerobic threshold. This was followed by two-minutes of rest, before a two-minute maximal effort block, wherein as many rotations as possible had to be scored. Results indicated no significant differences in T:C ratios for the first 7 tests (P>0.05), despite large ranges reported for some testing sessions (timepoint 1 = 0.68 to 1.11, timepoint 2 = 0.43 to 2.11, and timepoint 6 = 0.82 to 2.33). Mean ± SEM for the respective timepoints were 1.07 ± 0.16, 1.05 ± 0.25, and 1.31 ± 0.26. A significant decrease (P<0.05), however, was reported between tests 8 and 9 (mean ± SEM, 0.75 ± 0.06 *vs.* 1.25 ± 0.19, respectively). Interestingly, the three samples collected during the two-week training camp (samples 3, 4, and 5), were lower than those reported for the other three of the first seven samples, despite a lack of significance (mean ± SEM, 0.85 ± 0.10, 0.87 ± 0.09, and 0.95 ± 0.10, respectively). No significant differences (P>0.05) were reported for any samples when compared to the baseline sample taken at timepoint 1. When comparing the samples collected during the intensified training camp, to the first test, in twelve out of fifteen cases (80% of the rowers), a decrease in the T:C ratio ranging from 5 to 50% was reported. This is in line with previous data outlined by Cunniffe *et al.* (2011) which reported a significant decrease in T:C ratios after competitive match-play in elite rugby union players. As the players' samples were collected after a day with no intensified training, these values could be associated with the intensified training period the players were exposed to. A reference value of below 0.35 was used as a criterion measure for overtraining in the study by Vervoorn *et al.* (1991), however the lowest ratio reported was 0.43 during sample 2.

This was further confirmed by numerous other studies including Banfi *et al.* (1993), where T:C was shown to be a reliable and valid marker of training response and recovery in Italian National Team speed skaters, with a mean ± SD age of 20.8 ± 1.9yrs for males and 22.7 ± 3.8yrs for females. Banfi *et al.* (1993), measured the free T and C ratio (FTCR) in the 8 elite speed skaters; three females and five males. The purpose of the study was to monitor the effectiveness of the ratio in determining overstrain or incomplete recovery across an eight-month season. Across the eight months, six resting serum samples were collected between June 1991 and January 1992, with all samples collected during a fasted state at 10am on all collection points to control for the circadian rhythm. The first sample was taken on June 29th with the second sample taken after the first training on the ice rink on August 20th. The third sample was collected on September 1st after a period of training at 2000m altitude and sample four on September 12th after an intensive training camp at sea level. Sample five was collected on November 20th and sample six collected on January 8th after the national and international competition period prior to the Olympic training camp in preparation for the Olympic games and

World Championships. Samples at timepoint 1 for both males and females, which acted as a baseline measure, reported mean ± SD FTCR derived from the equation of 0.668 ± 0.183 and 0.050 ± 0.022, respectively. Results indicated a significant increase in FTCR in males, but not in females, at timepoints 3 and 5 from timepoint 1 (P<0.01). Mean ± SD FTCR results derived from the equation for timepoints 3 and 5 were 2.203 ± 0.787 and 2.590 ± 0.757 in males. Increased FTCR were also observed at timepoint 6 for males but not females (mean ± SD, 1.230 ± 0.536). No significant changes were reported across timepoints for females in FTCR derived from the equation. Variations were more pronounced in males due to the wider variations of T concentrations.

Researchers in team sports have also determined the T:C ratio as a valid and reliable marker of fatigue, as outlined in Appendix A, as it significantly reduced post-game in field sport athletes, specifically elite male rugby players, before returning to baseline levels at 38h post-game (Cunniffe *et al.,* 2011) and in research with a similar population at 60h (West *et al.,* 2014). These findings by Cunniffe *et al.* (2011) and West *et al.* (2014) oppose the findings by Banfi *et al.* (1993). Banfi and colleagues (1993) reported increases in FTCR immediately after intensive periods of training and competition (timepoints 3 and 5), while both Cunniffe *et al.* (2011) and West *et al.* (2014) reported decreases in TC ratios from pre- to post-match before returning to baseline 38- and 60h post-match, respectively. It is interesting to note that Banfi *et al.* (1993) reported a significant decrease in C concentrations in both males and females at timepoints 3 and 5 for both males (mean ± SD, 0.279 ± 0.077 and 0.192 ± 0.012, respectively) and females (mean ± SD, 0.406 ± 0.059 and 0.195 ± 0.061, respectively) when compared to baseline for males and females (mean ± SD, 0.566 ± 0.100 and 0.626 ± 0.077, respectively). Conversely, Cunniffe *et al.* (2011) reported increases in C concentrations post-match. Reductions in TC ratios have previously been associated with incomplete recovery (Vervoorn *et al.*, 1991), with a reduction of 30% indicating incomplete recovery. The criteria of a decrease in FTCR of 30% or more first appeared in the research conducted by Adlercreutz *et al.* (1986) to distinguish between nonoverstrain and overstrain. The threshold was also used to distinguish between athletes in the uncertain group, to identify a distinguishing factor. All athletes in the uncertain group reported a decrease of 30% or more in their respective FTCR (Adlercreutz *et al.,* 1986). A possible explanation for the discrepancies reported in the results of the papers by Cunniffe *et al.* (2011), West *et al.* (2014), and Vervoorn *et al.* (1991), when compared to Banfi *et al.* (1993), may be the frequency of sampling in the latter. Eight sampling points across an eight month period, may not have been sensitive to the acute changes reported by Cunniffe *et al.* (2011) and West *et al.* (2014) whereby a significant reduction in TC ratios was reported from pre- to immediately post-match before returning to baseline 38- and 60h post-match, respectively.

In addition to the research by Cunniffe *et al.* (2011), research into the use of the T:C ratio to distinguish between players who were intentionally overreached, and those that took part in normal training, was also conducted (Coutts *et al.,* 2007). Eighteen male rugby union players (mean ± SD, age 23.3 ± 3.3yrs; VO_2 max 50.5 ± 3.5ml.kg^{-1}.min^{-1}, body mass index 27.5 ± 1.8kg.m^{2}), were randomly allocated into either a deliberately overreached group (IT) or a normal training group (NT). Both groups took part in six-weeks of overload training, with the IT group completing 21.6% more training load than the NT group. Results indicated that the T:C ratio significantly decreased with six weeks of progressive overload training, however, no between group findings were reported. Body mass also reduced in both groups from pre- to post-training, which the authors suggest could be as a result of the reduced anabolic state, indicated by the reduced T:C ratios in both groups. Interestingly, after a one-week taper following the six-week overload training, the T:C ratios did not return to baseline in the IT group, while in the NT group, T:C ratios surpassed baseline ratios after the taper. These findings may corroborate the research suggesting maladaptation may occur (Crewther *et al.,* 2011).

2.3 Physiology of Overtraining

2.3.1 Overreaching and Overtraining

The definitions and differentiation of overreaching and overtraining (Keider, Fry & O'Toole, 1998) were discussed in the review by Halson and Jeukendrup (2004). Kreider, Fry and O'Toole (1998) defined overtraining as the accumulation of training and/or non-training stress that if left unaddressed, can result in athletes experiencing a chronic decrease in performance capacity. This decrement in performance may or may not be associated with physical or psychological symptoms of overtraining. The authors reported that these signs and symptoms may take weeks to month to recover fully (Keider, Fry & O'Toole, 1998). GAA players are exposed to both training and non-training related stress associated with playing at the elite amateur level, as previously discussed by Sarkar and Fletcher (2014), and as a result athletes must be monitored effectively by coaches and support staff.

There are two clinical forms of overtraining; the sympathetic and parasympathetic forms. Sympathetic overtraining is defined by increased sympathetic activity at rest and is reflective of thyroid hyperexcitability patterns. This is usually exhibited through hyperexcitability, restlessness, and impaired exercise performance. This form of overtraining may reflect excessive psychological stressors that are commonplace in normal living. Emotional stress coupled with the stress of training and competition is what leads to the development of sympathetic overtraining. Parasympathetic

overtraining on the other hand is much more common and reflects adrenal insufficiency patterns. This form of overtraining is characterized by the predominance of vagal activity during rest and exercise (Keider, Fry & O'Toole, 1998).

Parasympathetic overtraining is more properly termed, overreaching in the acute stage, however it is qualitatively similar in symptoms when chronic, called overreaching syndrome. Overreaching has since been subdivided into the acute and chronic conditions (Meeusen *et al.,* 2013) . The short-term or acute overreaching syndrome (OS) was divided by Meeusen *et al.* (2013) into 'functional overreaching syndrome (FOR)', and 'non-functional overreaching syndrome (NFOR)'. FOR is utilized by practitioners during certain training cycles to elicit specific effects on their athletes. Inducing FOR in the short-term, coupled with sufficient recovery, can cause a 'supercompensation' to occur. This supercompensation results in an athlete exhibiting enhanced performance when compared to their baselines measures. In practical terms, practitioners would usually look for this to occur after an intensive training camp. Recovery timelines were suggested by Meeusen *et al.* (2013) with FOR requiring days to weeks for recovery and NFOR requiring weeks to months to fully recover. Overtraining Syndrome (OTS) on the other hand requires months to fully recover from. Budgett (1998) highlighted several prevention strategies for OTS that must be considered by practitioners during intensified periods; adequate nutrition, maintaining hydration status and sufficient rest between training sessions. Additional commitments such as work, and family may also impact the recovery of athletes. A combination of monitoring tools may need to be employed in order to adequately monitor the progress of each of these strategies.

2.3.2 Strategies for Diagnosing OTS

The early review of over-reaching by Urhausen and Kindermann (2002), identified the need for a diagnosis process which was inexpensive, and may be measured either at rest or during submaximal exercise. Another very important element of a diagnosis process is ensuring that it causes minimal disruption to the training process. Appropriate monitoring strategies that can identify early risk factors are key in trying to prevent athletes experiencing the sustained poor exercise performance associated with both FOR and NFOR. In addition to diminished performance, altered sleep patterns, persistently high ratings of perceived exertion (RPE), mood disturbances, altered immune functions and acute and chronic alterations in systemic inflammatory responses can also be seen. It is important therefore, for coaches to ensure the proper implementation of overload training to ensure a systematic improvement in performance, without resulting in overreaching or overtraining. A balance must exist between training load and recovery to reduce the risk of a chronic imbalance, which subsequently

may negatively impact performance (Kreider, Fry & O'Toole, 1998). Halson (2014) examined other tools used to measure training load, both subjectively and objectively reported. Two main forms of training loads were identified; internal and external training load. In the following sections, a variety of measurement tools for both internal and external training load monitoring will be analysed.

2.4 Monitoring Training Load

2.4.1 Internal Training Load Monitoring

As discussed previously, internal load is described as an athletes' psychophysiological response to the external workload they complete during physical preparation and competitive match-play (Impellizzeri *et al.,* 2019). While it is important to utilise a range of load monitoring tools, as outlined by Halson (2014), coaches still strive to find the 'perfect' metric to monitor their players. In this section, a variety of internal load monitoring tools will be discussed, from the basic, sRPE, to the complex, heart rate monitoring. While there are pros and cons to all load monitoring tools, it is important to understand the clarity that a broad range of tools can provide as practitioners look to ensure their athletes are responding appropriately to the external load that the coach is manipulating (Impellizzeri et al. 2019), and attempting to prevent overtraining (Halson, 2014).

When measuring internal load, Profile of Mood State (POMS) questionnaires have long been used to determine athletes' responses to training load. As will be outlined later, Morgan *et al.* (1987), concluded a dose-response relationship exists between mood disturbance and training load in collegiate swimmers. Alternatively, training load (AU) quantified as sRPE x training time, provides practitioners with a unit that represents the internal load experienced by the athlete and was first proposed by Foster *et al.* (1996). Moreover, studies in more recent years have reported strong positive correlations between heart-rate derived measures of internal load and sRPE as they analysed the criterion validity of sRPE (Alexiou and Coutts, 2008).

The first step in diagnosing both FOR and NFOR is having the ability to quantify the load experienced by athletes to prevent excessive spikes in training volume and intensity. Despite the plethora of different monitoring tools out there, coaches may be driven to find 'the best' one to use with their players. Weaving *et al.* (2017), explored the influence of various training modalities, conditioning and skills, on the capability of a single training load measure in determining overall training load. The authors concluded that, as previously highlighted by Halson (2014), single measures when monitoring training load are insufficient. Using a single measure may not provide coaches with a comprehensive

insight into the players overall training load, especially when the training modality is considered as well.

2.4.1.1 Session RPE

The Rating of Perceived Exertion (RPE) was first introduced by Borg (1970) and was proposed as a measure of internal fatigue during a cycle ergometer incremental test. The scale was initially designed to increase in proportion to increases in heart rate (HR) and perceived muscle soreness. Additionally, muscle biopsies were conducted to determine muscle lactate. Later studies by Borg *et al.* (1985), utilized a modified scale that ranged from 0 to 10 in contrast to the 6 to 20 that was originally proposed. This research (Borg *et al.,* 1985) further investigated the psychophysical functions for physical exertion, aches and pain in the legs, HR and blood lactate, with larger groups of participants. An incremental cycle ergometer test was implemented whereby twenty-eight (n=28) healthy male students in good physical condition, who volunteered to take part in the study, warmed up for five-minutes before a stepwise increase in power output. Participants pedalled at sixty revolutions per minute (rpm), before a 40W increase every five-minutes until a voluntary maximum had been reached. Measurements for RPE and ratings of aches or pain in the legs were obtained during the last-minute of exercise at each power-level to determine variation across intensities. The final measure was recorded immediately before the participant stopped pedalling, with blood samples collected immediately post-test. Results concluded that, while there were low concentrations of blood lactate and low HR, the terminal values for ratings of aches or pain in the legs and RPE were both high. Despite varying levels of acceleration, the means increased systematically. Interestingly, the lower power outputs related less to perceptions of pain and more to exertion. Moreover, as power outputs increased and a subsequent increase in blood lactate ensued, perceptions of aches and pain increased accordingly. Resting HR values also increased gradually with increases in power. Blood lactate demonstrated a monotonic relationship with power. Pearson's correlations displayed strong positive relationships between ratings of perceived aches and pains in the legs and both HR and blood lactate (r = 0.82 and r = 0.76, respectively) and between RPE and both HR and blood lactate (r = 0.91 and r = 0.81, respectively). The relationships between RPE, HR, blood lactate and session intensity are key findings when determining the validity of the session-RPE measure used to quantify internal load in athletes.

It is important to consider, however, that the participants reached a voluntary maximum which may not be representative of an absolute maximum. It is important to note that psychological factors as well as motivation may contribute to the level of intensity reached by participants when asked to

reach a voluntary maximum (Djaoui *et al.,* 2017). Djaoui *et al.* (2017) explained that while sRPE may be reflective of session intensity, it is not independent of an athlete's psychological state or motivation for continuing to a maximum. This is where the recommendations for implementation of multiple monitoring tools stem from as depending on the session type, a single measure may not suffice (Weaving *et al.,* 2017).

In a more recent study conducted by Gaudino *et al.* (2015), the factors that influence sRPE in elite soccer players were explored. Twenty-two elite soccer players (mean ± SD; age 16 ± 6yrs, height 182 ± 7cm, body mass 79 ± 7kg) participated in the study, all of whom competed in the English Premier League. Player's RPE was collected in isolation ~20 minutes post-session before being multiplied by the session duration to equate the training load (RPE-TL), which will be discussed in more detail in the next section. External workload for the players was quantified using 10Hz (hertz) GPS units (STATSports, Belfast, Northern Ireland). Due to the collinearity of external load measures such as total distance, very high-speed running distance, and high metabolic power distance, only a few metrics were used. Total high-speed running distance (HSR; >14.4km.h^{-1}) and number of impacts and accelerations (>3m.s^{-2}) remained after the final multivariate model (*P*<.001). Several measures of external workload were significantly related to sRPE (*P*<.001) including HSR per minute, impacts per minute, and accelerations per minute. Despite the significant relationship that exists, the correlations described weak correlations with both HSR per minute and accelerations per minute (*r* = .141 and *r* = .249, *P* <.001, respectively). No relationship was reported for impacts per minute (*r* = .095, *P*<.001). These findings indicate that there are additional factors influencing the sRPE and associated perceived effort of competitive match play in elite soccer players.

2.4.1.2 Training Load

Further to RPE as outlined above, another common form of quantifying the load accumulated by athletes at any given time is by calculating their training load. Training load is equated by multiplying session-RPE (sRPE) by session duration. This method was used by Foster *et al.* (1996) whereby fifty-six competitive athletes, comprised of forty cyclists/speed skaters and sixteen runners, were monitored over twelve weeks of training. Session load value and weekly totals were then summated for the duration of the study. The training comprised of six weeks of baseline training at a subjectively moderate intensity, followed by six weeks of training at a self-selected training load increase. Available data indicated that there was no significant difference in the average weekly training time from moderate intensity phase (baseline) to self-selected training load (second evaluation) (mean ± SD 61 ± 12 *vs.* 71 ± 12min) , however, there was a significant increase in the mean training intensity as

determined by sRPE (mean ± SD, 3.8 ± 0.1 *vs.* 4.0 ± 0.1, respectively). Additionally, Foster *et al.* (1996) reported there was a significant increase in mean weekly training load as well as a significant decrease in mean time to completion on a standardised time-trial. Foster *et al.* (1996) demonstrated the quantifiable performance improvements associated with a relative increase in 'training load'. This model was the first to provide practitioners with the ability to quantify the work completed by their athletes and implement gradual increases to elicit improvements in performance.

Lovell *et al.* (2013) explored the relationships between sRPE and training load. The authors explored various training load measures, both internal and external, and analysed the relative contribution to sRPE. The researchers identified that sRPE has a very large correlation with total distance (r = 0.82) and a large correlation with HSR (r = 0.62). Intensity measures of relative TD ($m.min^{-1}$) and HSR were also analysed. These metrics quantify the absolute total and HSR distances covered by the athlete, relative to the number of minutes they were on the pitch for (Buchheit and Simpson, 2016). These metrics were reported to have moderate correlations with sRPE with moderate correlations reported for both relative TD (r = 0.47) and relative HSR (r = 0.30). These findings suggest that absolute measures of both total and HSR distances covered, relate better to sRPE, while the relative metrics do not reflect the differences in differences in subjective sRPE scores. Session-RPE has been previously correlated with blood lactate measures, showing a large correlation during cycle ergometer testing (Borg *et al.,* 1985). Together, these conclusions are relevant to the current research topic as the relationship between commonly collected internal and external load monitoring measures is explored further. It is important, however, to distinguish between positional demands when analysing any given sport when referring to distances covered, as will be discussed later.

As previously discussed, the research by Gaudino *et al.* (2015) reported that HSR, and number of impacts and accelerations, were significantly related to RPE-TL. Interestingly, HSR recorded a weak correlation with RPE-TL (r = .114), which would indicate that alone, HSR is not sufficient in determining the extent of the physical demands endured by a player during soccer training or match play. Number of accelerations however, reported a moderate correlation with RPE-TL (r = .371). These findings further support the idea that there are multiple contributing factors when determining the physical and psychological demands of team sports that no one metric can accurately quantify (Weaving *et al.,* 2017).

While RPE-TL may not be appropriate in providing practitioners with a clear indication of the external workload completed by athletes, as measured by HSR, impacts and accelerations (Gaudino *et al.,* 2015), it has been related illness incidence in elite academy rugby players (Tiernan *et al.,* 2019). Nineteen elite male rugby union players volunteered to take part in the study (mean ± SD, age 19.7 ±

1.1yrs, height 184.5 ± 7.7cm, and body mass 96.2 ± 12.5kg). Players trained 4-5 days per week with multiple sessions occurring per day. Players provided saliva samples an hour after waking, and prior to completing any training sessions, to determine their salivary immunoglobulin A (IgA) concentrations. Samples were collected on Monday and Friday mornings over a 10-week period. RPE-TL was also collected for all players to determine their weekly training load. Logistic regression determined that as salivary IgA significantly decreased, the risk of contracting an URTI increased (P = 0.046). There was a significant decrease (65% or more) in salivary IgA 1-2 weeks before players contracted an URTI (P < 0.001). While no significant association (P>0.05) was reported between salivary IgA concentrations and RPE-TL, it was noted that players' training load increased on average by 49% from the previous week of training, prior to contracting an URTI. While there was no significant associations between RPE-TL and contracting an URTI, the results still indicate that combining measures in internal load, as measured by salivary IgA, and external load, as measured by RPE-TL, creates a clearer picture for practitioners on how their athletes are responding to training.

2.4.1.3 Heart Rate Monitoring

When considering objective measures, which are usually a measure of external load, there are numerous methods available. Some of the most effective and widely used tools are Global Positioning Systems (GPS) and HR monitors, both worn by players during training and games. Alexiou and Coutts (2008) conducted a study on fifteen elite female soccer players over sixteen-weeks of training and matches. HR, sRPE and session duration were recorded for seven hundred and thirty-five individual training sessions and matches. Players took part in an average of eight sessions per week during the study; three technical sessions, two high-intensity resistance training sessions, one aerobic conditioning session, one core stability session, one pool 'recovery' session, and a competitive match. Three commonly collected HR derived measures of internal load were analysed; the TRIMP method, the Edwards HR-based method, and the HR-based approach based on lactate thresholds. From each player a minimum of twenty sessions of RPE and HR-based training load data were used. All three HR-based measures of internal training load demonstrated strong positive correlations with sRPE; Bannister's TRIMP (r = 0.84), Edward's (r = 0.83) and lactate threshold derived HR (r = 0.85). These data indicate that sRPE could be a reliable and valid measure of internal load due to its relationship with HR-derived internal load measures. The ease at which sRPE can be implemented with a large group of players is also an appealing factor when approaching the problem of training load monitoring as a practitioner. Furthermore, the relationships described by Alexiou and Coutts (2008) between sRPE

and commonly collected HR derived measures, shows that sRPE is a valid marker of internal training load.

2.4.1.4 Mood Disturbances and OTS

In addition to objectively measuring the internal load players experience from session to session, subjective measures allow another avenue to be explored by practitioners. Early research in American collegiate swimmers analysed POMS across a season (Morgan *et al.,* 1987). In 16 male swimmers, who completed the POMS questionnaire at the beginning, middle and end of a given indoor season, significant changes in global mood were observed. The authors reported significant increases in fatigue and decreases in vigour during the mid-season when training demands were highest. In another sample from the same study by Morgan *et al.* (1987), in fifteen female swimmers who were administered the POMS questionnaire in September and again after an overtraining stimulus in January, a significant disturbance in global mood was revealed, which is a combination of scores for tension, depression, anger, fatigue and confusion, before subtracting the score for vigour. Increase in global mood scores reflect poor psychological state, while reduced scores reflect an improved psychological state. The fifteen female swimmers demonstrated significant increases in depression and anger ($P<0.01$). In a third sample of athletes, twenty-two male and eighteen female swimmers completed the POMS questionnaire during a selected macro-cycle. For this period in males, spanning from September to March, mood disturbance demonstrated a dose-response relationship with training load. Average training load for the swimmers during the course of the study was approximately 3,000 yards per day in September at the start of the season which increased to a peak load of 11,000 yards per day in January when demands were highest. The peak load was followed by a taper that consisted of 5,000 yards per day prior to the conference championship. The female swimmers reported identical results, however there was an important finding whereby during a periodic taper included in the sampling period, the global mood for the female swimmers decreased in line with their training load. This taper occurred during an intensified block of training and resulted in improved performance at the following swim-meet. The reduction in global mood scores in the middle of this intense block when a taper was administered is an important finding for practitioners as it demonstrates that the increased perceived stress as a result of overreaching during an intensified training block, can be ameliorated by a properly administered taper. This may be an important consideration for conditioning coaches when designing training macrocycles, ensuring that they include a taper at the end of intensified blocks to ensure athletes are given sufficient time to recover and super compensate, prior to entering a new phase of training (Mujika, 2010). Another two samples

in the study by Morgan *et al.* (1987), reported similar dose-response relationships between training load and POMS scores. Mean POMS scores for both men and women's teams were 115 at the beginning of the season and increased to 135 for men and 140 for women with increased training and competition demands. While there were no significant differences reported between male or female swimmers for POMS scores, the way in which their scores were comprised differed (male; significant increase in fatigue and vigour *vs.* female; significant increase in depression and anger), as outlined in the first sample in this paper (Morgan *et al.,* 1987). While there may be individual psychological responses to training load, the results remain the same whereby a dose-response relationship appears between training load and POMS scores. The final sample in this study, carried out analysis on forty-four swimmers and eighty-six controls. The swimmers' group in the final study was combined following the results indicating no significant differences between POMS scores in male or female swimmers. This investigation intended to determine whether the previously outlined mood disturbances, were due to the training load associated with being a collegiate swimmer and the intense competition period, or the psychosocial effect of their academic responsibilities. Results demonstrated no significant changes in global mood scores in the control group throughout the thirteen-week sampling period (from September to early December), whereas the competitive swimmers experienced significantly higher global mood scores from the fifth to the eleventh week of the semester ($P<0.05$). These findings provide additional support to the idea that increased mood disturbances with overtraining are associated with the training stimulus rather than external stressors.

2.4.2 External Training Load Monitoring

While there is a myriad of ways to monitor your athletes as a coach, it is imperative that the modalities used are both valid and reliable, but also seamlessly integrate into the schedule of the team environment to minimise disruption and distraction. Coaches will run their teams differently and as a member of the backroom staff, employing effective athlete monitoring protocols that complement the systems implemented by coaches is essential.

A study was conducted by Akenhead and Nassis (2016), where high-level soccer clubs filled out surveys relating to their training load monitoring protocols. Forty-one surveys were included in the study (59% compliance rate), seventeen of which, were from the English Premier League. The most commonly used monitoring process was the analysis of time-motion, external training load variables. Variables relating to acceleration activity and distance covered above certain thresholds used to differentiate running, HSR and sprinting, were the most common. Akenhead and Nassis (2016) suggested that as injury prevention is an established objective of the monitoring process, that injury-mechanism studies

have identified areas which put soccer players at more risk, and it is these intense activities that contribute to the injury. For example, Gabbett and Ullah (2012) described the distances covered at mild, moderate and maximal accelerations, and at a moderate intensity movement velocity were the most significant risk factors for no-time-loss injuries. Time-loss injuries have been identified as any injury that results in any time lost in training or matches; ie. A player being removed from the field of play due to an injury, that prevents the player from returning that day (Williams *et al.,* 2016). Williams and colleagues (2016) reported that increases in the number of time-loss injuries has been negatively associated with team success. The same factors were discussed by Gabbett and Ullah (2012), were also said to predict the incidence rates for time loss injuries. No-time-loss injury risk was 2.7 times higher when very high-intensity running (> 7 $m.s^{-1}$) exceeded 9 m per session compared to less than or equal to 9m. Similarly, when very low-intensity running (1-3 $m.s^{-1}$) exceeded 542m per sessions and when the distance covered in moderate acceleration activity (1.12-2.78 $m.s^{-2}$) was >217m, there was a 60% lower risk of time-loss injury reported. These data demonstrate the protective effect that distances covered at various speed-thresholds can have. When the demands that are put on players are considered, especially when exploring individualised speed-zones and the inherent injury risk that is associated with this HSR, it must then be asked; under what conditions are these thresholds being applied.

Weaving *et al.* (2017) explored the effectiveness of a single load monitoring measure in determining the loading experienced by rugby league players during both skills and conditioning sessions. Four measures of loading were included in this study, two internal measures, namely, Heart Rate Exertion Index (HREI) and sRPE, and two external measures, namely, PlayerLoad™ and individualised HSR. HREI is a manufacturer-derived HR exertion index which follows the same principles as Edwards but utilises arbitrary exponential weighting zones to differentiate between the time spent in each zone, assuming zone 5 (above 90%) is more taxing than zone 1 (50-59%). Twenty-three professional rugby league players were monitored during the study. When specifically monitoring conditioning, the authors suggested that one of either PlayerLoad™, HREI or sRPE should be used in addition to individualised HSR. These recommendations by Weaving and colleagues (2017) for the use of a combination of internal and external measures of workload are evident throughout research. Halson (2014) first highlighted the importance of collecting multiple measures of both internal and external workload, like sRPE and GPS monitoring. Research conducted by Twist and Highton (2013) explored the effects of fatigue on rugby league players. Twist and Highton (2013) reported that in conjunction with psychological measures and blood-borne markers, there also appears to be marked changes in neuromuscular performance and performance tests. CMJ flight time was reported to decrease in addition to both power and force. Furthermore, running velocity was also noted as being reduced

post-match. HR was reported to stay the same post-match in rugby league players. The findings highlighting the inability for a single measure to accurately convey the load experienced by athletes, to the support staff working to effectively monitor both internal and external training load. Practitioners are encouraged to use a blend of measures to create a clearer picture of their athlete's workload and subsequent load tolerance..

2.4.2.1 Reliability and Validity of GPS in Team Sports

When the research by Akenhead and Nassis (2016) is considered, suggesting that GPS monitoring is one of the most common tools utilised by practitioners for load monitoring, in addition to the injury prevention strategies reported by Gabbett and Ullah (2012), the reliability and validity of the GPS units being used using to quantify these data, must be considered. Scott *et al.* (2016) completed a review on the reliability and validity of GPS in team sports and its application in quantifying the external workload of athletes. GPS units are categorised by the rate at which they sample per second. For example, a 1 hertz (Hz) unit would sample once per second, in comparison to a 10Hz unit which samples ten times per second. Increased sampling rates allows for more accuracy in determining how far and how fast the GPS unit moves. This information is then classified as distances covered at varying speeds, total distance or sprint distance for example, or simply a measure of velocity measured using metres per second ($m.s^{-1}$) or kilometres per hour ($km.h^{-1}$). Technological advancements led to the integration of triaxial accelerometers into the devices which use the sum of accelerations in 3 planes (X, Y and Z) to produce composite vector magnitude (expressed as G-force). Certain metrics like player or body load are the collation of all forces acting on an athlete and are measured using the accelerometer. The reliability of GPS units is an important factor to consider as there needs to be an element of reproducibility of values on repeat occasions. If two players run 100m, the measurement should be the same between units (interunit reliability), and, if the same unit was used for the same test on different occasions (intra-unit reliability). In this study by Scott *et al.* (2016), intra-unit reliability and validity are rated as acceptable or good if the variance is <5%. The authors suggested that all GPS units, regardless of their sampling rates, were capable of athlete tracking for distance with adequate interunit reliability. The 10 and 15Hz units appear to be more reliable and valid than the 1 and 5Hz units, particularly with regards to the measurement of mean and peak speed during team sport running (Johnston *et al.,* 2014), and also for distance reliability over 30m (Castellano *et al.,* 2011). There are, however, still limitations associated with shorter, high intensity running, subsequently affecting the validity of the measure of instantaneous velocity (Varley *et al.,* 2012). Practitioners are

advised to interpret data with caution and are advised to use broader velocity bands rather than having 5 or 6 smaller bands (Scott *et al.,* 2016).

The reliability and validity of the STATSports (Belfast, Northern Ireland) Apex 10 and 18Hz units in measuring distances and maximum velocity (Vmax) were explored by Beato *et al.* (2018). The 10 and 18Hz units were tested using twenty university students (mean ± SD, 21 ± 2yrs). Criterion validity was tested by comparing distances recorded by the units with ground truth reference for a 400m trial, a 128.5m circuit, and a 20m trial. Vmax was compared against the gold standard (Stalker ATS Radar Gun; Texas, United States of America), during a 20m sprint. The bias for the 10Hz unit for the 400m trial, 128.5m circuit, and 20m trial was mean ± SD 1.05 ± 0.87, 2.3 ± 1.1, and 1.11 ± 0.99%, respectively. Vmax bias for the 10Hz unit was mean ± SD 2.36 ± 1.67%. All of these biases were deemed as acceptable, based on research by Hopkins *et al.* (2009) whereby the bias was interpreted as poor, (>10%), moderate (5-10%), or good (<5%). The validity results for the 18Hz unit revealed similar results with the bias for the three distance tests being reported as mean ± SD 1.17 ± 0.73, 2.11 ± 1.06, and 1.15 ± 1.23%, respectively. Vmax bias was deemed to be mean ± SD 2.02 ±1.24% which was also reported as being acceptable. No difference between the 10 and 18Hz units was reported for any of the three distance trials or the Vmax trial. These data support the validity of both Apex models which are important when interpreting the research in this current study.

Once the reliability and validity of the units have been established, it is then important to question the purpose of the metrics being used by practitioners and how they are going to influence their training methods. Buchheit and Simpson (2016), discussed the application of player-tracking systems and how the various variables are used in team sports. This study suggested that using variables that are simple to understand and, as a result, can be used by practitioners at various clubs and at variable levels, are most appropriate. Secondly, valid and reliable metrics that can be trusted when decisions need to be made, must be used. This is where the inter- and intra-unit reliability, as discussed by Beato *et al.* (2018), becomes important, as lower Hz units may be less reliable when measuring high-intensity movements. Buchheit and Simpson (2016) divided metrics into three distinct categories; level 1 includes distances covered in various speed thresholds, level 2 relates to movements including a change in velocity, i.e. metrics derived from acceleration and deceleration data, and level 3 are metrics derived from inertial sensors or accelerometers. Level 1, which includes total distance (TD) and HSR, has been described as an overall measure of training load, while acceleration and deceleration patterns, described using the high metabolic load (HML) distance metric, relates to a more neuromuscular-oriented type of load which has an inherent injury risk that is important for practitioners to manage. The authors also questioned the reliability of accelerometer and inertial

sensor derived data in level 3, as there has been limited research to suggest they are reliable and valid metrics.

2.4.2.2 GPS Application in Rugby

Once practitioners have deemed their units both reliable and valid, and have selected appropriate metrics for their training modality, the next step is to determine the locomotion demands of the sport in order to quantify the external load of the players in relation to benchmark data. Cunningham *et al.* (2016) conducted a study to determine the movement demands of both elite professional junior players (U20) and elite professional senior players, both from an international performance squad. Forty-three U20 players and twenty-seven senior players participated in the study. Players were broadly grouped into forwards and backs before being sub-divided into six positional groups; front row, second row and back row (forwards), and half backs, midfield/centres and back three (backs). Fifteen games from two 6 Nations tournaments and one Junior World Cup were provided by U20s players, while senior players provided eight games across two 6 Nations tournaments. Players must have played 60min of the game or more to be included in the study. The senior squad won all eight games and the U20s won eight of ten games in the 6 Nations and four of five games in the Junior World Cup. Seventy-nine 10Hz GPS units (Viper Pod, STATSports, Belfast, Northern Ireland) were used during the study. To avoid inter-unit variability, players wore the same unit for every match. Relative TD, HSR, HML, sprint number, accelerations and decelerations (at varying intensities) were collected relative to playing time (expressed as per minute) with HSR thresholds set at 18.1km.h^{-1} or 5m.s^{-1}. Game time included time-off time, but did not include sin bin, periods on the bench, or half-time. As a result, game-time may exceed 80min. There was a significant interaction between team (U20 *vs.* Senior) and between positions (forwards and backs) for relative TD, relative HSR, accelerations 2-3m.s^{-2}, accelerations 3-4m.s^{-2}, decelerations 3-4m.s^{-2}, decelerations >4m.s^{-2}, HML distance, HML efforts and sprint.min^{-1}. Seniors covered significantly greater relative distance than U20s for backs (mean ± SD, 73.3 ± 8.1 *vs.* 69.1 ± 7.6m.min^{-1}; $P<0.05$), but not in forwards (mean ± SD, 66.8 ± 7.1 *vs*. 61.5 ± 8.0m.min^{-1}; $P>0.05$). U20s played significantly more match time than seniors which could be explained by substitution strategies or by other factors influenced by discipline and referees. The U20s forwards performed significantly more relative HSR, accelerations in zones 2–3 & 3-4m.s^{-2}, decelerations 3-4m.s^{-2} and sprint.min^{-1} than the seniors, but significantly less HML distance. Senior backs covered significantly more relative TD, decelerations 2-3 and 3-4m.s^{-2}, accelerations 3-4m.s^{-2} HML distance and efforts, but the U20s performed significantly more relative HSR and sprint.min^{-1}.

Cunniffe *et al.* (2009) described similar findings to Scott *et al.* (2016) when analysing the absolute distances covered by two elite senior rugby union players, during an 83min game. The two players, an out-half (back) and back row (forward), covered on average of 6953m during the game. A significant main effect was observed for TD across quarters. These findings, however, must be reviewed with caution due to the low sample size. Research by Cahill *et al.* (2013) described the physical demands of English Premiership rugby union players from multiple clubs. Ninety-eight elite players participated in the study. These players were from eight premiership clubs and they provided samples across forty-four games in the 2010/2011 season. A similar positional breakdown to that of Cunningham *et al.* (2016) was utilised by Cahill *et al.* (2013); forwards and backs with further subdivision into front row, second row, and back row for forwards, and scrum half, inside, and outside backs for backs. Additionally, they were categorised by individual positions due to the larger sample size, resulting in more players from each position. Players wore 5Hz GPS units and the overall mean ± SD time on the pitch was 91 ± 8min. TD and relative TD were calculated for players in addition to Vmax and average speed. Locomotion thresholds were set based on players Vmax and categorised as follows; <20% (standing/walking), 20-50% (jogging), 51-80% (striding), 81-95% (sprinting) and 96-100% (maximum sprint). The employment of these thresholds must be considered when comparing papers. When comparing the general positional groups, backs covered greater absolute and relative distances than forwards (median, 6545 *vs.* 5850m, respectively), which equated to an 11.9% greater difference in absolute TD. This difference was reflected in the relative TD (10.9%) where backs covered significantly more distance per minute than forwards (71.1 *vs.* 64.6m.min^{-1}, respectively). With reference to the speed thresholds, there were some noteworthy findings when comparing distances covered when jogging and striding between positional groups. While no significant difference was reported, backs were described as covering less distance jogging than forwards (2559 *vs.* 2616m, respectively), as well as less distance striding (822 *vs.* 860m, respectively). While a general measure of HSR distance was not reported by the authors, total distance covered about 50% Vmax may be compared to other papers, but with caution, as the previously reported thresholds by Cunningham *et al.* (2016) equated to 5m.s^{-1}. Considering Vmax for forwards was 26.3km.h^{-1} and 30.4km.h^{-1} for backs (7.3 and 8.4m.s^{-1}, respectively), a threshold set to summate distance covered above 50% Vmax would result in HSR thresholds of 3.6m.s^{-1} for forwards and 4.2m.s^{-1} for backs, respectively, which may result in large discrepancies between data sets.

While Scott *et al.* (2016) did not include a measure for HSR, absolute HSR distances for elite rugby union backs and forwards were outlined by Jones *et al.* (2015) after collecting data from thirty-three professional rugby players across six European Cup games and seven Celtic League games during the 2012/13 season. Each player provided an average of four samples, wearing 10Hz GPS units. Positional

groups were assigned in line with previous research by Cunningham *et al.* (2016). HSR thresholds were set at between 5.0 and 5.5m.s^{-1} which is similar to previous reported data (Cunningham *et al.,* 2016). Data indicated positional differences for absolute TD covered as well as HSR distance. The findings discussed by Jones *et al.* (2015), support those of both Cahill *et al.* (2013) and Cunniffe *et al.* (2009). Jones *et al.* (2015) reported a significant difference in HSR was also reported between inside (mean ± SD, 586 ± 182m) and outside backs (mean ± SD , 566 ± 171m) compared to half-backs (mean ± SD, 381 ± 172m) and both tight (mean ± SD, 147 ± 80m) and loose forwards (mean ± SD, 306 ± 171m). These data provide insight into the importance of applying individualised approaches to both training and recovery in elite rugby union rather than adopting a one size fits all approach.

2.4.2.3 GPS Application in Australian Football

Wisbey *et al.* (2010) analysed the physical demands of elite Australian Football League (AFL) players. Four years' worth of data (2005-2008 inclusive), from eight of the sixteen AFL clubs, were utilized in this study. TD, HSR (>18km.h^{-1} or 5m.s^{-1}) distance, accelerations and Vmax were recorded using 1Hz GPS units. Players were divided into nomadic/midfielders, forwards and defenders. During the 2008 season where the largest sample of GPS data was collected (793 files *vs.* 632 (2005), 244 (2006) and 80 (2007)), midfielders covered significantly more TD than forwards (mean ± SD: 12.3 ± 1.9 *vs.* 11.7 ± 2.0km, respectively) but not defenders (mean ± SD, 11.9 ± 1.7km). Interestingly, midfielders also played significantly fewer total minutes compared to both forwards and defenders (mean ± SD, min:s, 99:02 ± 14:19 *vs.* 103:28 ± 14:43 and 104:02 ± 13:12, respectively). Midfielders spent significantly more time running at high-speed (>5m.s^{-1}) than all other positions (mean ± SD, min:s, 5:29 ± 1:30). On average, forwards spent slightly more time at high-speed than backs (mean ± SD, min:s, 4:26 ± 1:09 *vs.* 4:23 ± 1:10, respectively).

2.4.2.4 GPS Application in Gaelic Football

Current research in Gaelic Football is lacking, however, Malone *et al.* (2016) was one of the first papers to quantify average running demand data for elite players using GPS units. Data was collected over the course of two full seasons (February – September) and included games from both the National Football League and the All-Ireland Championship. Data was only included if players completed the full 70min game. Fifty elite male Gaelic with a mean ± SD age of 24 ± 6yrs, who were squad members for 5 ± 3yrs, volunteered to participate in the study. The mean ± SD TD recorded equated to 8160 ± 1482m with a mean ± SD intensity of 116 ± 21m.min^{-1}. Of this distance, a mean ± SD of 1731 ± 659m

was covered at high speed (≥ 17km.h^{-1} or 4.7m.s^{-1}). It is important to note that current literature by Malone *et al.* (2016) which has set HSR thresholds at ≥ 17km.h^{-1} (4.7m.s^{-1}), as may impact distances when analysing data from different sources. Mean ± SD Peak running velocity was 30.3 ± 1.8km.h^{-1} with a mean ± SD average velocity of 6.5 ± 1.2km.h^{-1}. A significant main effect was reported between positions. Midfielders covered significantly higher absolute (mean, 9523m) and relative TD than all other positions. Similar distances were also reported in AFL midfielders (Wisbey *et al.,* 2010). Malone and colleagues also reported that both Gaelic football half backs (8700m) and half-forwards (8952m) had higher values than both inside lines; full-backs and full-forwards (6892 and 7090m, respectively). Similar results were reported for HSR with Gaelic football midfielders (2228m), half-backs (1784m) and half-forwards (1884m) covering greater HSR distances than full-backs (1369m) and full-forwards (1366m). A similar trend was outlined for sprint distance. No significant main effects were revealed for playing position in the elite Gaelic football players for either peak or mean velocities. When comparing the first- to the second-half, significantly less HSR distances were covered in the second half. Gaelic football midfielders had the largest decrement in HSR distance from first- to second-half when compared to all positions. Additionally, half-forwards experienced a greater reduction in HSR distances than full-forwards and full-backs only. No significant main effect was observed by Malone and colleagues (2016) for playing position in elite Gaelic footballers for TD between halves. These data would indicate comparable results for relative distances between halves.

Subsequent research by Malone *et al.* (2017), further analysed the game demands of elite Gaelic football. Fifty players with a mean ± SD age of 24 ± 6yrs, who were squad members for 5 ± 3yrs, volunteered to participate in the study. Game data from three full seasons were used for analysis. Data was only used if a full 70min game was played. The first and second halves of the Gaelic football game were further broken down into quarter one and two and quarter three and four, respectively. A significant main effect for quarter of play was observed. Significant reductions in TD were observed in quarter two, three and four when compared to quarter one. Similar significant reductions were also reported for HSR distance, sprint distance and number of accelerations. Regarding positional differences in the elite Gaelic footballers, midfielders experienced the greatest decrement in both TD and HSR distance across quarters when compared to all other positions. This is in line with previous research by Malone *et al.* (2016) that reported Gaelic football midfielders experiencing the greatest decrement in both TD and HSR distance from the first to the second half when compared to all other positions. Gaelic football midfielders and half-backs experienced the greatest decrements in sprint distance when compared to both full-forwards and full-backs. The number of accelerations completed by midfielders decreased most across quarters when compared to all other positions in elite Gaelic football.

Moreover, in research conducted on twenty-six elite Division 1 Gaelic football players, seasonal variations in performance characteristics were observed (Kelly and Collins, 2017). The elite Gaelic football players were assessed at the start of pre-season (November), following early in-season (January) and mid-season (March). Performance was measured using a squat jump (SJ) and countermovement jump (CMJ), 5-, 10- and 20m sprint times, upper and lower body strength (1RM) and Yo-Yo intermittent recovery test: Level 2 (Yo-Yo IR2). Significant increases were observed between November and March for both SJ and CMJ (10.1 and 9.8%, respectively). Positional differences revealed that Gaelic football midfielders were significantly slower over 5m and 10m when compared to half-backs. Half-forwards were also significantly slower than all other positions in the 20m sprint. While all Gaelic football positions in the research by Kelly and Collins (2017) showed increases in Yo-Yo IR2 performance from November to March, half-forwards covered significantly more distance when compared to full-forwards, half-backs, and full-backs who covered the least distance (mean ± SD, 1840 ± 335 *vs.* 1200 ± 202, 1580 ± 305, and 1424 ± 209m, respectively). When comparing 5 and 10m sprint times, midfielders were significantly slower over both distances than all positions, while half-backs possessed the fastest times for both distances (Kelly and Collins, 2017). These data indicate that the positional differences in elite Gaelic football players reported by Malone *et al.* (2017) in Gaelic football games can also be observed when comparing pre- and in-season performance testing data (Kelly and Collins, 2017). Considering these results in conjunction with perceived effort and mood is important for practitioners when monitoring internal training load.

RPE and training load have previously shown moderate correlations (Foster *et al.,* 2001) as well as strong correlations with relative TD and relative HSR (Lovell *et al.,* 2013). Furthermore, increased POMS scores have also been reported during intensified periods of training (Morgan *et al.,* 1987). Having identified the seasonal variations in performance metrics in GAA athletes, as well as understanding the relationships that increased load and intensity has on players from a physical and psychological standpoint, it is essential for coaches to monitor a multitude of internal and external load measures to create a clear picture of players well-being across a season. Quantifying the external load that players have been exposed to as well as monitoring their psychological responses to the training, lays the foundations for a rounded athlete monitoring program.

2.4.2.5 Individualisation of HSR Thresholds

While current literature in the GAA (Malone *et al.,* 2016; 2017) utilises HSR thresholds set at ≥17km.h^{-1}, it is important to note that this absolute threshold may result in significant inter-individual differences when compared to relative thresholds. Reardon *et al.* (2015) discussed the application of

individualised speed thresholds in rugby union to categorise the locomotive demands of elite players. The primary issue addressed by the authors was the application of an absolute speed threshold. The authors suggested that when a relative or individualised speed threshold is applied, there is a significant shift in the interpretation of the HSR demands of both forwards and backs in rugby union. With the relative thresholds applied, it was concluded that there was a significant overestimation of HSR for backs and a significant underestimation of HSR for forwards. The authors suggested that utilising individualised thresholds, at 60% of a players' Vmax, has been identified as being more reliable than absolute thresholds. When the aforementioned data (Malone *et al.,* 2016; 2017) is compared to the suggestions in this literature, comparing 4.7m.s^{-1} to a player's top speed, equates to a peak running velocity of 7.8m.s^{-1}. Default HSR bands on STATSports (Belfast, Northern Ireland) GPS units are set at ≥ 5.5m.s^{-1}. This would equate to 60% of 9m.s^{-1} which would significantly impact the accumulation of HSR metres when compared to ≥17km.h^{-1} as previously used. For the purpose of this research, the default STATSports (Belfast, Northern Ireland) thresholds for HSR were used. This being considered, there is still a subsequent reduction in HSR demands for Gaelic Football. Moreover, these individualised thresholds may influence the positional differences observed in previous research in Gaelic football (Malone *et al.,* 2016; 2017). Further research is warranted to establish the differences between positions for elite Gaelic football players using individualised HSR thresholds.

While rugby union playing positions require significantly different anthropometric characteristics and body types, in comparison to sports like soccer or Gaelic Football, it is important to consider the relative demands of players in different playing positions. The nature of the sport, as well as tactical considerations, will have a significant impact on players' running demands. Most importantly, the introduction of relative speed thresholds will allow coaches to monitor the running demands of individual players as well as explore the positional demands of their players under certain tactical conditions. From an injury management and training load tolerance perspective, these relative thresholds for HSR will give benchmarks for younger or less experienced players who are attempting to make the step up in playing level. Having a better understanding of the HSR demands of the position will allow conditioning coaches to ensure there is sufficient exposure to these high-loads in training, as suggested by Gabbett and Ullah (2012), who established that low- and moderate-intensity running had a protective effect against injury. Furthermore, research by Duhig *et al.* (2016) indicated that increased playing experience in AFL reduced hamstring strain incidence.

Further to the Level 1 (distance covered in various speed zones) and Level 2 (changes in velocity) GPS metrics being utilized, as recommended by Buchheit and Simpson (2016), research by Tierney *et al.* (2018) introduces the novel concept of high-speed running density (HSR%). Its purpose in the literature is for interpreting a variety of return-to-play running sessions during the rehabilitation

process in Rugby Union. This metric can also be applied in Gaelic football as it has been shown that players in the middle-third accumulate significantly more TD and HSR than players in the inside; full back and full-forward lines (Malone *et al.,* 2016). These positional differences between forwards and backs are also evident in rugby (Cunniffe *et al.,* 2009; Cunningham *et al.,* 2016; Cahill *et al.,* 2013; Jones *et al.,* 2015) as well as in Australian Football League midfielders, forwards and defenders (Wisbey *et al.,* 2010). While the middle third may cover greater HSR distances, the way in which these metres are accumulated, as defined by the HSR%, may contribute to both the perceived and relative difficulty (ie. if two players cover 100m of HSR, was this accumulated through one long sprint, resulting in a lower HSR%, or twenty short sprints, resulting in a higher HSR%) for the players involved. The addition of this metric allows us to explore another dimension of the running demands imposed on players in various positions in Gaelic football.

Performance staff and coaches need to consider the positional running demands that their players experience in competitive games. This provides the basis for the question surrounding recovery both pre- and post-game. Research has demonstrated that there are positional differences in fitness levels (Kelly and Collins, 2017) and game running demands (Malone *et al.,* 2016; 2017), yet practitioners persist in applying the same recovery timelines to all players. The purpose of this research, aside from understanding the activity of immunoendocrine markers in response to competitive Gaelic football match-play, is to explore the relationships between these internal load measures, and commonly collected external load as measured by GPS. The metrics to be examined are TD and HSR, both absolute and relative, as well as HSR%. While absolute measures of distances accumulated in games have been identified as key factors in injury management through load monitoring (Buchheit and Simpson, 2016; Gabbett, 2016), it is hypothesized that relative GPS metrics (relative TD and HSR%) will dictate the recovery timelines of players better than their absolute counterparts (TD and HSR).

2.5 Effects of Competitive Match-Play on Athletes

2.5.1 Hormonal Fluctuations after Exercise

Further to the modalities discussed above, there has been a significant body of research that explored the hormonal response of athletes to both training and matches (Hoffman *et al.,* 2005; Elloumi *et al.,* 2003; West *et al.,* 2014; Cunniffe *et al.,* 2011; Coutts *et al.,* 2007). Those that analysed T and C as well as their ratios, are outlined in Appendix A. Fluctuations in the hormonal markers, T and C in response to fatigue, were first outlined by Adlercreutz *et al.* (1986). The authors explored the effects of

prolonged intense exercise on the hormonal markers of long-distance runners and discovered that intense exercise interventions induced a significant increase in plasma C concentrations and a significant decrease in plasma T concentrations compared to control days. The physiological variables investigated by the authors were plasma, free and saliva T:C ratios, as well as serum sex hormone binding globulin (SHBG) and GH concentrations. The results concluded that the plasma T:C ratio was the best test at categorising the runners into three categories; non-overstrain, overstrain and uncertain. Overstrain was classed as a decrease in free plasma T:C ratio by more than 30%. The ratio for participants with no symptoms of overstrain were negative, and positive for participants in the overstrain category. All other tests gave false positives, particularly the saliva T:C ratio. The false positive results are likely explained by the fact that a reduction in T concentrations and T:C ratios as well as an increase in C concentrations occur in participants who do not exhibit the qualitative effects that are seen in the overstrain group. These findings become more apparent in later research that is discussed in the latter sections of this review, whereby hormonal fluctuations are used as a monitoring tool rather than a diagnostic tool (Coutts *et al.,* 2007). Moreover, these data may have been affected by external factors, such as, diet or sampling times (Hayes *et al.,* 2016). Recent research reviewed the effects of competitive match-play on player hormonal status, and determined that hormonal status appears to return to baseline approximately 48h post-match, while markers of muscle damage as measured by CK, appear to remain elevated for 72h post-match (Doeven *et al.,* 2018). Furthermore, immunosuppression appears to peak 38h post-match as reported by Cunniffe *et al.* (2010). In addition to these immunoendocrine fluctuations, neuromuscular performance also appears to be compromised for between 48- and 60h post-match as measured using CMJ (West *et al.,* 2014) and maximal voluntary contractions (Doeven *et al.,* 2018).

2.5.2 Anticipatory Hormonal Responses

In addition to the fluctuations of hormones in response to acute exercise outlined above, there are additional factors which may contribute to the reported fluctuations in hormonal responses around competitive match-play, including the circadian rhythm (Passelergue *et al.,* 1995; Lange *et al.,* 2010). Moreover, pre-competition anxiety (Salvador *et al.,* 2003) and the motivation to win (Zilioli and Watson, 2013) have also been reported to affect hormonal responses. Interestingly, during a 24-week period of combined strength and endurance sessions, athletes who completed the sessions in the evening experienced great increases in muscle cross-sectional area from week 13-24, compared to those who completed the session in the morning ($P<0.05$).

Salvador *et al.* (2003), analysed the effect of pre-competition anxiety on hormonal responses to judo competitions. Seventeen male judo competitors provided samples over 8 resting sessions, once every fortnight, performed at the same time of day, during the second part of their season (February-June). Approximately half-way through the testing period, participants competed in a regional competition which occurred at the same times as the resting sessions. T and C concentrations were determined 1h before, as well as 30min before competition. Mood, anxiety and expectancies were also evaluated using POMS and State Anxiety Inventory (STAI-S) questionnaires. Participants warmed up after the second set of questionnaires were completed. No significant differences were reported between resting sessions, however, a non-significant reduction in both mean T and C concentrations were observed between samples one and two for all resting sessions (P>0.05; mean ± SD, 203.6 ± 15.4 *vs.* 190.0 ± 13.4, and 10.9 ± 1.0 *vs.* 8.1 ± 0.6pmol.L^{-1}, respectively). T concentrations reported significant negative correlations with fatigue (r = -0.63), while C concentrations described significant positive correlations to possibilities of winning (r = 0.64), in the second sample collected 30min before competition. C fight or flight response may explain the positive correlations observed between pre-competition concentrations and the perceived possibility of winning (Crewther *et al.,* 2011). The dual effects discussed by Crewther *et al.* (2011), outline the effects both T and C have at a cellular and system level, subsequently improving the function of the CNS and PNS which can positively contribute to neuromuscular performance. Moreover, research conducted by Parfitt and Pates (1999) determined that self-confidence had a significant effect on successful passes (R^2 = 0.51; P<0.01) and assists (R^2 = 0.65; P<0.01). Alternatively, state anxiety did not appear to effect successful passes or assists (R^2 = 0.15; P<0.01 and R^2 = 0.16; P<0.02, respectively). Salvador *et al.* 2003 concluded that C concentrations were significantly higher in competition than those for resting sessions. These findings are important considerations when comparing pre- and post-game hormonal responses later in this discussion as there may be implications for the magnitude of change observed.

Earlier research by Eubank *et al.* (1997) conducted a similar study using ten elite male canoeists which is outlined in Appendix A. The participants were competing in a marathon race (42km) and their state-anxiety was evaluated using a Competitive State Anxiety Inventory-2 (CSAI-2). Pre-race catecholamine concentrations were also measured by collecting urine samples from the participants. Additionally, blood samples were collected to investigate both serum T and C concentrations. All testing variables were collected, 24-, 2- and 1h before the race. Participants were split into facilitatory and debilitatory groups based on their scores from the CSAI-2. The former represented the participants who possessed a more positive cognitive and somatic state anxiety direction response compared to the debilitatory group. Moreover, the facilitatory group displayed consistently higher intensity self-confidence response compared to the latter. Significant interactions between group and time-to-event were

reported for T, but not C concentrations. T concentrations in the debilitatory group were significantly higher 24h pre-race than the facilitatory group. No significant difference was observed between groups at 2h pre-race, however, at 1h pre-race, the facilitatory group had significantly higher T concentrations than the debilitatory group. While no group by time interaction was observed for C concentrations, interestingly, plasma C concentrations did increase significantly from 2- to 1h pre-race for the debilitatory group. The facilitatory group exhibited consistently lower C concentrations than the debilitatory group which is similar to results reported by Salvador *et al.* (2003). The facilitatory group had a higher and faster rate of increase in all three catecholamines (epinephrine, norepinephrine and dopamine) 2h pre-race, while the debilitatory group remained stable with a slight trend for increasing. These data support those reported by Salvador *et al.* (2003) and would suggest that anxious, or a debilitatory group of players, would experience significantly lower T concentrations in the hour's pre-game with concomitant higher C concentrations, with the opposite being true for non-responders or a facilitatory group. The latter group also experience a sharp increase in catecholamines 2h pre-race. These findings are important considerations for researchers when analysing and comparing data between groups and time-points. Findings relating to the differences between facilitatory and debilitatory groups as outlined by Eubank *et al.* (1997), highlight the need for the inclusion of subject POMS questionnaires as well as objective hormonal measures to ensure state-anxiety can be accounted for when comparing pre- vs. post-competition hormonal concentrations. These data also emphasize the individual responses of athletes to competition stimuli, even prior to the external load accumulated as a result of competition.

2.5.3 Time-Course Response of Salivary Biomarkers in Soccer

Early research focused on the fluctuations of hormones across sporting seasons as researchers hypothesised that fluctuations, similar to those initially reported (Adlercreutz *et al.,* 1986), may be seen in athletes at various timepoints in the season when there is a variation in training load. Filaire and Lac (2003) examined changes in haematological, metabolic, immunological, hormonal and psychological markers, over the course of a competitive soccer season in a top French professional league. Sampling was conducted with twenty participants, all of whom competed at professional level in a top French league, providing samples at four-time points across the season. Baseline samples were collected at the beginning of pre-season, post-holidays (T_1). Follow-ups were collected at the end of the early season, at the end of September (T_2). Both serum and saliva samples were also collected in April, at the end of the competitive season (T_3), and again in July before the next season commenced (T_4). Serum samples were analysed for immunoglobulins A (IgA), G (IgG) and M (IgM), in

addition to leucocytes, neutrophils and lymphocytes. Glutamine (Gln) and uric acid were also measured using blood samples. Saliva samples were collected and subsequently analysed for T, C concentrations. The intensified period of training (between T_1 and T_2), commonly associated with 'pre-season' training, induced significant decreases in Gln concentrations and an increase in salivary C concentrations. Serum immunoglobulin A (IgA), G (IgG) and M (IgM) concentrations remained unchanged throughout the study. Despite the decrease in serum Gln concentrations, incidence of upper respiratory tract infection (URTI) only occurred twice during the season and thus, immune suppression related to the reduction in serum Gln concentrations is not obvious. As a result, this may not be a reliable marker of immune suppression in professional soccer players. Salivary C concentrations increased significantly between T_2 and T_3. The training during the same period induced a nonsignificant reduction in salivary T concentrations and the T:C ratio. In a study using a similar population, Moreira *et al.* (2009) collected saliva samples immediately before and after an internal training game with a top-level male professional soccer team. Twenty-two male professional soccer players participated in the study. Players competed in the Brazilian Division A league. Players provided saliva samples 10min prior to the warm-up as well as approximately 10min post-session. The training match took place during the competitive season. No significant difference was reported between Team-A and Team-B for either pre- or post-game C concentrations (P>0.05; mean ± SD, 7.6 ± 4.4 *vs.* 8.8 ± 3.0 and 12.1 ± 6.0 *vs.* 10.3 ± 6.2ng.mL^{-1}). Analysis was subsequently conducted to explore the fluctuations in individual samples from pre- to post-match. A significant difference was, however, reported between players (P<0.05). The authors examined individual changes pre- *vs.* post- game. No significant correlations between RPE and salivary C absolute changes between teams were reported (P<0.05). While no significant changes were observed for absolute C concentrations (P<0.05), there was a general trend to change between pre- and post-game. Seven players increased post-game while five players decreased post-game. These data are interesting when compared to those observed by Elloumi *et al.* (2003) collected samples before and after pre-season and reported significant increases in C, yet pre- *vs*. post- C concentrations for an internal-game yielded no significant results. While the RPE rated the game as above "hard", it may not have been intense enough to elicit significant C increases (Moreira *et al.,* 2009). The individualised approach taken by the authors is interesting as it demonstrates the individualised responses to intense exercise, reporting an increase in C concentrations in post-match samples when compared to pre-game samples (Moreira *et al.,* 2009). Furthermore, this was the first study to report hormone concentrations both pre- and post-competition. It is important to note that while Elloumi *et al.* (2003) highlighted a significant change between the start and finish of an intensified training block, the teams pre-season, data reported by Moreira *et al.* (2009) had confounding results when analysing samples pre- and post- an internal game.

This research suggested that chronic responses may be observed in professional soccer, however, results are unclear in an acute setting due to the individual nature of the hormone responses as a result of the variability in the exercise stimulus. Positional running demands have previously been discussed by Bloomfield *et al.* (2007), identifying a variety of differences between positions. These differences are not addressed in the research conducted by Moreira *et al.* (2009) but may have contributed to the hormonal responses to the match-play.

2.5.4 Time-Course Response of Salivary Biomarkers in Rugby Union and League

In addition to the research conducted across soccer seasons and match-play, studies investigating rugby have analysed the effects of competitive match-play on players' hormonal status. Early research by Elloumi *et al.* (2003), as seen in Appendix A, analysed the behaviour of C, T and the T:C ratio during a rugby match and the post-competition recovery days. Twenty Tunisian international rugby players participated in the study. Participants provided saliva samples on three rest days, two months prior to the study, to determine reference hormonal concentrations that were used as baselines for comparison. Samples were collected at 8am, 4pm and 8pm on these days. On the day of competition, four samples were provided at 8am, 4pm (post-game), 6pm and 8pm. Twelve samples were then collected across the six-day period (Monday - Saturday) of post-competition recovery at both 8am and 8pm each day. Interestingly, mean ± SD reference C concentrations on rest days ranged from 17.0 ± 1.4nmol.L^{-1} at 8am to 5.9 ± 0.6nmol.L^{-1} at 8pm. These changes represent the effect circadian rhythm has on C concentrations (Lange *et al.,* 2010), and must be considered when interpreting results. C concentrations recorded at 4pm on competition day were significantly higher (148%) than at 4pm on rest days which reflects the effect competitive match-play has on homeostasis. No significant difference was observed at 8pm on competition day, however the post-competition C concentrations at 8pm were statistically lower than those observed at 8pm on a rest day which would suggest players had recovered from the strenuous exertion during competition. Furthermore, C concentrations were statistically lower during the first four days of recovery (Monday-Thursday) than during the rest day at the same time. T concentrations at 8pm on the rest day were significantly lower than those reported at both 8am and 4pm on the same day. T concentrations at 8am and 8pm on the day of competition were similar to those reported on the resting days. Post-game T concentrations were significantly reduced (-16%) when compared to the reference values. T concentrations followed a similar recovery timeline to C concentrations with no significant differences being observed at 8pm on competition day when compared to 8pm on the rest day. During the recovery period, 8am T concentrations were

significantly higher than reference concentrations from rest days, on Monday, Wednesday and Saturday, while 8pm T concentrations were significantly higher on Monday and Tuesday and Saturday. T:C ratios measured on the day of competition were similar to those recorded on rest days. Post-match (4pm) T:C ratios were significantly lower than rest day values. During the first 4 days of recovery (Monday-Thursday), 8am T:C ratios were significantly higher than rest days, while 8pm T:C ratios were significantly higher on Tuesday and Wednesday only. Notably, the pre-competition anxiety and anticipation outlined by Salvador *et al.* (2003), was not evident in this research. Elloumi *et al.* (2003) suggested that this may have occurred as the 8am concentrations were representative of 6h pre-game rather than immediately pre-game or 1h pre-game as implemented by Salvador and colleagues (2003). Six hours pre-game may be too early to observe the higher C concentrations associated with pre-game anxiety and anticipation or the increased T concentrations.

More recent research by Cunniffe *et al.* (2011), adopted similar methodology to Elloumi *et al.* (2003), however, the authors collected samples over a three week period, including samples from two competitive games. Samples were also collected at fewer timepoints to see a general trend in training load tolerance and recovery. A summary of timelines and data can be observed in Appendix A. Cunniffe *et al.* (2011) collected less samples as they mapped the hormonal responses of the Welsh international players during a three-week international series. Serum samples were collected from eight players at various time points around two games over a three-week block. There was one game played between games one and three where no samples were collected. A baseline measure was collected at camp-entry, after a minimum of 48h rest from rugby activity, while game samples were taken immediately before and after the game, as well as 14- and 38h post-game. Samples were analysed for changes in serum C-reactive protein (CRP), T, C, blood leukocytes, interleukin-6 (IL-6) and creatine kinase (CK). When T and C concentrations were analysed, a significant main effect of time was observed for both T and C concentrations. C concentrations significantly increased after both games and returned to baseline after 38h. T concentrations significantly decreased post-game and recovered to within pre-game concentrations after 38h. T:C ratios increased throughout the three-week series which suggests that this could represent the accumulated fatigue associated with competition. T:C ratios were significantly higher than camp-entry at 38h post-game. CRP concentrations decreased gradually with pre- and post-game concentrations significantly lower than at camp-entry. The authors suggested that the higher pre-camp values may have been as a result of an intensified period of training that was undertaken in the lead up to the three-week series.

The most recent hormone study conducted in professional club rugby was conducted by Scarlets Rugby in Wales (West *et al.,* 2014). The hormonal responses observed in this study can be seen in Appendix A, alongside the data for research by Cunniffe *et al.* (2011) who reported changes in

hormonal concentrations across varying recovery periods. The novelty of the study by West *et al.* (2014) was the inclusion of a subjective measure of fatigue, as well as an objective, neuromuscular test to assess physiological fatigue. The subjective measure used was a brief assessment of mood state (BAMS) questionnaire which allowed the author to consider the athletes perceived level of fatigue in addition to their hormonal responses. Baseline samples were collected 3h pre-game with sampling repeated at 12-, 36- and 60h post-game. Saliva samples were used to measure both T and C concentrations and subsequent T:C ratios. CMJs were performed by the athletes to quantify both peak power output (PPO) and jump-height, both of which were used to determine lower limb neuromuscular fatigue (NMF). Results for the fourteen rugby union athletes, indicated that an increase in NMF can be expected for up to 36h post-game with NMF returning to baseline levels at 60h post-game. There was a significant effect for time in post-match changes for both PPO and jump-height. PPO decreased below levels recorded 3h pre-game, at both 12- and 36h post-game but had returned to pre-game levels at 60h post game. In addition to the NMF observed, there was a significant effect for time in player mood disturbance scores. The psychological effects, however, were evident for 36h post-game in comparison to 60h for NMF. It is important to note that mood disturbances were not related to changes in PPO or any salivary hormone concentrations at any time point post-match, despite being significantly altered at 12h and returning to pre-game concentrations after 36h. Regarding salivary hormonal concentrations, T, C and T:C ratios followed similar trends to those observed by Cunniffe *et al.* (2011), who described disturbances in all three variables at 38h post-game. T concentrations reduced significantly post-game and remained low even at 36h post-game. T concentrations returned to baseline at 60h post-game. The inverse was true for C concentrations as they increased 12- and 36h post-game when compared to initial concentrations recorded pre-game. Concentrations similar to 3h pre-game were observed 60h post-game. T:C ratios followed a similar trend to T concentrations and decreased below baseline concentrations 12- and 36h post-game but had returned to pre-game concentrations at 60h post-game. Lindsay *et al.* (2015) contributed to the aforementioned body of research (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2011; West *et al.,* 2014) when reporting a significant increase in salivary C concentrations that returned to baseline 17h post-game. The authors did not measure T concentrations in this study (Lindsay *et al.,* 2015). Eleven rugby players from two senior men's division one rugby teams in New Zealand participated in this study. Post-game effect sizes were measured and presented using partial eta squared (ηp^2). A large effect size for C concentrations immediately post-game was reported ($\eta p^2 = 0.583$). The authors identified the negative impact mean concentrations can have on significance and as a result included the partial eta squared (Lindsay *et al.,* 2015). These data outlined by Lindsay *et al.* (2015) are extremely noteworthy as they are the first to suggest that the P value masks the real findings of the research. As discussed by Hopkins

et al. (2009), the significance of findings as reported by the P value is drastically impacted by the sample sizes which have been small in practical research, especially those reporting the hormonal status of both rugby (Elloumi *et al.,* 2003; Coutts *et al.,* 2007; Cunniffe *et al.,* 2011; West *et al.,* 2014) and soccer players (Moreira *et al.,* 2009; Lindsay *et al.,* 2015) in response to competitive match-play. While early research described significant alterations in both T and C concentrations post-game (Cunniffe *et al.,* 2011; West *et al.,* 2014), some research reported no significant changes in hormonal concentrations across timepoints (Moreira *et al.,* 2009; Lindsay *et al.,* 2015). The latter data that described a lack of significance in their findings, further analysed the data and reported the individual data that described some players as having an unexpected decrease in C concentrations. Lindsay *et al.* (2015) went on to report the partial eta squared which described a large effect size as outlined above for post-game C concentrations. The inclusion of the effect size has been suggested as more pertinent as it reports the effect rather than the significance, allowing real world research to explore the effect rather than misinterpreting a non-significant result by accepting the null-hypothesis and reporting no differences in findings (Hopkins *et al.,* 2009). Furthermore, the inclusion of a magnitude-based inference, allows researchers to explore the positive and negative effects as reported by the confidence intervals, as substantially beneficial or harmful which provides a much clearer practical context for findings.

A unique study in this area was conducted by Coutts *et al.* (2007) utilising an intervention study with eighteen semi-professional rugby league players, to explore the effects of overtraining and has remained the only randomised control trial (RCT) in the discipline. Participants were divided into a control and an intentionally overreached group. Analysis of the physiological and hormonal effects caused by NFOR in comparison to normal training was, therefore, possible within this study design. Various measures were tested both pre- and post-intervention. The tests used were, a multistage fitness test (MSFT), $\dot{V}O_2$max, peak aerobic running velocity (MAS), maximal HR, vertical jump, 10-s cycle sprint performance and body mass. In addition to the performance tests, hormonal, haematological and immunological parameters were measured, some of which matched those used by Elloumi *et al.* (2003). T, C and the T:C ratio, were used as in the aforementioned study, while plasma glutamate (Glu), creatine kinase (CK) and Gln to Glu (Gln:Glu) ratio were also used. Interestingly, the only physical parameters that showed a significant difference between the intensive and normal training group were results in the MSFT and $\dot{V}O_2$max, as they were significantly reduced in the IT group. While significant decreases in T concentrations and T:C ratio were reported, no significant differences were reported between groups. No significant differences were observed for C concentrations across time or group.

Each study contributed in its own way into progressing this area of research, examining and reporting the time-course of hormones and recovery in response to competitive match play. The improvements in study design between Cunniffe *et al.* (2011) and West *et al.* (2014) was the inclusion of a measure of NMF as well as the additional time point at 60h. The introduction of the brief assessment f mood state (BAMS) questionnaire by West *et al.* (2014) built on the early research conducted by Filaire and Lac, (2003), and analysed the mood disturbances around a single match rather than a competitive season, where acute changes could not be observed. These, alongside several other studies are outlined in Appendix A where comparisons can be made. Hormonal analysis provides coaches with an insight into the internal demands placed on players from both an acute and a chronic perspective. It is evident that players are affected by intense training periods and this is reflected in their hormonal status, measures of NMF, and their responses to BAMS questionnaires.

The evidence suggests that players must be monitored using a number of load monitoring tools to ensure that both the objective and subjective data collected, represents the individual nature of training load tolerance and the subsequent recovery. These findings echo the suggestions of Halson (2014) who suggested that coaches implement a range of monitoring tools to adequately manage their players as they strive to optimise recovery and maximise adaptation to training and match-play demands. Moreover, while researchers have contributed to specific areas of athletes' recovery post-match, the subsequent interpretation of these results must also be considered. Crewther *et al.* (2011) discussed the implications that training status and exercise type can have on the reported hormonal concentrations, in addition to genetic variation which can be masked by mean concentrations. Further research is warranted before coaches can fully understand the influence that the external load from specific match demands places on players and how this affects their recovery.

2.5.5 Glutamine:Glutamate Ratio

Walsh *et al.* (1998) suggested that plasma Gln concentrations decrease as a result of increased gluconeogenesis and associated increase in hepatic, gut and renal Gln uptake in response to catabolic stress, which includes prolonged exercise. A reduction in plasma Gln concentrations at rest have been exhibited by athletes experiencing the effects associated with OTS, when compared to active healthy controls. As previously discussed, increased salivary C concentrations on the other hand promote the circulation of proteins associated with glucose mobilisation and subsequent uptake in response to intense exercise (Buckingham, 2006). Additional ratios have also been analysed and the Gln and Glu were likely adopted as it was suggested that plasma Gln concentrations decrease because of an increase in gluconeogenesis. A reduction in plasma Gln concentrations at rest have been exhibited by

athletes who experience the effects associated with OTS, when compared to active healthy controls (Walsh *et al.,* 1998). Glu circulation on the other hand increases as a result of glucocorticoid release to stress and the subsequent circulation of C as a result of cortisone. The release of these glucocorticoids will result in an increase in Glu release from the hippocampus. Glu is a key intermediate metabolite in the detoxification of ammonia and a building block used in the synthesis of peptides and proteins (Popoli *et al.,* 2012). Moreover, it is a key immunomodulator in the initiation and development of T-cell-mediated immunity (Pacheco *et al.,* 2007). Determining the Gln:Glu between the decreased Gln concentrations and increase Glu concentrations in response to intense exercise can give an insight into the magnitude of stress that athletes experience. Higher ratios determine the athlete is better recovered in comparison to decreased ratios, whereas lower ratios indicate the athlete is experiencing the effects of OTS as highlighted above (Coutts *et al.,* 2007). The results reported by Coutts *et al.* (2007), described a significantly lower Gln:Glu ratio after six weeks of training in the overtraining group when compared to the normal training group (mean ± SD, 3.73 ± 0.76 *vs.* 4.47 ± 0.53 and 4.75 ± 0.72 *vs.* 5.24 ± 0.70, respectively). Furthermore, decreased Gln:Glu ratios have been posed as a prognosticator of muscle atrophy (Castell, 2003) which would be an unfavourable condition for athletes who are required to maintain optimal performance during the competitive season.

2.5.6 Creatine Kinase

The enzyme creatine kinase (CK) is an indirect measure of muscle damage and this is suggested to be as a result of both metabolic and mechanical causes which were discussed by Brancaccio *et al.* (2007). Metabolically exhausted muscle fibres can exhibit a decrease in the membrane resistance following an increase in the internal free calcium ions which promotes the activation of the potassium channel. Another mechanism is the local tissue damage with sarcomeric degeneration from Z-disk fragmentation (Brancaccio *et al.,* 2007). CK has also been reported to be significantly correlated with contacts in rugby when comparing pre- *vs.* post-game concentrations (Jones *et al.,* 2014). Jones *et al.* (2014) collected serum samples from 28 rugby players, 2h pre-game, as well as 16 and 40h post-game. HSR markers and number of physical contacts were also measured. Players must have played a minimum of 60min in the match to be included in the study. Significant moderate correlations were reported for both absolute and relative percentage change in CK concentrations and sprint number, sprint distance and HSR (>5m.s^{-1}) in backs at 40h post-game (r = 0.42, r = 0.42 and r = 0.43 *vs.* r = 0.33, r = 0.36 and r = 0.44, respectively), while no correlations were observed for forwards. Furthermore, moderate to large effect size correlations were observed for both backs and forwards for various

contact measures at both 16- and 40h post-game. Moderate correlations were recorded between mean absolute changes in CK concentrations and hit-ups at 16h (*r*=0.45), and 40h post-game (*r*=0.41) for forwards. While mean absolute changes in CK concentrations and tackles revealed moderate correlations with tackles, contacts hit, and total impacts at 40h post-games for backs (*r*=0.58, *r*=0.52, and *r*=0.64, respectively). Furthermore, moderate correlations (*r*=0.41) were described between mean absolute changes in CK concentrations and hit-ups at 16h post-game in backs which is similar to the results reported for forwards. The CK responses observed by Cunniffe *et al.* (2011) reported peak concentrations occurred at 14h post-game. These values remained elevated at 38h post-game which is similar to the response initially described by Brancaccio *et al.* (2007).

Lacome *et al.* (2018) also analysed the effect of external workload across a competitive under 20s rugby union competition and its effects on subjective and objective measures of internal workload as measured by sRPE and serum CK, respectively. Additionally, NMF was monitored using CMJ height. Twenty-four elite junior rugby union players (19.8 ± 0.5yrs, 99.1 ± 9.1kg, 185.4 ± 7.0cm) from a single European international team participated in the study. During the nineteen-day tournament period, five matches and ten training sessions took place. Four days (94-98h) separated matches one, two and three, while five days (118-120h) separated matches three, four and five. Players were subdivided into two groups relative to their match-play time. The high exposure group (HEG, *n* = 13) played 276 ± 44min which accounted for 69 ± 11% of total playing time. The low exposure group (LEG, *n* = 11) played mean ± SD 132 ± 52min which accounted for 33 ± 13% of total playing time. There was a relatively even positional split among the groups with the HEG group comprising of seven forwards and six backs, with the LEG group containing five forwards and six backs. External workload was measured using 16Hz GPS units and data was collected for TD and HSR. HSR thresholds were individualised and set at speeds above the players maximal aerobic speed (MAS). Both sRPE and players' perception of fatigue were assessed to subjectively measure fatigue. Serum CK samples were collected 20-24h before and after the game. These times correlated with the evening before and after the game (7-8pm). Data indicated a likely moderate difference in cumulated TD between groups across the tournament (mean ± SD, 39030 ± 8061 *vs*. 33923 ± 5797m, +15 ± 14%), while an unclear difference was reported between groups for HSR (mean ± SD, 3427 ± 1865 *vs*. 3260 ± 1416 m, +5 ± 35%). However, match-play data revealed a likely very large difference in TD and a likely moderate difference in HSR between groups (mean ± SD, 20240 ± 4231 *vs*. 10040 ± 3662m, +54 ± 14% and 1886 ± 1110 *vs*. 1002 ± 1481m, +44 ± 29%, respectively). No progressive decrease was observed for either TD or HSR, however inter-game differences were reported. Match-day 1 wellbeing scores were used as a benchmark value and further analysis revealed unclear to possibly small increases in well-being scores across the tournament for the LEG. Possibly small increases were observed in wellbeing scores

recorded for the HEG for match two and three. Well-being scores two days post-match reported unclear to small increases for both groups. Possibly small decreases in CMJ performance were observed for the LEG two-days before both match three and four with unclear results prior to match five. In the HEG a possibly small decrease in CMJ performance was observed before match three and five, with a likely trivial decrease before match four. CK concentrations prior to match two, three and five for the LEG demonstrated unclear changes in comparison to the concentrations recorded at match one. A possibly small increase in match four was reported for the LEG. The HEG reported possibly small increases in CK concentrations pre-game for matches two, three, and four with unclear changes before match five. Confounding results were reported for CK concentrations post-game in the HEG group as unclear variations were observed after match two, likely moderate increases after match three, and likely small decreases after match four.

These data were similar to those reported by Coutts *et al.* (2007) where while no significant difference was observed between the normal training group and the intentional over-trained group, the CK concentrations showed large differences between groups at week six, despite a lack of significance as reported by the P value (*P*=0.06). When looking at the CK concentrations reported for both groups, there is a notable difference between both the IT and NT groups (mean ± SD, 1402 ± 1107 *vs.* 664 ± 423$\mu.L^{-1}$, respectively). If the results reported in the study by Coutts *et al.* (2007) were considered, they would merely deem them as insignificant, however upon further analysis, results would suggest this was not the case. The large inter-individual differences as described by the large SD in the IT group masks the true effects that the training block had on both groups (Coutts *et al.,* 2007). Lacome *et al.* (2018) also reported that the LEG showed small increases after matches two and three while results were unclear after match four. While only small decreases in CMJ performance were reported, these results do correspond with previously reported findings in professional rugby union (West *et al.,* 2014). Moreover, the largest decrease in CMJ performance (-5.5%) was reported after match four which would suggest that a trend for the accumulation of fatigue is apparent in players with greater exposure (HEG) to match-play during an intense competition. Interestingly, the authors noted that the decrease in CK concentrations prior to game five, occurred after the team played the lowest ranked team in the competition. The highest CK concentrations were also reported after match three which was against the top ranked team in the competition.

As previously reported by Cunningham *et al.* (2016), there are significant differences in movement demands between U20 and Senior international players, unfortunately however, no contact measures were included in this study. Lacome *et al.* (2018) suggested that the differences in CK concentrations between players after playing the lowest ranked teams and the highest ranked teams could be explained by the level of physicality of the higher ranked teams. It could be hypothesised, that in

addition to differences in locomotion demands in Senior international rugby which have previously been reported by Cunningham *et al.* (2016), there could also be differences in contact measures, or 'physicality', between playing levels. Increased CK concentrations have previously been positively correlated with collision numbers as measured by GPS (Jones *et al.,* 2014), therefore further research may be warranted to explore the differences in CK concentrations reported by Lacome *et al.* (2018), after playing teams of various rankings.

2.5.7 Analysis of Salivary Biomarkers in an Athletic Population

In terms of the athletic population, the effects of both C and T can impact performance and thus have been of interest to researchers. The acute stress that sparks the production of C can be either training or match-play, in addition to the psychological stress associated with performing at a high-level. T on the other hand can represent the other end of the spectrum as it fluctuates as an expression of the motivation and excitement associated with positive performances (Salvador *et al.,* 2003; Eubank *et al.,* 1997). Crewther *et al.* (2011) suggested that T and C are important in terms of skeletal muscle size in athletes as the research accepts that the force-generating capacity of muscle is related to its cross-sectional area. Moreover, the roles of T and C have largely been considered anabolic and catabolic, respectively, which contribute to regulating muscle adaptation following resistance training (Herbst and Bhasin, 2004). In addition to their effects on performance the inter-individual changes reported in rugby (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2011; West *et al.,* 2014; Lindsay *et al.,* 2015) and soccer (Filaire and Lac, 2003; Moreira *et al.,* 2009) players would suggest it is an appropriate measure of internal responses to competitive match-play.

The practicality of measuring immunoendocrine markers in the athletic population was improved by the switch from blood sampling to collect plasma for research purposes, to being able to use saliva for the same purposes (Crewther *et al.,* 2011). Steroid hormones were traditionally measured in plasma, but research indicated that these can be detected in oral fluid as well (Gröschl, 2009; Wood, 2009). These hormones are circulated as a result of passive diffusion or active transport (Gatti and De Palo, 2011). For athletes, the effects of C may not be ideal during intense periods of training where recovery is a priority. An athlete manifesting high concentrations of C may suffer long-term, detrimental effects on their ability to perform at high levels, as elevated serum C concentrations have previously be described in athletes after a 6-week intensified training period, coupled with a significant reduction in serum T concentrations (Coutts *et al.,* 2007). The effects of reduced protein synthesis coupled with an increase in amino acid availability for gluconeogenesis and the increase in adipose tissue synthesis creates unfavourable conditions for athletes, however, protein metabolism is a necessary function of

C (Viru and Viru, 2004). Moreover, a significant increase in lean muscle mass was reported in normal male participants who were administered exogenous T over a 12-week period (20% increase; $P<0.02$), which highlights the importance of maintaining elevated T concentrations in athletes. C turnover is also an important consideration when discussing the effects on athletic performance as C is not produced at constant rates in response to exercise. Exercise duration and intensity results in considerable variability in C concentrations when analysed in weightlifters (Passelergue *et al.,* 1995). In addition to exercise duration and intensity, fitness levels, nutritional status and circadian rhythm will all affect the rate of C production (Passelergue *et al.,* 1995). Endurance events like marathon running and intense weight sessions may result in higher C concentrations than moderate-intensity exercise. Jürimäe *et al.* (2001) reported no significant increases in plasma T or C concentrations ($P>0.05$) from pre- to post-event in male competitive rowers. Mean rowing duration for participants was 2h 17min (mean ± SD; 7891 ± 761s), over a mean ± SD distance of 22.6 ± 2.5km, with mean ± SD heart rates of 136 ± 7 beats.min^{-1}. While no significant changes were reported in either plasma C or T concentrations, plasma C concentrations reported a moderate positive relationship with distance covered ($r = 0.49$), indicating that athletes who covered more distance, experienced greater increases in C concentrations, further supporting the comments by Passelergue *et al.* (1995). Moreover, free plasma T concentrations recorded a moderate negative correlation with distance covered. These findings are similar to those reported in rugby union matches, whereby by salivary and serum T and C concentrations decrease and increase, respectively, from pre- to post-match (Cunniffe *et al.,* 2010; West *et al.,* 2014).

Early studies in weightlifters determined that hormone concentrations, particularly the stress hormone, C, are dependent on exercise type, intensity and duration (Passelergue *et al.,* 1995). Another variable that can affect these salivary hormone concentrations is psychological stress (Salvador *et al.,* 2003). The study by Salvador *et al.* (2003) reported an anticipatory increase in C concentrations prior to Judo competitions in young male judo competitors. This elevated C concentrations pre-fight was a physiological response to increase energy availability and has been linked with an increased motivation to win. Additionally, athletes showed individualised salivary T concentrations, with some athletes experiencing an anticipatory rise in salivary T concentrations, while others did not. These increases in salivary T concentrations which were larger than 15% in T-responders (those who experienced a pre-competition increase in salivary T concentrations), were coupled with a greater motivation to win. T-responders also presented higher salivary C concentrations just before competition, compared to non-responders. These findings further suggest that motivation and psychological factors can influence pre-match T concentrations as previously outlined (Zilioli and Watson, 2013).

Salivary hormone responses induced by preparation and pre-competition training loads were also analysed in twenty-four elite level track and field athletes by Guilhem *et al.* (2015). Of the twenty-four athletes, fifteen were female and the remaining nine were male. The athletes participated in a range of events, including, short and long-distance sprinting, long jump, middle distance running and combined events. During the preparation phase, samples were collected three times in three months. In the pre-competition phase samples were collected five times in five weeks. Salivary T and C concentrations were collected at these time points in addition to alpha-amylase, IgA, chromogranin A and serum CK. Similar principles underlie the secretion of alpha-amylase and catecholamines (epinephrine and norepinephrine) during physiological stress, both of which are co-stored and co-secreted in saliva with chromogranin A (Montero-Hadjadje *et al.,* 2008). A measure of total mood disturbance and fatigue perception was implemented in the form of a POMS questionnaire which was filled out at each time point by the athletes (Guilhem *et al.,* 2015). The pre-competition period acted as a baseline, whereby basal hormonal profiles were determined. Participants were instructed to refrain from intense physical activity for 24h prior to testing days. It is important to consider the training focuses for these periods which were strength and aerobic development during the preparation phase, and anaerobic development during the competition period, where movements were performed at maximum velocity. The high-intensity nature of the pre-competition period has been previously associated with a higher injury incidence than in the preparatory phase (Papacosta and Nassis, 2011). Results concluded that there was no main effect of training period for T concentrations, with the same being observed for C concentrations (P=0.15). Salivary IgA concentrations significantly increased from the preparatory period to the pre-competition period which would suggest impaired immune tolerance as a result of an increase in competition demands (P<0.05). In addition to salivary IgA, CK activity increased significantly for males between the preparatory period and pre-competition period. Training load and perception of fatigue increased from the preparatory to the competition phase, yet only some of the measured hormone concentrations were affected. Interestingly, Salivary T concentrations were similar in both the preparatory and competition phases in the current study, despite changes in training load and psychological perception of fatigue between these periods (Guilhem *et al.,* 2015). Interestingly, analysis discovered that none of the psycho-physiological parameters were significantly correlated to training load during the pre-competitive period. The researchers described a significant decrease in mood disturbance as competitions approached. These findings are important considerations when comparing the acute and the chronic effects of training stimuli on hormonal homeostasis.

Exploring this adaptation across a season could provide practitioners with even more information relating to scheduling and load tolerance during a competitive season. Further research that

implements the protocols utilised by Cunniffe *et al.* (2011), whereby multiple games are analysed, would greatly improve the understanding of practitioners. Currently, a gap exists between the analysis of the chronic fluctuations of hormones over the course of a season and the acute effects observed in responses to a single game. Future research should attempt to replicate the methodology utilised by Cunniffe *et al.* (2011) to provide a clear outline of immunoendocrine response of athletes to multiple games in an acute setting.

2.6 Inflammation and Immune Responses to Exercise

Athletes are prone to several common infections such as influenza, viral sore throats and post-viral fatigue syndrome (Sharp, 1989). Coaches and performance staff must ensure that players are not exposed to extended periods of increased risk where they may be more susceptible to contracting such infections. Tiernan *et al.* (2019) reported that significant decreases in salivary IgA occur one to two weeks after an increase of ~49% in training load. Moreover, reductions in T:C ratios are apparent after both competitive rugby union match-play (Cunniffe *et al.,* 2010), and after six weeks of overload resistance training in rugby league (Coutts *et al.,* 2007). Monitoring of hormonal status across a competitive season, did not report significant differences in T:C ratios in competitive soccer players, despite a significant increase in C concentrations after a block of intensified training (Filaire and Lac, 2003). As a result, identifying hormones and inflammation markers that fluctuate in response to intense training blocks as well as competitive match play is essential in developing recommendations for practitioners to appropriately manage the well-being of their players.

2.6.1 The Immune System

While the immune system is a complex system, there are several key points that underlie the principles to be discussed in the coming sections. While the immune system in general refers to the collection of cells, chemicals and processes that work together to protect all areas of the human body from skin, respiratory passage, intestinal tract and others, from foreign antigens, there are two key divisions that are responsible. The innate immune system represents the first line of defence against pathogens. It is a non-specific, antigen-independent defence mechanism that acts in the acute phase of antigen infection (Turvey and Broide, 2006). In contrast, the adaptive immune system acts as a memory bank to limit the effects of future infections. This specific, antigen-dependant system involves a lag-time between the exposure to the antigen and maximal response from relevant areas of the

immune system. During subsequent exposures, the adaptive immune system can fight the antigen more quickly and efficiently, alongside the innate immune system, as it calls upon the stored memory of the antigen (Bonilla and Oettgen, 2010). It is important to note that the two branches of the immune system are not mutually exclusive, as they complement each other to combat the pathogen. Deficiencies in either system can result in an impaired response and increased host vulnerability (Marshall *et al.,* 2018).

The innate immune system as described by Turvey and Broide (2006), is comprised of four main components; the anatomic barrier which includes skin and the mucous membrane, the physiologic barrier which consists of temperature, low pH and chemical mediators, the endocytic and phagocytic barriers and the inflammatory barrier. A key element of the innate immune system and its ability to detect pathogens, is the pattern recognition receptors. These pattern recognition receptors allow a limited range of immune cells to detect and respond to a myriad of pathogens that share common structures (Akira *et al.,* 2006). Pathogens can be identified by their pattern associated molecular pattern. Another key element of innate immunity is the ability to produce cytokines and chemokines used to fight infection and inflammation (Feghali and Wright, 1997). Cytokines can mobilize many defence mechanisms as well as activating local cell responses to combat the infection or injury caused by acute stress. Three key cytokines involved in the inflammatory process associated with bacterial infections are tumour necrosis factor (TNF), interleukin 1 (IL-1) and interleukin 6 (IL-6). These cytokines are essential in the recruitment of the cells involved in initiating a response, as well as the local inflammation of the affected site which are both required for the clearance of many pathogens. The improper function of these cytokines is often associated with autoimmune of inflammatory diseases (Langrish *et al.,* 2005). In addition to8 cytokines, cells like phagocytes (macrophages and neutrophils), dendritic cells, mast cells, basophils, eosinophils, natural killer (NK) cells and innate lymphoid cells, all play important roles in innate immunity (Ravichandran, 2011). Both macrophages and neutrophils of the phagocyte family, are responsible for phagocytosis which is the engulfing of microbes to destroy them in the body (Wynn *et al.,* 2013). Neutrophils are short-lived cells, unlike macrophages which are long-lived cells, and contain granules and enzymatic pathways that assist in pathogen elimination (Nathan, 2006). Macrophages are also responsible for presenting pathogens to T cells. Mast cells, also known as sentinel cells, are the early producers of cytokines and are found in connective tissue surrounding blood vessels and mucosal surfaces (Stone *et al.,* 2010). NK cells, as the name suggest, play an important role in the rejection of tumours, and the destruction of infected cells. The innate system can also assist the adaptive system through the mobilization of antigen-specific cells (Bonilla and Oettgen, 2010). The adaptive immune system is aided by the actions of the innate immune system and is essential for preventing and minimising the effects of future infection as a result of its memory.

Antigen specific T-cell have the ability to distinguish between 'self' and 'non-self' antigens, to develop pathogen-specific immunologic effector pathways to combat specific infections, and the capability of developing an immunologic memory (Bonilla and Oettgen, 2010).

2.6.2 Immunity in Athletes

Early research into the acute effects of exercise on the immune responses of athletes was conducted by Nieman (1997). This review explored the acute immune response to prolonged (>90min) and intensive (>70% $\dot{V}O_2$max) endurance exercise. The suppression of NK cell activity, T and B cell function, upper airway neutrophil function and salivary IgA concentrations, has been identified in the hours post-intensive endurance exercise (Margeli *et al.,* 2005b). During this window of suppressed immunity is when it is hypothesized that viruses and bacteria can attack the weakened immune system and infect the host cells. While this is a fitting hypothesis, research indicates that while there is a reduction in immune markers, there appears to be little to no relationship between the incidence of illness and immunological markers (Papacosta and Gleeson, 2013). The elevation of stress related hormones like C, result in an inhibitory effect on inflammation, primarily by inhibiting the production of cytokines (Papacosta and Gleeson, 2013). Cytokines are protein signal molecules that regulate inflammation and typically act locally as paracrine hormones. Suppression of cytokines has a dampening effect on cell-mediated responses. After periods of intensified training, athletes require sufficient recovery in order to adapt to the training stimulus they have been exposed to. Cytokines are essential in modulating inflammation and cell-mediated responses (Turvey and Broide, 2006). Cytokine activity is supressed post-exercise (Papacosta and Gleeson, 2013), and so athletes must be monitored in the days after intense exercise to ensure they are not exposed to prolonged periods of immunosuppression which could lead to increased incidence of common illnesses as outlined by Sharp (1989).

2.6.3 Serum Amyloid-A

The comprehensive review by Sack Jr, (2018) explored the complex functions of Serum Amyloid A (SAA) and its function in amyloidosis. SAA is a precursor protein in inflammation-associated reactive amyloidosis that is released by the liver. Amyloidosis refers to the infiltrative histopathologic changes found in the kidney, liver and heart as a result of the deposition of an array of microfibrils or protein deposits. The constituent protein in amyloid deposits is important for distinguishing the pathologic context. For example, the protein known as "amyloid of unknown origin" (AOU) was found in cases of

"secondary" amyloidosis which was associated with chronic or recurrent inflammation of a tissue. The fibril-derived proteins, of varying lengths, were discovered in various laboratories and became known as "AA" (Amyloid-A). Amyloid A was originally discovered in tissues samples from patients with amyloidosis secondary to chronic inflammatory diseases and was later defined as "the precursor protein in AA amyloidosis and as a liver-derived, high-density lipoprotein (HDL) associated apolipoprotein" (Benditt *et al.,* 1971). As it was the first non-immunoglobulin serum protein identified as a precursor of amyloid disease deposits, it was called 'Serum Amyloid-A".

There are both primordial and adaptable mechanism associated with the protein, however, it is the primordial mechanism associated with the "acute-phase response" (APR) that is most prominent. Yoo and Desiderio, (2003) defined the APR as a transient deviation from homeostasis when an organisms' integrity is breached. The changes that occur are as a consequence of various events including inflammation, infection and trauma, with inflammation being particularly of interest in a sporting context. There are numerous physiological responses that occur as a result of the APR, some of which include fever, hormonal changes and metabolic alterations. During the APR, serum protein levels are dramatically altered. CRP and SAA concentrations are generally quite low in the blood of healthy individuals. SAA is normally present in the range of 20-50$\mu g.mL^{-1}$ (Wilkins *et al.,* 1994). Serum concentrations for both SAA and CRP concentrations rise as much as 1000-fold 24h after the onset of APR before falling rapidly as the APR pattern resolves (Sack Jr, 2018). Of the various forms of the SAA proteins, SAA 1 and SAA 2 were most strongly related to the primordial host defence and inflammation associated with the APR. SAA 3 was discovered to be a pseudogene while SAA 4, also an apolipoprotein of HDL, is synthesized constitutively and, is thus not induced in the APR.

SAA gene expression is regulated by several complex molecular mechanisms, including proinflammatory cytokines IL-6 and IL-1, that act either alone or in various combinations. The cytokines that regulate its expression have been explored in recent training load research in both team sports (Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015) and endurance sports (Gleeson *et al.,* 2008; Nieman *et al.,* 2001). Exhaustive endurance exercise results in muscle damage and immune suppression which contributes to inducing leucocytosis (Nieman, 1997; Suzuki, 2018). The training stimulus and trauma associated with training and match-play are key stimuli in the release of pro-inflammatory cytokines and subsequent production in SAA.

2.6.4 Immunoglobulin Responses to Exercise

Studies have reported upper respiratory tract infections (URTI) as being one of the most common illnesses suffered by athletes due to their impaired immune responses after intensive, exhaustive exercise (Nieman, 1997). Nieman *et al.* (1990) This can particularly be seen in the in the 1- and 2-week after marathon and ultramarathon events, in endurance athletes. Moreover, the relationship between exercise and URTI may be modelled as a "J" shaped curve (Nieman and Pedersen, 1999), with sedentary individuals being exposed to a greater incidence of URTI compared to regularly active individuals. However, Nieman and Pederson (1999) reported that athletes who are very highly active, are suggested to experienced increased risk of URTI.

The production of secretory IgA is the major effector function of the mucosal system providing the 'first line of defence' against pathogens (Papacosta and Nassis, 2011). Researchers have analysed immune markers, including IgA, in addition to logging how an athlete is feeling to monitor any pre-defined medical signs (Filaire and Lac, 2003). Filaire and Lac (2003) concluded that the immunological factors IgA, IgG and IgM remained unaltered throughout the study. These results are corroborated by those described in response to a rugby union game where salivary IgA values remained unchanged in pre- *vs.* post-game samples (Lindsay *et al.,* 2015). Interestingly, Lindsay *et al.* (2015) highlighted that mean results masked the individual responses which displayed a general trend for post-game increases that remained altered for up to 86h. These are important considerations when interpreting the data by Filaire and Lac (2003). Mean plasma Glu concentration decreased significantly (mean ± SEM, 577.6 ± 23.8 *vs*. 480.8 ± 15.2umol.L^{-1}) between T_1 and T_2 during preseason, where training load was at its highest. Despite these altered Glu values, only two URTI instances occurred throughout the season which suggests Glu is not a useful marker of physiological stress. While IgA, IgG and IgM concentrations remained unchanged and were within normative ranges in the research by Filaire and Lac (2003), recent research by Tiernan *et al.* (2019) reported a significant association between incidence of URTI and low salivary IgA concentrations in Academy Rugby Union players, monitored over a ten-week period. Furthermore, the authors explored relationship between training load and URTI infections. Data indicated that while no significant increases between training load and salivary IgA were reported, however, in thirteen out of fifteen incidences of URTI, players had a mean increase in training load of 49% in the preceding week. Interestingly, during the 70-day testing period, no differences in mean salivary IgA concentrations were reported between players who experienced an URTI and players who did not (mean ± SD, 254.22 ± 140.98 *vs.* 257.23 ± 153.79µg.mL^{-1}, respectively). Interestingly, however, mean ± SD salivary IgA concentrations dropped to 110.80 ± 152.57µg.mL^{-1} two weeks before an URTI.

Early research by Gleeson *et al.* (1995) in twenty-six elite youth swimmers (fifteen males and eleven females), aged between 16-24yrs concluded contrasting results to those reported by Filaire and Lac (2003) regarding the salivary IgG and IgM responses across a training period and in response to individual sessions. In this research, the swimmers were compared against controls consisting of twelve staff members (seven males and eleven females). Samples were collected once a month for the duration of the study with pre-exercise samples collected 1h post-prandially and post-exercise samples collected at least 24h after the previous training session. Data indicated that a consistent downward trend for both pre- and post-competition salivary IgA concentrations was evident across the seven-month testing period for the elite swimmers when compared to controls. Furthermore, after individual exercise sessions there was a significant decrease in salivary IgA levels for athletes when compared to controls. These findings corroborate the findings in the more recent study by Tiernan *et al.* (2019) whereby reduced salivary IgA concentrations were reported two weeks prior to contracting an URTI. The findings by both Gleeson *et al.* (1995) and Tiernan *et al.* (2019) indicate that both individual training sessions and competitive seasons, as well as increases in training load, can result in decreased salivary IgA concentrations, increasing the risk of contracting an URTI.

A similar study analysed blood samples for thirty-seven different blood parameters, serum IgA and CK being the most pertinent, at five time-points over a six-month period in a professional soccer season (Heisterberg *et al.,* 2013). Twenty-seven professional soccer players playing in the top Danish league provided five blood samples over the six-month period. Thirty-seven variables were analysed for the purposes of the research. The first sample was collected before the winter break in mid-December (BS1) and the final sample was collected after the final game of the season in July (BS5). Three more samples were collected around the pre- and mid-season. The second sample was collected in the middle of January during the early stages of the pre-season (BS2) and the third sample in the middle of February, half-way through the pre-season (BS3). The fourth sample was collected in mid-March after the pre-season, three weeks into the regular season (BS4). Parameters related to immune function displayed varying results. Leukocytes were significantly lower at BS3 when compared to BS1 while lymphocytes were significantly lower at BS5 in comparison to BS1, whereas monocytes significantly decreased at BS5 compared to BS2. The only changes in immunoglobulins in the two studies was detected by Heisterberg *et al.* (2013) who reported significantly higher IgA values at the beginning of preseason (BS2) when compared to those obtained pre-winter break (BS1) and at the end of the competitive season (BS5). Similarly, IgM was higher at the beginning of preseason (BS2) and end of the competitive season (BS5) compared to during preseason and regular season. The authors suggested that the dramatically increased training load from a low-load maintenance phase during the Christmas holidays, to high-loads, compiled of both strength and endurance work, during

the preseason conditioning period, likely contributed to these changes observed in IgA and IgM. The absence of elevated IgA and IgM concentrations at BS3 and BS4 indicate that these spikes were transient rather than detrimental to performance, and further suggests that higher concentrations are observed after the acute onset of an intensified training block but not when training load is substantially less in season (BS4). The infrequency of their sampling times was highlighted as a potential limitation in both studies. It is suggested that more regular testing takes place and in relation to significant events, such as, matches and training during various phases of the season.

Mortatti *et al.* (2012) conducted a study to determine the incidence of URTI in elite youth soccer players during a twenty-day period during which seven games were played. Players played one game every three days, with two days recovery between matches. During this two-days recovery period, two sessions were completed, one per day, to facilitate physical and psychological recovery. Players completed a weekly log to track their signs and symptoms of URTI, which included coughing, a runny nose, and nasal congestion, as well as the number of days that the symptoms lasted for. Players were also required to rate the severity of their symptoms from 1 to 3. TO be classified as an URTI, symptoms must have persisted for two or more consecutive days. Saliva samples were also collected at 9am at rest, the morning of each match, to determine salivary IgA and C concentrations. RPEs were also collected after each match. Compared to match one, there was a significant increase in RPEs in matches 4-7 (P<0.05; 180% to 202% increase from 4-7 when compared to match one). Resting C concentrations did not change significantly across the twenty-day competition period (P>0.05). When compared to the first period (match 1-2), a significant increase in URTI incidence (P<0.05)was highlighted during period 2 (match 2-3) and period 6 (match 6-7). Interestingly, significant decreases in salivary IgA concentrations were reported when compared to period 1, during the period 2 and period 6 (P<0.05; -15% and -12%, respectively). These findings would support previous research that observed an increase in fatigue in elite rugby union players across a three-week competition period (Cunniffe *et al.,* 2011). Practical recommendations for practitioners, as suggested by Mortatti and colleagues (2012), suggest that salivary IgA may be useful in predicting URTI during intensified short-term competition periods. Coupling the findings of Mortatti *et al.* (2012) with those of Tiernan *et al.* (2019) whereby URTI incidence was associated with an increase in training load two weeks prior to the first reporting of symptoms, may allow practitioners to better monitor their athletes, thus preventing URTI. Further to managing training load of athletes, appropriate nutrition, which includes the ingestion of carbohydrates before and during exercise (Bishop *et al.,* 1999) and ingestion of the free amino acid glutamine (Walsh *et al.,* 1998), has also been reported to positively influence post-exercise immunity.

2.6.5 Interleukin-6

As previously outlined, IL-6 is one of the three key cytokines in the innate immune systems of which the majority of its circulation can be contributed to muscular contractions (Turvey and Broide, 2006). Fischer (2006) described the responses of IL-6 to exercise, making specific reference to its effect on the modulation of immunological and metabolic responses to exercise. Fifty-one percent of the variation in plasma IL-6 increase can be explained by the duration of exercise, as explained by the log_{10}-log_{10} linear relation. Moreover, IL-6 concentrations are not linear over time, they accelerate in a more exponential fashion which is an important consideration when analysing post-game responses in athletes. IL-6 increases are limited to the muscle mass. Exercise limited to the upper body may not increase plasma concentrations above pre-exercise levels in comparison to total body work as undertaken in rugby. IL-6 concentrations reported by Cunniffe *et al.* (2011) significantly increased from pre- to post-game and returned to baseline at 14h post-game in professional rugby players over a three-week international rugby tournament. While the magnitude of response was higher for game one in comparison to game three, there were no significant differences between games despite longer ball in play times for game three. In contrast, contact events were higher for game one than game three.

Interestingly, however, no significant difference in inflammatory cytokines including IL-1β, IL-6 and IL-8 was reported in male and female swimmers aged 18-35 years old, who were divided up into illness-prone and illness-free swimmers (Gleeson *et al.,* 2012). While IL-6 concentrations increase post-exercise, much like IgA concentrations, they do not appear to be associated with an increased risk of URTI. These findings may give context to the results outlined above by Heisterberg *et al.* (2013). In endurance athletes, the general downward trend for these immune markers may suggest that a training adaptation occurs over time as athletes adapt to their training and competition demands. Immune markers like IgA, IgM and IL-6 concentrations may increase during the onset of intense training period but do not directly result in increased risk of illness. Early research by Hooper and Mackinnon (1995), acknowledged that even in cases where there is no presence of OTS, hormonal imbalances can be observed. As a result, researchers must be weary of reporting causation when collected data is observational rather than cross-sectional. Reported hormonal responses may be insufficient in determining if over-training or even over-reaching is present and may simply be a description of hormonal responses to competition unless additional psychological measures are included.

While immune markers may not sufficiently predict impending overtraining, they are a useful addition to salivary biomarkers of fatigue as when exercise is repeated frequently, the immune system may not

be given sufficient time to recover (Papacosta and Gleeson, 2013). Similarly, as reported in studies that analyse the hormonal fluctuations in response to individual competition periods, rather than across whole seasons, acute changes in immunological parameters can be observed in response to intense bouts of exercise (Cunniffe *et al.,* 2011). Analysing SAA may provide a general insight into the effects of exercise on inflammation due the 'housekeeping' role that it provides (Urieli-Shoval *et al.,* 2000). SAA can act as a first line of defence by controlling the adhesion, migration and tissue infiltration of monocytes, lymphocytes, neutrophils and mast cells. Another key function of SAA is the modulation of inflammation through pro- and anti-inflammatory activities which is of importance to practitioners in the days following competition.

2.7 Practicality of Using Hormones as an Indicator of Overtraining

2.7.1 Limitations Associated with Research in Athletic Populations

Current research on the topic of monitoring athletes' hormonal status for signs of overreaching has centred around team sports such as rugby (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2011; West *et al.,* 2014; Lindsay *et al.,* 2015), and soccer (Filaire and Lac, 2003; Moreira *et al.,* 2009; Heisterberg *et al.,* 2013). Endurance sports have also been discussed due to the intense nature of the training and competition schedule (Gleeson *et al.,* 1995; Eubank *et al.,* 1997; Drenth *et al.,* 1998; Margeli *et al.,* 2005; Papacosta and Gleeson, 2013). While there is a general consensus that C and T are sufficiently sensitive to depict the dose-response relationship of competitive match play and thus training load tolerance, there has been varying degrees of efficacy. Hayes *et al.* (2016) address the limitations associated with research using team-sport athletes as participants which may explain some of the variance across studies and the lack of standardised methodology.

Considering team-sports athletes as participants means there is limited access and control available to researchers. As a result, there are limited number of RCT and Intervention studies in this research discipline. The difficulties associated with having half of a team acting as a control group by not participating in training manifests several issues that are not feasible for coaches. Additionally, there are ethical considerations that must be considered when intentionally overtraining a group of participants. Coutts *et al.* (2007) utilised an intervention study with rugby league players, to explore the effects of overtraining and has remained the only RCT in the discipline. Participants were divided

into a control and an intentionally overreached group. Analysis of the physiological and hormonal effects caused by NFOR in comparison to normal training was, therefore, possible within this study design. Various measures were tested both pre- and post-intervention. The only biochemical parameter that was significantly different was Gln:Glu. From a methodological standpoint, however, Coutts *et al.* (2007) noted that different glutamine assays can yield significantly different results and determines it unwise to cross-compare studies using different analytical methods. The inter-assay differences and ability to cross-compare results using different assays was further discussed by Castell (2003). The author highlighted the differences between enzymatic assays that use asparaginase or glutaminase to start the reaction versus bioassays or high-performance liquid chromatography. Results indicated the two former assays yielded lower normative ranges than the two latter, which produced much higher values for normal samples. Furthermore, it is interesting to note that in the only RCT study published in this field of research, no differences in commonly collected hormone concentrations, such as C and T, were reported between groups. These results suggest that whether athletes are exposed to normal or intensified training, they ultimately respond the same (Coutts et al. 2007). However, despite the lack of inter-group differences, the findings are corroborated in cross-sectional studies that analyse athletes either over the course of a season to determine the longitudinal effects to intense training (Filaire and Lac, 2003), in addition to studies that analyse acute effects of either intense training or match-play (Cunniffe *et al.,* 2010; West *et al.,* 2014). While intensified training may not elicit significantly greater increases in hormones in comparison to normal training, inter-individual differences are still apparent (Moreira *et al.,* 2009) and should be considered by practitioners when prescribing load (Halson and Jeukendrup, 2004).

Further research is warranted to fully understand the effects of these hormones across longer periods and in conjunction with measure of NMF such as CMJ and mood scores (West *et al.,* 2014) or measures of muscular activity (Brownstein *et al.,* 2017). These differences may be explained by the fact that exercise intensity and accumulated fatigue, result in increases from pre- to post-exercise in hormones like T and C, as well as immunoinflammatory markers such as SAA, CRP, IgA and IL-6. While they may not be practical, more RCT would be advantageous in determining whether C and T are valid measures of training adaptation in athletes. Moreover, where RCT are not practical, the use of appropriate statistical techniques such as partial eta squared (West *et al.,* 2014; Lindsay *et al.,* 2015) and the use of critical differences (Hayes *et al.,* 2014), could assist in helping to better understand whether the observed immunoendocrine responses are directly related to competitive match-play exposures. Due to the individual nature of hormone fluctuations, further research is necessary to create a more detailed picture of hormone responses in athletes across a multitude of sports.

2.7.2 Sample Collection Considerations

Since it has been established that salivary biomarkers of fatigue are sensitive enough to quantify the fatigue associated with exercise, it is important to consider the methodology in collecting these samples. Papacosta and Nassis, (2011) discussed ways to reduce variance and chances of methodological error. Pre-sampling guidelines must be considered by all researchers to improve the validity and reliability of their studies. These guidelines become even more important when working outside of laboratory conditions due to the lack of control researchers can have on research participants, for example, in a team sport environment.

To minimise acute variations in saliva, food and drink intake must be avoided for at least 2h prior to sampling (Granger *et al.,* 1994). High sugar and caffeine content, as well as acidity, can stimulate saliva flow which can negatively affect results. Acidity will also lower the pH of the mouth which can compromise enzyme and antibody-antigen binding activity (Granger *et al.,* 2004). Alcohol consumption must also be avoided in the 24h prior to sampling (Granger *et al.,* 1994).

Another important consideration, especially when the research participants are athletes, is that microinjuries and abrasions in the mouth may result in blood contamination which will significantly affect results. Blood hormone concentrations are an order of magnitude greater than salivary levels (Kivlighan *et al.,* 2004). These circumstances may arise as a result of direct contact in a game but could also be caused by the wearing of gum shields. As a result, it is recommended to avoid the wearing of gum shields where possible. Participants should be instructed to wash their mouths with water at least 10min before collection (Hansen *et al.,* 2008). However, Kivlighan *et al.* (2004) also reported that minor-to-moderate level microinjuries will not significantly impact the concentrations in quantitative salivary measurements. The first amount of saliva that accumulates in the mouth should then be swallowed before attempting to provide a sample by way of passive drool. While saliva samples can provide researchers with information on steroid hormones including T and C, it is necessary to take caution when collecting saliva samples. Salivary C concentrations are significantly lower than plasma C concentrations; 3-30nmol.L^{-1} *vs.* 200-800nmol.L^{-1}, respectively (Schwartz and Granger, 2004). The presence of blood in saliva can, therefore, drastically affect the C concentrations of a saliva sample. It is important to ensure that microtrauma and abrasions in the mouth are reported to prevent any interference in sampling. Similarly, food and drink should be avoided prior to providing saliva samples as certain foods can stimulate saliva flow as well as increasing the acidity which subsequently lowers the pH in the mouth (Granger *et al.,* 2004). Both of these effects can compromise the antibody-antigen binding and enzyme activity leading to invalid immunoassay results (Granger *et al.,* 1994). It is

important for researchers to consider such factors that may affect the methodology of their research study.

Despite best efforts in controlling for and limiting the occurrence of the outlined contaminating processes and confounding variables, there is still potential that samples may be inconsistent due to the diurnal variation of salivary hormones (Hayes *et al.,* 2016). C is especially sensitive to the light-dark cycle and thus, higher concentrations are observed in the morning compared to the evening (Lange *et al.,* 2010). Sampling times must be kept consistent to control for such variations, in addition to improving the accuracy of data by increasing the frequency of samples similar to the methods employed by Elloumi *et al.* (2003).

2.7.3 Sample Analysis

Further to the above recommendations that must be considered when collecting samples, the method used by researchers to quantify the hormonal changes must also be acknowledged. Papacosta and Nassis, (2011) explored the use of salivary analysis as a tool for monitoring steroid, peptide and immune markers of sport training. Saliva is suggested as a convenient alternative to serum or plasma samples due to the non-invasive nature of the sample as well as the speed and frequency at which it can be collected. The conveniences associated with the collection and subsequent analysis of saliva samples have been well researched, however, new research indicated that liquid chromatography–tandem mass spectrometry-based (LC-MS-based) tests that measure circulating total C and T using a finger stick volumetric absorptive micro-sampler are both reliable and valid in addition to being more practical, and its' use should be considered in future research. When traditional venepuncture collection was compared to finger stick collection for LC-MS-based tests, Deming regression and Pearson correlation indicated good test accuracy for both C and T between the two methods (Fragala *et al.,* 2018). These results could significantly impact the methods of future studies as it eliminates a large portion of the limitations currently associated with salivary analysis as outlined above.

2.8 Conclusion

2.8.1 Predicted study outcomes

This study aims to explore the immunoendocrine responses of elite Gaelic games players to competitive match-play. The analysis of salivary hormones namely C and T, collected in the days

preceding, proceeding, as well as samples immediately before and after the game itself, are intended to explain the responses of athletes to competitive match-play. Understanding the hormonal implications of match-play on athletes and how they prepare and recover for the game, will allow practitioners to monitor their training to optimise performances of the players. Furthermore, the addition of the serum immune marker, SAA, will further enhance the understanding of the inflammatory response to match-play, with particular interest in the post-match time course of recovery. Moreover, analysing these responses in conjunction with the external load, as measured using GPS, will provide practitioners with an understanding of how external workload dictates the recovery timelines of their athletes.

2.8.2 Research Questions

There are a number of questions this research study aims to explore:

1. How do players immunoendocrine markers respond to the external load of competitive match-play in elite Gaelic football?
    - How do players internal biomarkers of fatigue present pre-match?
    - How long does it take for athletes immunoendocrine markers to return to baseline post-match?
2. Does game volume and intensity determine the magnitude of change in players immunoendocrine markers?
    - Which GPS metrics best correlate to the delta changes in immunoendocrine markers?

Chapter 3

3.0 Methods

3.0 Methods

3.1 Participants

Thirteen elite male Gaelic football players (*n* = 13; mean ± SEM, age 25.7 ± 1.0yrs, height 184.2 ± 1.6cm, body mass 87.1 ± 1.3kg) provided samples across five timepoints over four games for the purpose of this study. Participants were provided with an information leaflet and informed consent was obtained (Appendix B and C).

A breakdown of the descriptive data for all participants included in the study can be seen in Appendix D. Eleven individual players provided saliva samples across five timepoints over the four games (*n* = 11; mean ± SEM, age 24.4 ± 0.8yrs, height 186.1 ± 1.5cm, body mass 88.0 ± 1.1kg). Some of these players provided samples for multiple games, while others provided samples for only one game. In total, there were seventeen sets of data for saliva samples (*n* =17), the breakdown of which can be seen in detail in Appendix F-H. These seventeen data sets were pooled and analysed to explore the effects in multiple players, across a series of games. The pooling of data was done to increase the sample size. Serum samples were collected for three of the four games (game 1, 2 and 4). Eight individual players provided serum samples across the three games (*n* = 8; mean ± SEM, age 26.0 ± 1.0yrs, height 187.2 ± 1.9cm, body mass 87.7 ± 1.8kg). In total there were eleven set of data (*n* = 11), the breakdown of which can be seen in Appendix I. These eleven samples were used for statistical analysis. The results for this pooled analysis can be seen in Section 1 of the results.

An overview of the number of samples provided by each player can also be seen below:

Provided saliva samples for one of four games – Players 3, 4, 7, 9, 10, and 13
Provided saliva samples for two of four games – Players 2, 5, 6, and 8
Provided saliva samples for three of four games – Player 1

Provided serum samples for one of three games – Players 2, 5, 6, 11, and 12
Provided serum samples for two of three games – Players 1, 8 and 13

In addition to the pooled samples outline above, a subset of samples was analysed from a single game (game 1) to explore the effects of a single game, in an attempt to control for variables not accounted for in the pooled analysis. Seven individual players (players 1, 2, 3, 5, 8, 9 and 10) provided saliva

samples (*n* = 7; mean ± SEM, age 25.1 ± 1.3yrs, height 184.7 ± 2.5cm, body mass 88.0 ± 1.7kg). Of these seven players, three also provided serum samples (players 1, 2, and 8). One player provided serum samples but no saliva samples (player 12). In total, four serum samples (*n* = 4; mean ± SEM, age 25.8 ± 1.8yrs, height 188.0 ± 3.5cm, body mass 89.0 ± 2.2kg) were collected and subsequently analysed. The results for this pooled analysis can be seen in Section 2 of the results.

3.2 Inclusion & Exclusion Criteria

All players were males aged between 18 and 40 years old and were members of the 2018 Dublin Senior Football Panel. Players were included in the analysis if they provided either saliva or serum samples across the five timepoints. All players who played a part in the match, whether a starter or if they can on as a substitute, were included in the analysis. Players must also have worn a GPS for the duration of the game to determine their external load. Individuals suffering from any musculoskeletal injury that had prevented participation in training within the sample collection period or, deemed unfit to participate by the medical practitioner on completion of a medical examination were excluded from pre- and post-training sample collection. Participants who were suffering from minor sporting injuries were allowed to participate and performed exercises within the limits of their injuries and saliva samples were collected accordingly. Athletes must have completed the required sample collection and monitored training period which included five timepoints.

Participants with any metabolic disorders, chronic sports injury or any other contra-indicatory symptoms were excluded from sample collection completely.

3.3 Experimental Design

This cohort study collected both saliva and blood samples at five time-points around a competitive game. Samples were collected 46h and 2h pre-game as well as immediately (0h), 48h and 72h post-game. Participants wore GPS units for all training and match-days, worn either in specialised vests, or fitted into pouches in training jerseys so that the GPS sat between the players shoulder blades. Participants were asked to refrain from food or drink in the 30min prior to providing saliva samples. Strenuous exercise outside of the teams normal training routine was also to be avoided.

All participants were subjected to the same training regime over the sporting year. Players participated in one 1h long gym session per week, two 1-1.5h long pitch sessions per week, as well as

one competitive game per week. RPEs were collected and multiplied by training time to quantify the athletes training load as measured in arbitrary units (AU). Four games were included in the study; three games during the National Football League (NFL) and one game from the Leinster Football Championship (LFC). NFL games were games one, two and three of the season, respectively. Games one and three were home games and game two was an away game. The LFC game was game nine of the season and was an away game. Saliva and blood samples were collected for games one, three and nine, while saliva samples only were collected for game two. Individual game analysis was conducted on game one of the NFL.

As this was a free-living study, players continued with their normal diets, as monitored by the team's sports nutritionist. Players had access to caffeine supplements and sports drinks at training and matches.

3.4 Sample Collection

3.4.1 Saliva Samples

Saliva samples were collected from all players (*n* = 24) that were on the match day panel for the game. Eppendorf tubes were labelled with a number, corresponding to the player, the game and the sample number. Players provided a saliva sample via passive drool into their respective labelled SafeSeal 1.5mL tube (Sarstedt, Numbrecht, Germany) at each sampling point. Players were asked to refrain from consuming any food or liquids in the hour prior to avoid sample contamination.

Samples were stored in an Igloo Profile 16 Quart Cooler (Igloo, Texas, USA) during transportation to the laboratory. They were then frozen at -80°C until analysis. Prior to analysis, samples were thawed at room temperature and subsequently centrifuged using the VWR Compact Star CS4 (VWR, Dublin, Ireland) at 2500 rev.min^{-1} for ten-minutes. As suspended particles in the saliva were pushed to the bottom of the Eppendorf tub, clean saliva was separated into clean, labelled eppendorf tubes for analysis. During the four games, only players that provided saliva samples for the five timepoints were included for further analysis.

3.4.2 Serum Samples

Blood samples were collected from a sample of players from the starting team on match day squad. Samples were collected under aseptic conditions from the medial cubital veins in the cubital fossa of the arm using the Vacutainer system. This system involved the use of a 21G (gauge) BD Vacutainer®

Eclipse™ Signal™ blood collection needle (Becton Dickinson, Oxford, UK) and an 8mL Z-Serum tube (Greiner, Kremsmünster, Austria). Blood was drawn from alternate arms on each visit. Upon completion of sample collection, the eppendorf tubes were inverted five times to allow complete mixing of clotting agent.

Samples were thawed at room temperature before being centrifuged for 10-minutes at 1800 rev.min^{-1} (revolutions per minute) using the Smart R17 Micro Refrigerated Centrifuge (Hanil Scientific Inc., Gimpo, South Korea). Once centrifuged, the serum was separated into clean, labelled eppendorf tubes and frozen at -80°C for subsequent batch analysis of SAA .

3.5 Sample Analysis

3.5.1 Salivary Cortisol

Saliva samples were analysed using Salivary C Enzyme-linked Immunosorbent Assay (ELISA; DRG Instruments GmbH, Marbug, Germany). A standard curve was included with each assay plate and all standards, controls and samples were run in duplicate. The standard curve included known C concentrations; zero, 0.1, 0.5, 1.5, 4.0, 10 and 20ng.mL^{-1}. Controls of high and low C concentrations were also included. 100µl of standard, control and samples were dispensed in duplicate into the 96-well C ELISAs plate. 200µl of enzyme conjugate (C conjugated to horseradish peroxidase) was then dispensed into each well and subsequently mixed for 10s. The plate was then incubated at room temperature for 1h. After 1h the plate was briskly shaken to remove the contents of all wells. 400µl of wash solution was used to rinse the wells five times. After each wash process the plate was dabbed against absorbent paper to remove residue. 200µl of the substrate solution (tetramethylbenzidine) was added to each well and subsequently incubated for 30min at room temperature. The enzymatic reaction was stopped by adding 100µl of stop solution to each well. Analysis was completed using a FilterMax F5 platereader (Molecular Devices, California, USA) at 450nm.

3.5.2 Salivary Testosterone

The T ELISA followed a similar procedure where the standard curve included known T concentrations; zero, 10, 40, 80, 160, 400 and 1000ng.mL^{-1}. Controls of high and low T concentrations were also included. 100µl of standard, control and samples were dispensed in duplicate into the 96-well T ELISAs plate. 200µl of enzyme conjugate (T conjugated to horseradish peroxidase) was then dispensed into each well and subsequently mixed for 10s. The plate was then incubated at room temperature for 1h.

After 1h the plate was briskly shaken to remove the contents of all wells. 400µl of wash solution was used to rinse the wells 5 times. After each wash process the plate was dabbed against absorbent paper to remove residue. 200µl of the substrate solution (tetramethylbenzidine; TMB) was added to each well and subsequently incubated for 30min at room temperature. The enzymatic reaction was stopped by adding 100µl of stop solution to each well. Analysis was completed using a FilterMax F5 platereader (Molecular Devices, California, USA) at 450nm.

3.5.3 Serum Amyloid-A

Serum samples were analysed for SAA using Human SAA ELISAs (Abcam, Cambridge, UK). This assay employs an antibody specific for Human SAA coated on a 96-well plate. To create the reagents, all reagents were equilibrated to room temperature prior to use. Assay Diluent B was diluted 5-fold with deionized water before use. Wash Buffer Concentrate was centrifuged and mixed to ensure no crystals remained. 20mL of Wash Buffer Concentrate was diluted into deionized water to yield 400mL of Wash Buffer. The Biotinylated SAA Detection Antibody vial was briefly spun prior to dilution. 100µL of Assay Diluent B was subsequently added to the vial to prepare a detection antibody concentrate. The concentrate was pipetted up and down to ensure complete mixing prior to an 80-fold dilution with Assay Diluent B for use in the assay procedure. Finally, the HRP-Streptavidin Solution was spun and pipetted up and down to ensure complete mixing before it was diluted 800-fold with Assay Diluent B prior to its use in the assay procedure. After the standards were centrifuged and mixed, 100µL of standards were pipetted into the wells, and 100µL of the samples were pipetted into the remaining wells. Once standards and samples were placed in the appropriate wells, the plate was covered and incubated for 2.5h to allow SAA in a sample to be bound to the wells by immobilized antibody. Wells were then washed four times using 300µL of the wash solution. The plate was aspirated against a clean paper towel after each wash step to ensure complete removal of the liquid. 100 µL of Biotinylated anti-Human SAA antibody was added to each well and subsequently incubated for 1h at room temperature with gentle shaking. The washing procedure was then repeated to remove the unbound biotinylated antibody before 100µL of HRP-conjugated streptavidin was pipetted into the wells. A third wash procedure is completed before 100µL of TMB substrate solution was added and colour develops in proportion to the SAA bound in the well. To complete the assay procedure, 50µL of stop solution is added and the colour changes from blue to yellow. The intensity of the colour is measured at 450nm using a FilterMax F5 platereader (Molecular Devices, California, USA).

3.6 GPS Data Collection

STATSports™ GPS units (STATSports Apex; Belfast, Northern Ireland) were worn by the players for the duration of the warm-up and the game. Additionally, all players wore GPS units during training sessions. The same unit was worn by each player for the duration of the study. Units were powered on 30min to 1h before the beginning of the warm-up in line with manufacturers recommendations. Data were downloaded and further analysed by the STATSports (Belfast, Northern Ireland) Apex Software (Apex 18 Hz version 3.0). Start times for all sessions were entered before data for the relevant players was selected for analysis. The relevant data was selected and exported to Excel (Microsoft, Washington, USA) where it was further analysed. Only data that returned a consistent speed graph upon downloading were included in the analysis for this study.

Performance metrics for both volume and intensity were analysed following the game. Metrics used included TD, HSR, relative TD, as well as HSR%. The threshold for HSR was set at ≥ 5.5m.s^{-1}. HSR% was calculated by dividing the number of high-speed efforts, entries above 5.5m.s^{-1}, by the HSR total.

Data was downloaded using the Stats Sports™ software (version 3.0) where it was edited prior to being exported in csv. format. Once exported, it was uploaded to a master sheet on Excel (Microsoft, Washington, USA) on the Windows 10 operating system where it was stored until statistical analysis. All data was stored on a password locked PC in a password protected folder for data protection purposes.

3.7 Overview of Testing Protocol

Test	Details
Saliva Sample	• Passive drool sample • Analysed via ELISA for T and C concentrations • Pre-game (2h, 46h) • Post-game (immediately, 48h, 72h)
Blood Sample	• Anterior cubital fossa sample • Analysed via ELISA for SAA • Pre-game (46h, 2h) • Post-game (immediately, 48h, 72h)
Global Positioning System (GPS)	• Worn for duration of the game to quantify 'match-play demands' • Measures total distance (TD), high-speed running distance (HSR), total distance per minute (relative TD) and high-speed running density (HSR%).

3.8 Statistical Analysis

Data were initially screened using JASP software (version 0.9.2.0) for normality of distribution using Shapiro-Wilk test. Data were subsequently analysed using a repeated measures ANOVA to explore the fluctuations in hormone concentrations across the five measured timepoints. Bonferroni *post-hoc* tests were performed on significant data to determine where the significance occurred between samples.

Pearson Product-Moment Correlations were performed to describe the relationships between measures of internal and external load, specifically, hormonal concentrations and various GPS metrics. The results were graded against the Pearson Product Moment Correlation scale, which graded the

strength of the relationships as well as describing the direction they followed (Schober and Schwarte, 2018).

Linear regression analysis was conducted to explore the ability of individual GPS metrics to quantify the variance of Δ immunoendocrine concentrations. Multiple regression analysis was conducted to explore the ability of grouped GPS metrics to quantify the variance of Δ immunoendocrine concentrations. Volume metrics were grouped together (TD and HSR, TD and minutes, and HSR and minutes), while a combination of HSR and HSR% were combined to explore absolute and relative intensity measures. Finally, HSR% and relative TD were combined to explore intensity measures on their own.

***R* value**	**Direction of Correlation**
-1	Perfect negative correlation
-0.7	Strong negative correlation
-0.5	Moderate negative correlation
-0.3	Weak negative correlation
0	No correlation
0.3	Weak positive correlation
0.5	Moderate positive correlation
0.7	Strong positive correlation
1	Perfect positive correlation

Statistical analysis was performed using JASP software (version 0.9.2.0; Amsterdam, The Netherlands), with significance set at $P<0.05$. Results are presented as means with variation presented as standard error of the mean (SEM). Due to the low sample size, effect size was also measured and was reported using partial eta squared (partial η^2). The magnitude of effect size was reported as 0.01 being a small effect size, 0.06 as a moderate effect size and above 0.14 as a large effect size (Cohen, 1973). Both linear and multiple regression analysis was conducted using Excel (Microsoft, Washington, USA). The coefficient of determination was presented as R^2. Delta (Δ) immunoendocrine concentrations were calculated to represent the absolute changes between timepoints. They were calculated by subtracting the earlier timepoint, from the later timepoint, to quantify the increase or decrease in immunoendocrine concentrations, ie. 0h post-match minus 2h pre-match, to determine the increase or decrease reported from pre- to post-game in the given immunoendocrine measure.

Chapter 4

4.0 Results

4.0 Results

4.1 Pooled Data

4.1.1 T Concentrations

Repeated measures ANOVA revealed no significant effect for time (P<0.05) for salivary T (F = 0.859, P=0.494, partial η2 = 0.051), as outlined below in Figure 4.1. A breakdown of individual samples is outlined in Appendix F. Partial η2 results indicate a large effect size.

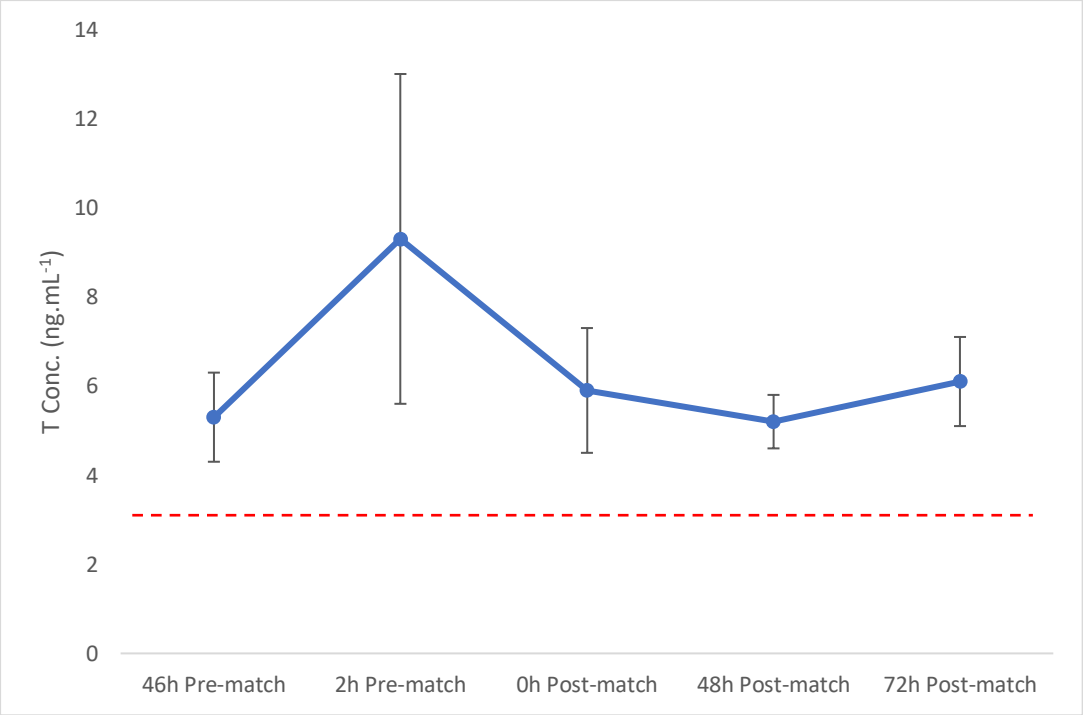

Figure 4.1 – Mean ± SEM T concentrations across sampling timepoints. Dashed line represents normative concentrations as per Hayes *et al.* (2016) (*n* = 17)

4.1.2 C Concentrations

Statistical analysis revealed a significant effect for time for salivary C (F = 16.57, P=0.001, partial η2 = 0.509). Partial η2 results indicate a large effect size. Bonferroni post-hoc analysis revealed samples

collected immediately post-match were significantly higher than all other timepoints (P<0.05). Results are presented below in Figure 4.2. Individual responses can be seen in Appendix G.

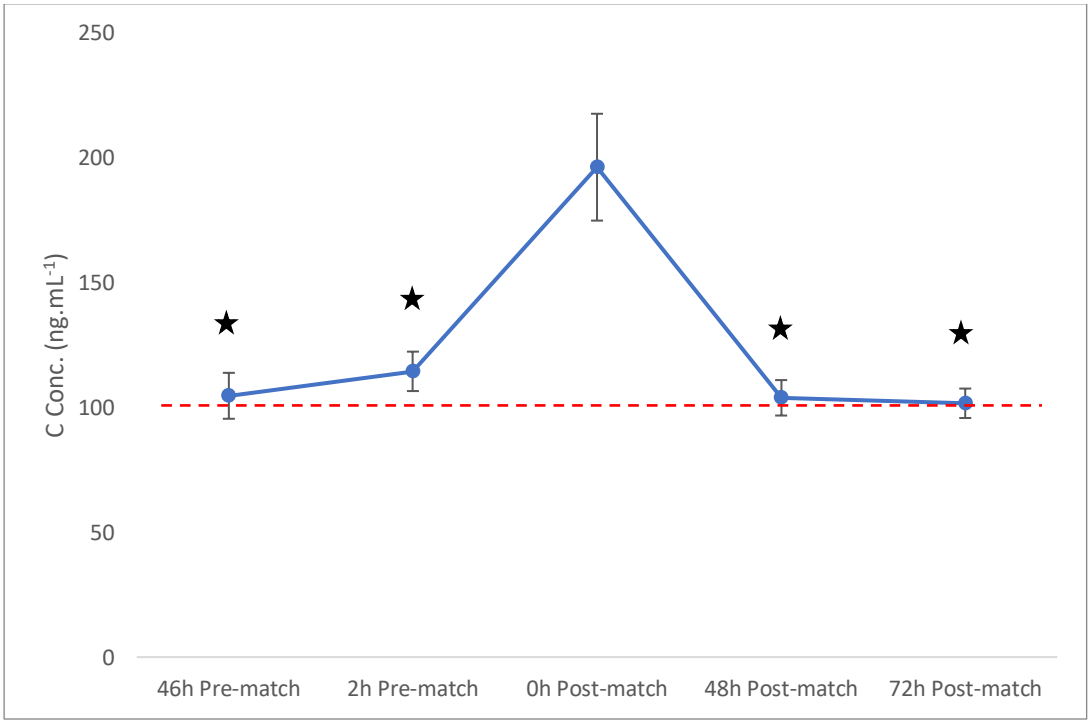

Figure 4.2 – Mean ± SEM C concentrations across sampling timepoints. Dashed line represents normative concentrations Hayes *et al.* (2016) (n = 17).★ Significantly different to 0h post-match

4.1.3 T:C Ratio

Statistical analysis revealed no significant effect for time (P<0.05) for T:C ratios (F = 1.109, P=0.360, partial η2 = 0.065). Partial η2 results indicate a small effect size. The timeline of changes can be seen below in Figure 4.3.

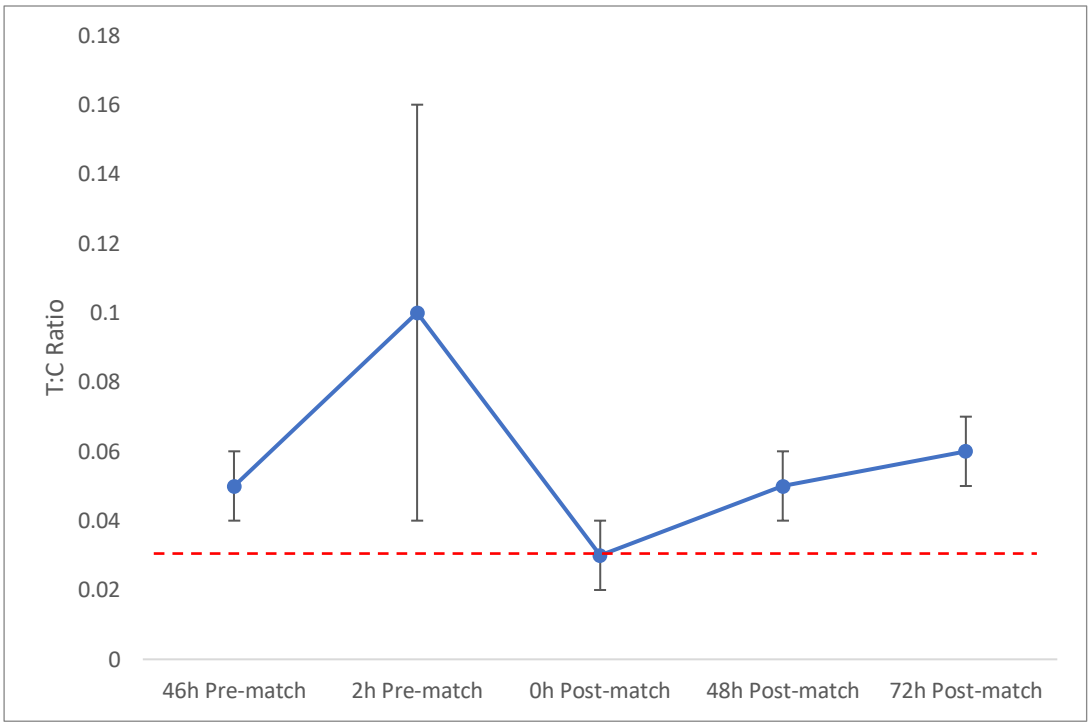

Figure 4.3 – Mean ± SEM T:C ratios across sampling timepoints. Dashed line represents normative concentrations Hayes *et al.* (2016) (*n* = 17).

4.1.4 SAA Concentrations

For SAA concentrations (see figure 4.4), no significant effect for time was observed (F = 0.281, *P*=0.889, partial η2 = 0.027). Partial η2 results indicate a small effect size.

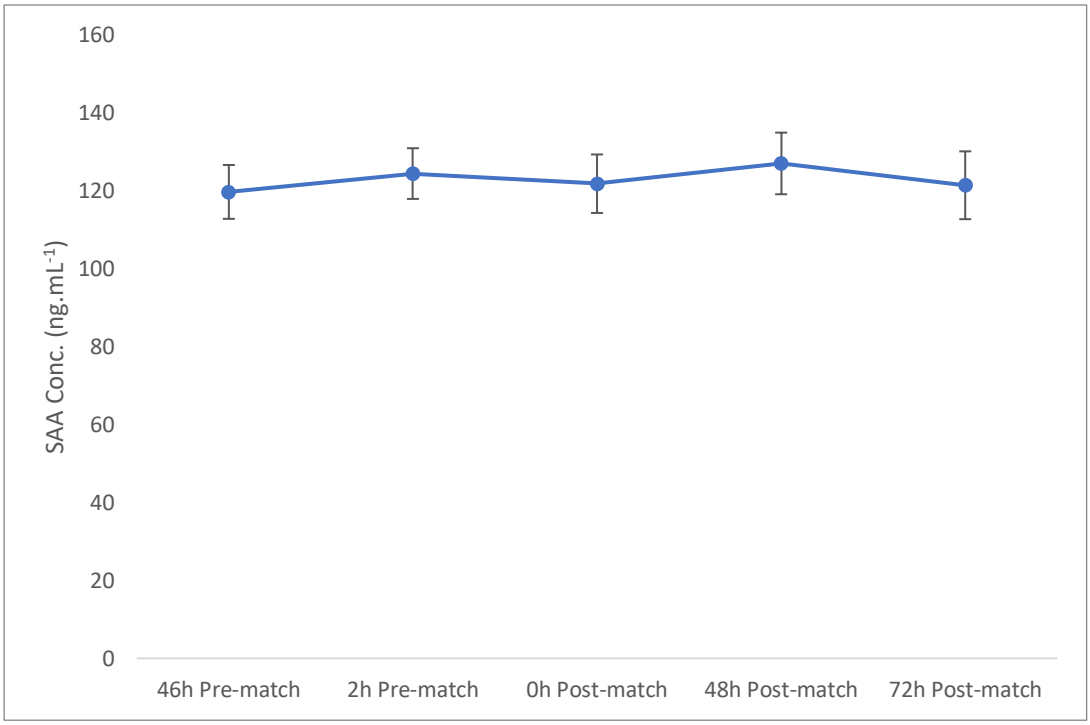

Figure 4.4 – Mean ± SEM SAA concentrations across sampling timepoints (*n* = 11).

4.1.5 GPS metrics

GPS metrics for the pooled games (*n* = 4) are outlined below in Table 4.1. GPS metrics for individuals from each game can be found in Appendix E.

Table 4.1 – Mean ± SEM Pooled GPS data from four games (*n*=20)

	TD (m)	HSR (m)	Min (mins)	Relative TD (m.min^{-1})	HSR% (%)
Mean ± SEM	7701.0 ± 623.1	809.1 ± 82.0	64.1 ± 4.9	122.1 ± 3.9	11.1 ± 0.8

TD = Total distance; HSR = High-speed running; Min = Minutes played; HSR% = High-speed running density

4.1.6 Training Load

Mean ± SEM training load (AU) was collected on the day of each sample and was calculated by multiplying session time by RPE. The mean ± SEM training load for the pitch sessions that occurred prior to the sample at 46h pre-match was 204 ± 23, with a mean ± SEM RPE of 4.3 ± 0.3. Mean ± SEM training load for games was 484 ± 39.6, with a mean ± SEM RPE of 7.5 ± 0.4. The gym sessions that

occurred after players provided the sample at 48h post-match had a mean ± SEM training load of 328 ± 11.5, with a mean ± SEM RPE of 5.5 ± 0.2. Finally, after providing a sample at 72h post-match, the players participated in a pitch session with a mean ± SEM training load of 486 ± 34.8, with a mean ± SEM RPE of 6.9 ± 0.4.

4.1.7 Pearson's Correlations

Pearson's correlations were performed to explore the relationships between GPS metrics and Δ immunoinflammatory marker concentrations. Results are outlined below in Table 4.2.

Table 4.2 – Exploring relationships between pooled game GPS metrics and Δ immunoendocrine marker concentrations

	TD (m)	HSR (m)	Min	Relative TD ($m.min^{-1}$)	HSR% (%)
TΔ46-0	0.22	0.04	0.34	-0.40	-0.28
TΔ2-0	-0.09	-0.39	0.01	-0.29	0.25
TΔ0-48	-0.40	-0.39	-0.48	0.29	0.27
TΔ0-72	-0.31	-0.22	-0.37	0.17	0.31
CΔ46-0	0.26	0.17	0.32	-0.19	-0.45
CΔ2-0	0.18	0.12	0.25	-0.20	-0.32
CΔ0-48	-0.24	-0.20	-0.28	0.14	0.34
CΔ0-72	-0.20	-0.16	-0.24	0.11	0.37
TCΔ46-0	-0.09	-0.15	-0.04	-0.12	-0.12
TCΔ2-0	-0.16	-0.43	-0.10	-0.16	0.29
TCΔ0-48	-0.20	-0.23	-0.23	0.09	0.28
TCΔ0-72	-0.05	-0.08	-0.06	-0.08	0.23
SAAΔ46-0	0.10	0.07	-0.03	0.22	-0.17
SAAΔ2-0	0.07	0.13	-0.05	0.19	-0.32
SAAΔ0-48	-0.13	-0.17	0.10	-0.36	0.38
SAAΔ0-72	0.14	0.07	0.35	-0.34	0.24

Results presented as R^2 values

4.1.7.1 Δ T Concentrations and GPS metrics

GPS metrics did not present any significant correlations with Δ T concentrations (Table 4.2). A weak-moderate correlation was described for relative TD (*r* = -0.40). HSR displayed a weak-moderate negative correlation with TΔ2-0 concentrations (*r* = -0.40). Negative weak-moderate correlations were

described for TD and HSR metres (*r* = -0.40 and -0.39, respectively), while a negative moderate relationship was described between TΔ0-48 concentrations and minutes played. Similar findings were described for relationships between TΔ0-72 concentrations and HSR% displaying weak-moderate correlations (*r* = 0.31). Weak-moderate negative relationships were reported for TD and minutes played (*r* = -0.31 and -0.37, respectively).

4.1.7.2 Δ C concentrations and GPS metrics

GPS metrics did not present any significant correlations with Δ C concentrations (Table 4.2). CΔ46-0 concentrations reported a moderate negative relationship for HSR% (*r* = -0.45). A weak-moderate positive correlation was also reported for CΔ46-0 concentrations and HSR% (*r* = 0.37, respectively).

4.1.7.3 ΔT:C Ratios and GPS metrics

No significant relationships were reported between Δ T:C ratios and GPS metrics (Table 4.2). The largest correlation reported was between TCΔ0-2 with HSR where a weak-moderate negative relationship was reported (*r* = -0.43).

4.1.7.4 Δ SAA Concentrations and GPS metrics

No significant correlations were reported for Δ SAA concentrations and GPS metrics (Table 4.2). A weak negative relationship was recorded between SAAΔ0-2 and HSR% (*r* = -0.32). A weak positive relationship was reported between SAAΔ0-48 and HSR% (*r* = 0.38). A weak positive relationship was reported between SAAΔ0-72 and minutes played (*r* = 0.35), in addition to a weak negative relationship was reported between SAAΔ0-72 and relative TD (*r* = -0.34).

4.1.8 Variance in Δ immunoendocrine concentrations explained by GPS metrics

To explore the effect that multiple metrics have on immunoendocrine variances across timepoints, multiple regressions were used to analyse the Δ immunoendocrine concentrations. The coefficient of determination was collected and subsequently reported to determine the percentage of the variance in immunoendocrine concentrations that each metric explains. Multiple regressions were then run to determine which combination of metrics best explained the variance.

4.1.8.1 Individual GPS Metrics

Table 4.3- Linear Regressions analysis of Δ immunoendocrine marker concentrations and GPS metrics

	All GPS	TD (m)	HSR (m)	Mins	Relative TD ($m.min^{-1}$)	HSR% (%)
TΔ46-0	0.56	0.05	0.00	0.12	0.16	0.08
TΔ2-0	0.42	0.01	0.15	0.00	0.08	0.06
TΔ0-48	0.43	0.16	0.09	0.23	0.08	0.07
TΔ0-72	0.48	0.10	0.05	0.14	0.03	0.10
CΔ46-0	0.46	0.07	0.03	0.10	0.03	0.20
CΔ2-0	0.34	0.03	0.02	0.06	0.04	0.11
CΔ0-48	0.25	0.06	0.04	0.08	0.02	0.11
CΔ0-72	0.31	0.04	0.03	0.06	0.01	0.14
TCΔ46-0	0.14	0.01	0.02	0.00	0.01	0.01
TCΔ2-0	0.34	0.02	0.18	0.01	0.03	0.09
TCΔ0-48	0.22	0.04	0.05	0.05	0.01	0.08
TCΔ0-72	0.20	0.00	0.01	0.00	0.01	0.05
SAAΔ46-0	0.14	0.01	0.01	0.00	0.05	0.03
SAAΔ2-0	0.15	0.00	0.02	0.00	0.04	0.10
SAAΔ0-48	0.50	0.02	0.03	0.01	0.13	0.14
SAAΔ0-72	0.34	0.02	0.00	0.12	0.12	0.06

Results presented at R^2 values

4.1.8.1.1 Δ T Concentrations and GPS metrics

56% of changes in TΔ46-0 concentrations could be attributed to all GPS metrics. Analysis of the variance in TΔ2-0 concentrations determined that 42% of the variance could be explained by all GPS metrics. 43% of variance in TΔ0-48 concentrations was explained by all GPS metrics. All GPS metrics could explain 48% of variance in TΔ0-72.

4.1.8.1.2 Δ C Concentrations and GPS metrics

All GPS metrics explained 46% of variance in CΔ46-0 concentrations. Thirty-four percent of variance at CΔ2-0 could be explained by all GPS metrics. Thirty-one percent of variance at CΔ0-72 was explained by all GPS.

4.1.8.1.3 Δ T:C Ratios and GPS metrics

Only 34% of pre-post game variance as measured by TCΔ2-0, could be explained by all GPS metrics. Similarly, only 22% of variance at TCΔ0-48 could be explained by all GPS metrics. Finally, 20% of variance at TCΔ0-72 was explained by all GPS metrics and 0% of variance was explained by both TD and minutes played.

4.1.8.1.4 Δ SAA Concentrations and GPS metrics

Variances in the 48h recovery window post-game, as described by SAAΔ0-48, could be explained by all GPS metrics (50%). All GPS metrics only explained 34% of variance in SAAΔ0-72.

4.1.8.2 Combined GPS Metrics

Table 4.4- Multiple regressions analysis of Δ immunoendocrine marker concentrations and GPS metrics

	TD x HSR	TD x Min	HSR x Min	HSR x HSR%	HSR% x Relative TD
TΔ46-0	0.11	0.27	0.25	0.08	0.33
TΔ2-0	0.32	0.12	0.40	0.16	0.11
TΔ0-48	0.17	0.27	0.25	0.11	0.23
TΔ0-72	0.10	0.17	0.15	0.11	0.17
CΔ46-0	0.07	0.13	0.12	0.20	0.31
CΔ2-0	0.04	0.11	0.08	0.11	0.20
CΔ0-48	0.06	0.09	0.08	0.12	0.18
CΔ0-72	0.04	0.07	0.06	0.14	0.20
TCΔ46-0	0.03	0.04	0.04	0.06	0.04
TCΔ2-0	0.30	0.06	0.32	0.20	0.09
TCΔ0-48	0.05	0.06	0.06	0.09	0.11
TCΔ0-72	0.01	0.00	0.01	0.05	0.05
SAAΔ46-0	0.01	0.05	0.01	0.03	0.06
SAAΔ2-0	0.03	0.04	0.05	0.10	0.10
SAAΔ0-48	0.03	0.14	0.09	0.14	0.20
SAAΔ0-72	0.03	0.19	0.15	0.10	0.13

Results presented as R^{2-} values

4.1.8.2.1 Δ T Concentrations and GPS metrics

The highest percentage of variance explained by GPS metrics was in TΔ2-0 where forty percent of the variance was explained by HSR and minutes played. All other combinations explained less than 40% of variance as outlined in table 4.4.

4.1.8.2.2 Δ C Concentrations and GPS metrics

Similar low percentages of variance were reported for Δ C concentrations with 31% of variance in CΔ46-0 was explained by relative TD and HSR%. All other combinations of GPS metrics explained 20% or less.

4.1.8.2.3 Δ T:C Ratios and GPS metrics

Similar low percentages of variances were explained in Δ T:C ratios. The highest percentage of variance was reported in TCΔ2-0 concentrations with TD and HSR with 30% of variance described.

4.1.8.2.4 Δ SAA Concentrations and GPS metrics

No GPS metric combinations described variances above 20% in Δ SAA concentrations. HSR% and relative TD described 20% of the variance in SAAΔ0-48 concentrations.

4.2 Individual Game Data

4.2.1 Participants

In addition to the pooled data outlined in section 4.1, a subset of this pooled data (*n* = 8; mean ± SEM, age 25.6 ± 1.2yrs, height 184.1 ± 2.2cm, body mass 87.7 ± 1.6kg), was selected and subsequently analysed, using data from only one of the four sampled games. Seven eligible saliva samples were analysed (*n* = 7; mean ± SEM, age 25.1 ± 1.3yrs, height 184.7 ± 2.5cm, body mass 88.0 ± 1.7kg), alongside four serum samples (*n* = 4; mean ± SEM, age 25.8 ± 1.8rs, height 188.0 ± 3.5cm, body mass 89.0 ± 2.2kg). Results for this subset are outlined below. An overview of descriptive statistics can also be seen in Appendix D.

4.2.2 T Concentrations

Repeated measures ANOVA revealed no significant effect for time (*P*>0.05) for salivary T (F = 1.720, *P*=0.178, partial η2 = 0.223). Partial η2 results indicate a moderate effect size.

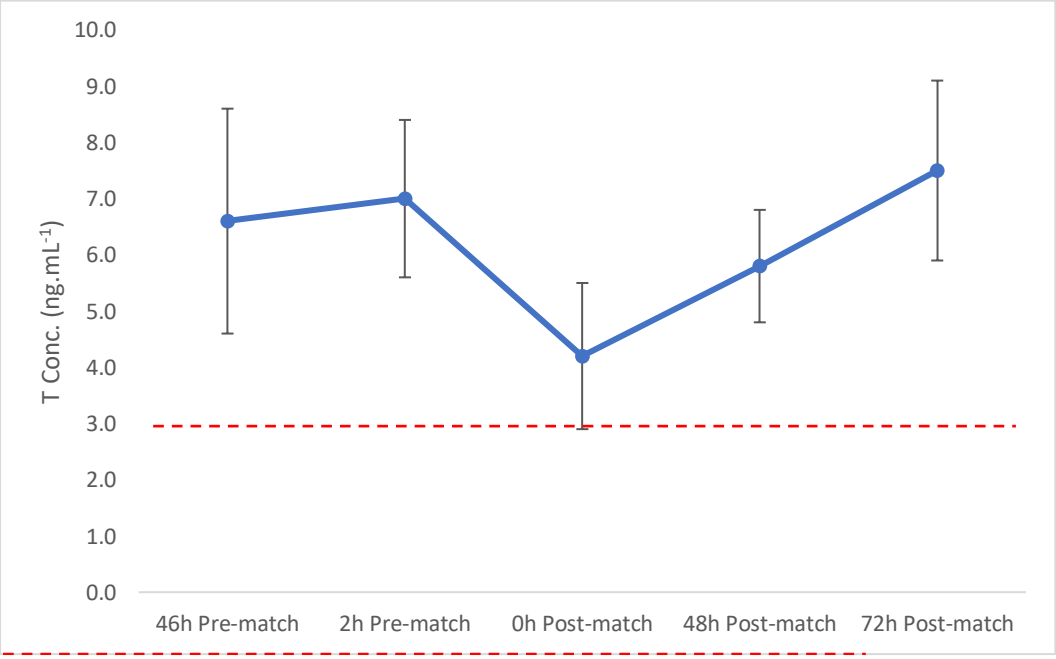

Figure 4.5 – Mean ± SEM T concentrations across sampling timepoints (*n* = 7). Dashed line represents normative concentrations as per Hayes *et al.* (2016)

4.2.3 C Concentrations

Repeated measures ANOVA revealed no significant effect for time (*P*>0.05) for salivary C (F = 2.519, *P*=0.068, partial η2 = 0.296). Partial η2 results indicate a large effect size.

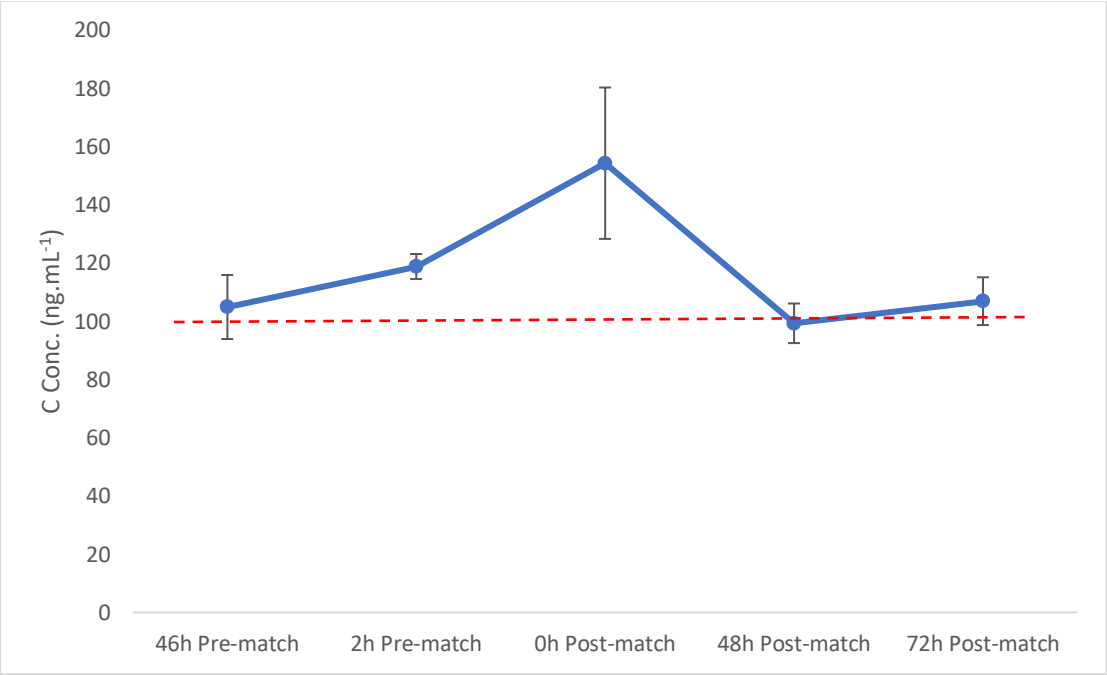

Figure 4.6 – Mean ± SEM C concentrations across sampling timepoints (*n* = 7). Dashed line represents normative concentrations as per Hayes *et al.* (2016)

4.2.4 T:C Ratio

Statistical analysis reported no significant effect for time (P>0.05) for T:C ratios (F = 2.760, P=0.051, partial η2 = 0.315). Partial η2 results indicate a large effect size.

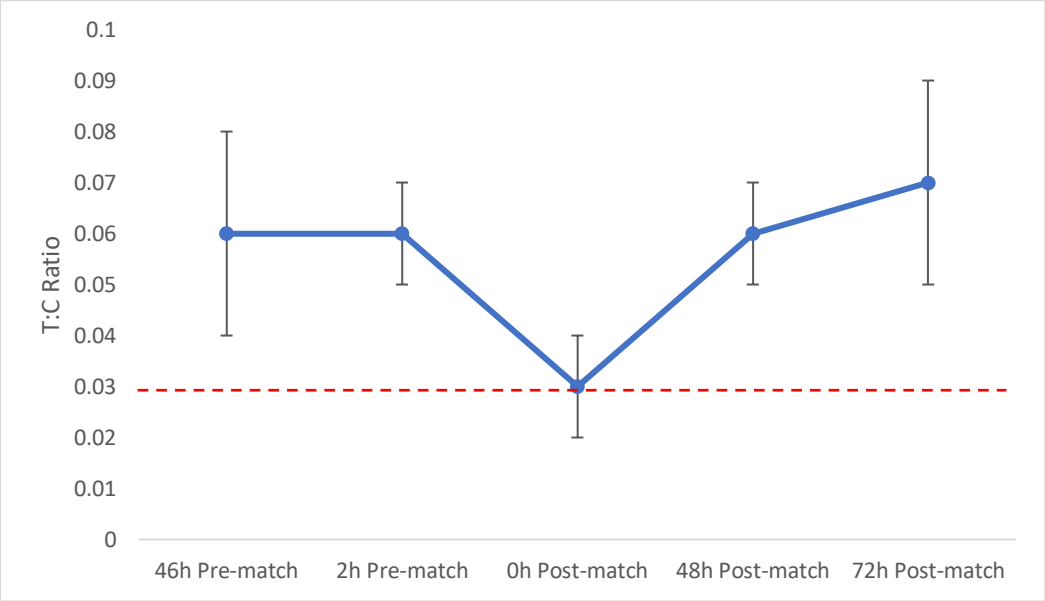

Figure 4.7 – Mean ± SEM T:C ratios across sampling timepoints (n = 7). Dashed line represents normative ratio as per Hayes *et al.* (2016)

4.2.5 SAA Concentrations

Statistical analysis revealed a significant effect for time for SAA (F = 4.071, P=0.026, partial η2 = 0.576). Partial η2 results indicate a large effect size. Bonferroni post-hoc results did not show significance across timepoints.

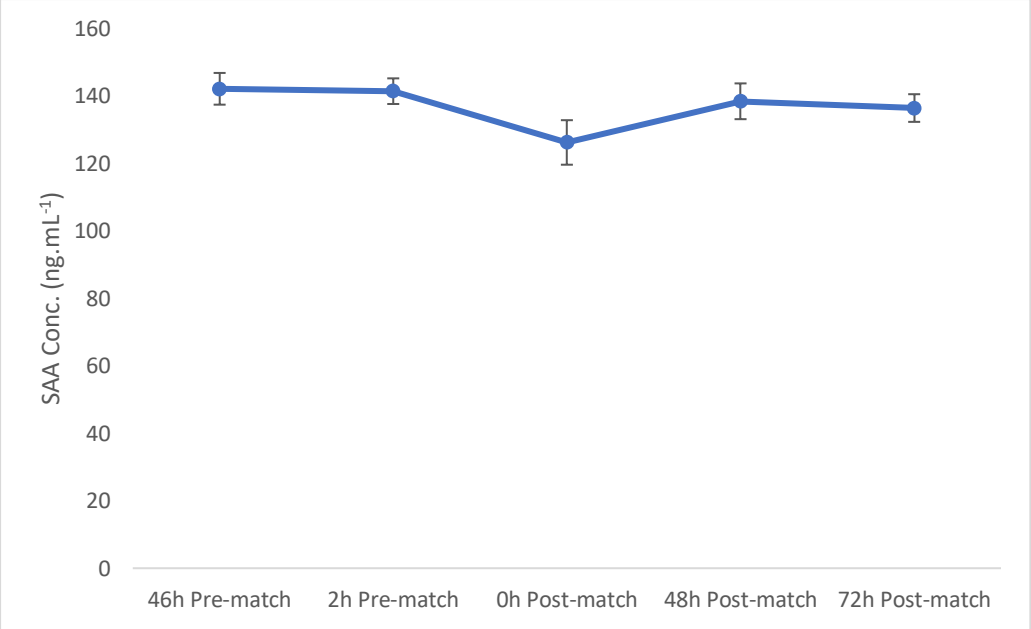

Figure 4.8 – Mean ± SEM SAA concentrations across sampling timepoints (*n* = 4).

4.2.6 GPS metrics

Individual game GPS metrics are outlined below in Table 4.5. GPS metrics for individuals from each game can be found in Appendix E.

Table 4.5 –

Mean ± SEM GPS data for individual game samples (*n*=8).

	TD (m)	HSR (m)	Min	Relative TD ($m.min^{-1}$)	HSR% (%)
Mean (± SEM)	7690.0 ± 853.3	806.5 ± 100.8	68.6 ± 5.9	112.4 ± 7.5	14.2 ± 0.7

4.2.7 Training Load

Mean ± SEM training load (AU) was collected on the day of each sample and was calculated by multiplying session time by RPE. The mean ± SEM training load for the pitch session that occurred prior to the sample at 46h pre-match was 135 ± 13, with a mean ± SEM RPE of 3.4 ± 0.3. Mean ± SEM

training load for the game was 529 ± 44, with a mean ± SEM RPE of 8.1 ± 0.2. The gym session that occurred after players provided the sample at 48h post-match had a mean ± SEM training load of 317 ± 17, with a mean ± SEM RPE of 5.3 ± 0.3. Finally, after providing a sample at 72h post-match, the players participated in a pitch session with a mean ± SEM training load of 429 ± 41, with a mean ± SEM RPE of 6.1 ± 0.3.

4.2.8 Pearson's Correlations

Pearson's correlations were performed on the data to explore to relationships between GPS metrics and Δ immunoinflammatory marker concentrations. Results are outlined in Table 4.6 below.

Table 4.6 – Exploring relationships between GPS metrics across four pooled games, and Δ immunoendocrine marker concentrations.

	TD (m)	HSR (m)	Min	Relative TD ($m.min^{-1}$)	HSR% (%)
TΔ46-0	0.07	0.29	0.34	-0.56	-0.59
TΔ2-0	-0.01	0.05	0.16	-0.38	-0.44
TΔ0-48	-0.56	-0.72	-0.65	0.03	0.83*
TΔ0-72	-0.63	-0.63	-0.73	0.03	0.68
CΔ46-0	0.33	0.41	0.55	-0.39	-0.60
CΔ2-0	0.16	0.33	0.35	-0.34	-0.41
CΔ0-48	-0.24	-0.41	-0.38	0.24	0.48
CΔ0-72	-0.30	-0.50	-0.46	0.27	0.58
TCΔ46-0	-0.16	0.05	0.03	-0.47	-0.31
TCΔ2-0	-0.06	-0.12	0.03	-0.21	-0.30
TCΔ0-48	-0.28	-0.39	-0.44	0.27	0.66
TCΔ0-72	-0.31	-0.27	-0.42	0.14	0.37
SAAΔ46-0	-0.90	-0.84	-	-0.90	0.57
SAAΔ2-0	-0.97*	-0.92	-	-0.97*	0.47
SAAΔ0-48	0.39	0.50	-	0.39	0.70
SAAΔ0-72	0.93	0.88	-	0.93	-0.39

* signifies significance at $P<0.05$

4.2.8.1 Δ T Concentrations and GPS metrics

A significant strong positive relationship was reported for HSR% and TΔ0-48 (r = 0.83, P=0.020), as outlined in Table 4.6. A moderate negative correlation was described between TΔ0-48 and both

intensity metrics relative TD (*r* = -0.56) and HSR% (*r* = -0.59). A negative moderate negative relationship was reported between TΔ0-48 concentrations and TD (*r* = -0.56), while a strong negative correlation was described for HSR (*r* = -0.72). A moderate-strong negative relationship was described for minutes played (*r* = -0.65). Similar results were reported for TΔ0-72, with moderate-strong negative correlations reported for both TD (*r* = -0.63) and HSR (*r* = -0.63). A strong negative relationship was reported for minutes played (*r* = -0.73). A moderate-strong positive correlation was described for TΔ0-72 concentrations and HSR% (*r* = 0.68).

4.2.8.2 Δ C Concentrations and GPS metrics

No significant relationships were reported between GPS metrics and Δ C concentrations. A moderate positive relationship was reported between CΔ46-0 and minutes played (*r* = 0.55), while a moderate-strong negative relationship was described for HSR% (*r* = -0.60). A moderate negative relationship was described for HSR (*r* = -0.50) and a weak-moderate relationship for minutes played (*r* = -0.46). A moderate relationship was described for HSR% (*r* = 0.5). Additional correlations are reported in Table 4.6.

4.2.8.3 Δ T:C Ratios and GPS metrics

No significant relationships were described between any GPS metrics and Δ T:C ratios, as outlined in Table 4.6. A moderate negative relationship was reported between TCΔ46-0 and relative TD (*r* = -0.47). A moderate-strong positive relationship was reported between TCΔ0-48 and HSR% (*r* = 0.66).

4.2.8.4 Δ SAA Concentrations and GPS metrics

Significant strong negative correlations were described between SAAΔ2-0 and both TD (*r* = -0.97; *P*=0.035) and relative TD (*r* = -0.97; *P*=0.035). SAAΔ46-0 correlations demonstrated strong negative relationships with TD (*r* = -0.90), HSR (*r* = -0.84), and relative TD (*r* = -0.90). Similar relationships were reported between SAAΔ2-0 and HSR with a strong negative correlation described (*r* = -0.92). A strong positive correlation was reported between HSR% and SAAΔ0-48 (*r* = 0.70), while strong positive relationships were also reported for TD (*r* = 0.93) and relative TD (*r* = 0.93) with SAAΔ0-72. Finally, a strong positive correlation was described between HSR and SAAΔ0-72 (*r* = 0.88). As all players played the same number of minutes and thus no correlations results were recorded, as can be seen in Table 4.6.

4.2.9 Regression Analysis

To explore the effect that multiple metrics have on immunoendocrine variances across timepoints, multiple regressions were run to analyse the Δ immunoendocrine concentrations. The coefficient of determination was collected and subsequently reported to determine the percentage of the variance in immunoendocrine concentrations that each metric explains. Multiple regressions were then run to determine which combination of metrics best explained the variance.

4.2.9.1 Individual GPS Metrics

Table 4.7 - Linear Regressions between GPS metrics and Δ immunoinflammatory concentrations

	All GPS	TD (m)	HSR (m)	Mins	Relative TD ($m.min^{-1}$)	HSR% (%)
TΔ46-0	0.93	0.01	0.09	0.11	0.32	0.35
TΔ2-0	0.42	0.00	0.00	0.03	0.15	0.20
TΔ0-48	0.84	0.32	0.52	0.43	0.00	0.70
TΔ0-72	0.64	0.40	0.40	0.53	0.00	0.46
CΔ46-0	0.56	0.11	0.17	0.30	0.15	0.36
CΔ2-0	0.36	0.03	0.11	0.12	0.13	0.17
CΔ0-48	0.34	0.06	0.17	0.14	0.06	0.23
CΔ0-72	0.47	0.09	0.25	0.21	0.07	0.34
TCΔ46-0	0.81	0.03	0.00	0.00	0.22	0.10
TCΔ2-0	0.26	0.00	0.13	0.00	0.05	0.09
TCΔ0-48	0.47	0.08	0.15	0.20	0.07	0.43
TCΔ0-72	0.30	0.10	0.07	0.18	0.02	0.14
SAAΔ46-0	-	0.80	0.71	0.52	0.80	0.32
SAAΔ2-0	-	0.93	0.84	0.52	0.93	0.22
SAAΔ0-48	-	0.15	0.25	0.32	0.15	0.50
SAAΔ0-72	-	0.86	0.78	0.49	0.86	0.16

Results presented as R^2

4.2.9.1.1 Δ T Concentrations and GPS metrics

The relationship between all GPS metrics and TΔ46-0 explained 93% of the variance in TΔ46-0 concentrations. Relationships between TΔ0-48 concentrations and all GPS metrics explained 84% of the variance seen in TΔ0-48. HSR% explained 70% of the variance in TΔ0-48 concentrations. Similar

results were reported for TΔ0-72 concentrations, with all GPS metrics explaining 64% of the variance. Minutes played explained 53% of the variance in TΔ0-72 concentrations. Additional relationships are outlined in Table 4.7.

4.2.9.1.2 Δ C Concentrations and GPS metrics

The variance in CΔ46-0, as outlined in Table 4.7, was best explained by all GPS metrics with 56% of the variance explained. Most of the variance in CΔ0-72 concentrations was explained by all GPS metrics (47%).

4.2.9.1.3 Δ T:C Ratios and GPS metrics

All GPS metrics explained 81% of the variance in TCΔ46-0 ratios. All other GPS metrics, as can be seen in Table 4.7, reported less than 50% of variance in other Δ T:C ratios.

4.2.9.1.4 Δ SAA Concentrations and GPS metrics

TD explained 80% of the variance in SAAΔ46-0 concentrations, while 72% of the variance could be explained by HSR. Minutes played accounted for 52% of the variance. Relative TD explained 80% of the variance in SAAΔ46-0 concentrations. Similar results were reported for variance in SAAΔ2-0 concentrations, with TD explaining 93% and HSR explaining 84%. Relative TD explained the same amount of variance in SAAΔ2-0 concentrations with 93%. TD explained 86% of the variance in SAAΔ0-72 concentrations and HSR explained 78% of the variance. Relative TD explained 86% of the variance. All GPS metrics could not be reported due to too small of a sample size, with too many metrics in the equation, hence their omission from Table 4.7.

4.2.9.2 Combined GPS Metrics

Table 4.8 - Multiple Regressions between GPS metrics and Δ immunoinflammatory concentrations

	TD x HSR	TD x Mins	HSR x Mins	HSR x HSR%	HSR% x Relative TD
TΔ46-0	0.12	0.47	0.12	0.36	0.58
TΔ2-0	0.01	0.19	0.03	0.29	0.30
TΔ0-48	0.52	0.44	0.56	0.75	0.70
TΔ0-72	0.47	0.54	0.56	0.53	0.47
CΔ46-0	0.17	0.50	0.30	0.36	0.45
CΔ2-0	0.12	0.27	0.14	0.18	0.26
CΔ0-48	0.17	0.21	0.18	0.25	0.26
CΔ0-72	0.26	0.30	0.28	0.36	0.37
TCΔ46-0	0.08	0.23	0.00	0.13	0.28
TCΔ2-0	0.01	0.05	0.04	0.25	0.12
TCΔ0-48	0.15	0.29	0.21	0.44	0.46
TCΔ0-72	0.10	0.21	0.18	0.14	0.15
SAAΔ46-0	0.90	0.98	0.93	0.94	0.95
SAAΔ2-0	1.00	0.94	0.86	0.99	0.99
SAAΔ0-48	0.93	0.17	0.27	0.82	0.99
SAAΔ0-72	0.92	0.88	0.79	0.88	0.89

Results presented as R^2 values

4.2.9.2.1 Δ T Concentrations and GPS metrics

The variance in TΔ46-0 concentrations was explained best by HSR% and relative TD (58%), while TD and minutes played combined explained 47% of the variance in TΔ46-0 concentrations. Results were reported for TΔ0-48 with 52% of variance in TΔ0-48 concentrations explained by TD and HSR. Intensity metrics explained the most variance in the 48h recovery window post-match with HSR and HSR% explaining 75% of the variance, while HSR% and relative TD explaining 70% of the variance. Both TD and HSR, as well as HSR% and relative TD explained 47% of the variance in TΔ0-72 concentrations. HSR% and HSR explained 53% of the variance in TΔ0-72 concentrations, with TD and minutes accounting or 54%, and HSR and minutes accounting for 56% of the variance as outlined in Table 4.8.

4.2.9.2.2 Δ C Concentrations and GPS metrics

Fifty percent of the variance in CΔ46-0 concentrations was explained by TD and minutes, while HSR% and relative TD explaining 45% of the variance. HSR% and HSR explained 36% of the variance in CΔ0-72, with 37% of the variance in CΔ0-72 explained by HSR% and relative TD. Further metrics and their ability to explain variance in Δ C concentrations can be seen in Table 4.8.

4.2.9.2.3 Δ T:C Ratios and GPS metrics

HSR% and relative TD explained 46% of the variance in TCΔ0-48 concentrations, while HSR and HSR% explained 44%. All other metric combinations described less than 30% of the variance in Δ T:C ratios.

4.2.9.2.4 Δ SAA Concentrations and GPS metrics

All GPS metric combinations described at least 80% of the variance in all Δ SAA concentrations, except for TD and minutes (17%), and HSR and minutes (27%) which explained the least amount of variance in SAAΔ0-48 concentrations.

Chapter 5

5.0 Discussion

5.0 Discussion

5.1 Overview

This study aims to determine the immunoendocrine response to Gaelic games match-play, and explore the effects commonly collected GPS metrics have on the fluctuations and subsequent recovery timelines of these immunoendocrine markers. Results indicated that immunoendocrine measures pooled from four games did not significantly differ across time, with the exception of salivary C concentrations. Immunoendocrine concentrations illustrated similar results around one observed game, however only SAA concentrations demonstrated a significant effect for time.

While there were no significant changes reported for T concentrations, T:C ratios and SAA concentrations in the pooled samples, C concentrations were significantly elevated from 2h pre- to 0h post-match. C concentrations at 0h post-match returned to concentrations similar to those observed at baseline, after 48h post-match. No significant relationships were reported between any GPS metrics for volume and intensity, and delta immunoendocrine concentrations. In conjunction with the lack of significance reported in immunoendocrine concentrations across time, these findings would suggest that individual variation in immunoendocrine responses outweighs the effects that external training load has on immunoendocrine responses to competitive match-play elite Gaelic football players.

Individual game analysis with no significant changes in salivary T or C concentrations, however, a significant effect for time was reported for SAA concentrations. Interestingly, no significance difference was reported in the Bonferroni post-hoc analysis, however, this may have been as a result of the limited sample size of $n = 4$.

5.1.1 The response of immunoendocrine markers to competitive match-play in elite Gaelic footballers

Pooled data indicated no significant effect for time for salivary T concentrations, T:C ratio, or SAA concentrations, however a significant effect for time was reported for C concentrations (see Figure 4.2). The significant increases from pre- to post-match in C concentrations in the pooled samples were similar to the findings previously reported in rugby union (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015). Moreover, the lack of significance reported for T concentrations and T:C ratios due to the individual nature of salivary immunoendocrine responses (Hayes *et al.,* 2015)

were previously reported in soccer players (Moreira *et al.,* 2009). Moreira *et al.* (2009) also described no significant changes in salivary C concentrations from pre- to post-match. The significant increases in pooled salivary C concentrations in response to competitive match-play may be due to the anti-inflammatory properties of C which suppress the immune response (Barnes, 1998), but also due to their role in maintenance of blood glucose (Gleeson *et al.,* 2004). Recovery timelines of salivary C concentrations were reported to be 48h post-match in the current research, with previous research reporting a range of recovery timelines for salivary T and C concentrations ranging from 4h post-game (Elloumi *et al.,* 2003) to 60h post-game (West *et al.,* 2014).

No significant effect for time was reported in pooled data for SAA concentrations. These findings are similar to those reported after a 5km run, whereby no significant changes in SAA were reported from immediately pre- to immediately post-run (Drenth *et al.,* 1998). A similar timeline was used by Drenth *et al.* (1998), with samples collected 3-, 24- and 48h after the 5km run. Interestingly, Drenth *et al.* (1998) reported a significant increase in CRP concentrations 24h post-race, before returning to baseline at 48h post-race. These findings by Drenth and colleagues (1998) echo the findings in previous rugby union research, whereby a significant increase in CRP concentrations were reported 38h after a competitive rugby union match (Cunniffe *et al.,* 2010). Research into acute-phase response proteins suggest that their increase can be seen 24h after the onset of the acute-phase response, in response to other cytokines such as IL-6 (Sack Jr, 2018). The lack of significant increases in the current study would indicate that competitive match-play does not induce inflammation associated with the APR in elite Gaelic footballers. Moreover, IL-6 concentrations have been reported to stimulate the increase in CRP concentrations at the onset of inflammation (Steensberg *et al.,* 2003), which is further supported by the research conducted in ultra-endurance marathon runners (Margeli *et al.,* 2005). Margeli *et al.* (2005) reported a significant increase in both IL-6 and SAA concentrations immediately post ultra-endurance marathon, before returning to baseline concentrations 48h post-race. These findings further support the findings that elite Gaelic football matches, do not appear to result in the same level of inflammation as both rugby union (Cunniffe *et al.,* 2010) and ultra-endurance marathon running (Margeli *et al.,* 2005), despite a significant increase in C concentrations immediately post-match.

No significant effects for time were reported for any salivary hormones for the individual game analysis, however a significant effect for time was recorded for SAA concentrations. The lack of significant changes reported for salivary T and C concentrations echo previous research that highlighted the individual nature of hormonal responses (Hayes *et al.,* 2015). However, it is interesting

to note that all players reported a reduction in T concentrations from 2h pre- to 0h post-match (range;3-94%), which would match the expected responses previously reported after rugby union matches (Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015). For salivary C concentrations, 4 out of 7 players in the individual game analysis increased in C concentrations as would be expected based on previous research in rugby union (Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015), but the fact that three players decreased in salivary C concentrations from 2h pre- to 0h post-match, is not surprising, as has been reported in previous research in soccer players (Moreira *et al.,* 2009).

5.1.2 Additional factors influencing immunoendocrine responses around elite Gaelic football match-play

5.1.2.1 T Concentrations

Interestingly, mean T concentrations (see Figure 4.1) increased from 46h pre-match to 2h pre-match (~75%), which may be explained by the anticipatory responses previously outlined by Eubank *et al.* (1997) in elite marathon canoeists, whereby an increase in T concentrations was reported 24h before competition in the debilitatory group. A subsequent reduction (~37%) in mean T concentrations from 2h pre- to 0h post-match were reported which was in line with previous data by Cunniffe *et al.* (2010) who reported a significant decrease in mean T concentrations (~43%) from the morning of a 5pm game, to immediately post-game. Despite the reported decrease in T concentrations from 2h pre- to 0h post-match, the T concentrations reported 0h post-match were actually higher than the concentrations recorded at 46h pre-match (mean ± SEM, 5.9 ± 1.4 *vs.* 5.3 ± 1.0ng.mL^{-1}, respectively). Moreover, an increase (~17%) in mean T concentrations was described from 48h to 72h post-match (mean ± SEM, 5.2 ± 0.6 *vs.* 6.1 ± 1.0ng.mL^{-1}, respectively). These findings may be explained by the gym session that took place immediately after providing the 48h saliva sample, however further research would be warranted. Kraemer *et al.* (2005) explored the factors that contribute to the reported elevation in T concentrations after a resistance training session. The research indicated that players who have previously followed a periodised strength program, have greater elevations in T concentrations than athletes who are typically endurance trained. Furthermore, completing a resistance training session which targeted larger muscle groups, resulted in higher T concentration elevations than a session focusing on smaller muscle groups. The players involved in the present research follow periodised strength and conditioning programmes throughout the year and at the time of testing, would have completed a total body session, containing compound exercises, after the

48h sample was collected. As a result, an acute elevation in T concentrations may have occurred. Despite the occurrence of this gym session, an increase in T concentrations was previously reported by Cunniffe *et al.* (2010) from 14h to 38h post-match when athletes adhered to a strict recovery window where no exercise took place. A mean increase (~20%) was reported by the authors which is similar to the findings of the current research.

Interestingly, Eubank *et al.* (1997) reported that a 15% increase in T concentrations coincided with an increased motivation to win. Individual analysis of the mean T concentrations for the individual game demonstrated the finding that the authors noted which is that some athletes respond to the anticipatory stress, while others do not. In the current research, analysis of the seventeen total samples across the four games revealed that 10 out of the 17 athletes that provided samples, increased T concentrations from 46h to 2h pre-game. Conversely, the 7 remaining players decreased from 46h to 2h pre-match. Interestingly, all of the 10 players who increased T concentrations from 46- to 2h pre-match, all exceeded the 15% which as outlined by Eubank *et al.* (1997) is reported to be accompanied with an increased motivation to win. Most notably, of the 7 players who were reported to be 'non-responders' pre-match, two players appeared twice, contributing to four of the seven samples. Moreover, another player who was reported as a non-responder in game 3, was identified as a responder in game 1. While evidence is limited, this player may have had a greater desire to win in game 1 of the season, compared to game 3 of the season, or this may have been associated with additional psychological influences such as motivational material used in the days preceding the match (Cook and Crewther, 2012). Moreover, greater individual T responses from 85min to 5min pre-match in rugby union players, following these motivational interventions, was associated with better performance ratings from their rugby coaches (Cook and Crewther, 2012). These findings echo those reported by Eubank *et al.* (1997), whereby an increase in pre-match T concentrations was associated with an increased desire to win. Conversely, Cook and Crewther (2012) reported that greater individual C concentrations were associated with lower performance ratings from coaches. These findings emphasise the individual nature of pre-match T responses. The individual nature of salivary C concentrations has previously been outlined in soccer players after a competitive training match (Moreira *et al.,* 2009). Future research should include questionnaires similar to those included by Eubank *et al.* (1997), to identify those that are responders, and those that are not. Despite the strenuous nature of the competitive match-play, players T concentrations returned to within baseline concentrations 48h post-match. These findings indicate that given 48h recovery, including one full day of rest, allows sufficient time to recover from match-play.

Specifically, in the individual game samples, mean T concentrations decreased (~40%) which is similar to pooled samples, as well as those reported by Cunniffe *et al.* (2010). The variance in magnitude of mean T concentration changes from 46h to 2h pre-match may be explained by the individuals included in this subset. These athletes may have been less prone to anticipatory stress, non-responders, as previously discussed by Eubank *et al.* (1997), when compared to the pooled samples. In the current research, individualised analysis revealed that 4 out of the 7 athletes that provided samples, increased T concentrations from 2h to 0h pre-game (range; 48-275%). Contrastingly, three players decreased from 46h to 2h pre-match (range; 21-35%). These findings emphasise the individual nature of pre-match T responses. The individual nature of salivary C concentrations has previously been outlined in soccer players after a competitive training match (Moreira *et al.,* 2009). Future research should include questionnaires similar to those included by Eubank *et al.* (1997), to identify responders and non-responders. However, while there can be some observations made on the individual data, the lack of significance would indicate that the individual nature of salivary T responses was too strong to yield significant results in these elite Gaelic footballers, as outlined for both pooled and individual game sample analysis. While T is associated with increased protein synthesis and increased lean body mass (Griggs *et al.,* 1989), and subsequently maintaining T levels would be favourable in an athletic population, it would not appear that T concentrations are directly influenced by competitive match-play in elite Gaelic footballers.

5.1.2.2 C Concentrations

Pre-match C concentrations in the pooled samples (see Figure 4.2) responded in a similar fashion to T concentrations, with an increase (~9%) in C concentrations from 46h to 2h pre-match (mean ± SEM, 104.7 ± 9.2 *vs.* 114.5 ± 7.9ng.mL^{-1}, respectively), possibly due to the pre-competition anticipatory response previously outlined by both Eubank *et al.* (1997) and Salvador *et al.* (2003). These responses would also have contributed to the increased T:C ratio that was also observed from 46h to 2h pre-match (mean ± SEM, 0.05 ± 0.01 *vs.* 0.10 ± 0.06), despite C concentrations being the only ones that changed significantly across time. Individual game analysis did not reveal any significant effect for time for C concentrations, which may have been affected by the broad range of changes from pre- to post-match, with four players experiencing increased C concentrations, and three players experiencing a decrease. The responses in the pooled samples corroborated previous findings in rugby union athletes for both C concentrations (Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015) and T:C ratios (Cunniffe *et al.,* 2010). An inverse response was reported in C concentrations when compared to T concentrations, with a significant increase (~71%) from 2h pre- to 0h post-match (mean ± SEM, 114.5

± 7.*9 vs.* 196.3 ± 21.4ng.mL^{-1}, respectively. *P*=0.009). These acute increases are likely due to the anti-inflammatory effects of C as previously outlined by Barnes (1998). These increases in C concentrations coincide with the increase in protein oxidation (Braun *et al.,* 2005) and increased neuromuscular fatigue (Duffield *et al.,* 2012) associated with exercise. C concentrations reduced significantly (~47%) to return to within baseline concentrations 48h post-match (mean ± SEM, 103.9 ± 7.1ng.mL^{-1}; *P*=0.005). With both T and C concentrations returning to baseline concentrations 48h post-match, the players in the current study appear to tolerate the training stimulus associated with competitive match-play, when given 48h to recover in line with previous research in rugby union (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015). These findings are also supported by the research conducted by Coutts *et al.* (2007) whereby significant changes in T and C concentrations were reported across six weeks of progressive overload training in both a normal training and an intentionally overstrained group. These findings relate to the current study as the post-match reductions in T:C ratios returned to baseline at 48h post-match, unlike the T:C ratios in the research by Coutts *et al.* (2007), which remained reduced for the duration of the intensified six week block. It could be inferred that if the players T:C ratios did not return to baseline after 48h post-match, they may have been subjected to FOR, however, it appears the players in the current study, are sufficiently recovered after 48h, as per the significant reductions in C concentrations from 2h pre- to 0h post-match. Interestingly, ingestion of a 6% carbohydrate drink, both before and during a marathon have shown to significantly reduce C concentration increases from pre- to post-race when compared to a placebo group (Nieman *et al.,* 2001). As outlined in the methods, this was a free-living experiment and the athletes included in this study had access to exogenous carbohydrate supplements both before, during, and after matches, which may have altered the reported responses. Future research could monitor the carbohydrate intake of athletes to better understand their 2h pre- to 0h post-match C responses, as well as the subsequent recovery timelines of C concentrations.

Another factor which may have influenced the salivary T and C concentrations reported in the current study is the fact two games were home (game 1 and 3), and two were away (2 and 4). Previous research has identified home advantage as having a significant effect on salivary C concentrations, but not salivary T concentrations, in elite academy soccer players (Fothergill *et al.,* 2017). Fothergill *et al.* (2017) reported that salivary C concentrations collected 1h prior to a home game were significantly lower than 30min after the match, while no significant changes were reported from pre- to post-match for away figures. Interestingly, while no significant differences were reported in C concentrations from pre- to post-match in away games, C concentrations pre-match in home games were significantly different to the respective samples in away games. In an additional study included in the same article,

the authors included an STAI-6 questionnaire to measure the athletes mood. Fothergill *et al.* (2017) concluded no relationship between mood states and salivary T or C concentrations, despite similar significant differences reported for time in C concentrations for home games, but not away games. No significant effect for T concentrations was reported for time or venue. These findings may give further context to the findings reported in the current study whereby no significant effect for time was reported for T concentrations.

5.1.2.3 SAA Concentrations

Exercise results in adaptive microtrauma associated with the movement demands of team sports, which subsequently results in local inflammation and a cytokine response (Kreher and Schwartz, 2012). With continued intensified training and its' associated psychological stress, combined with a lack of recovery, athletes may be susceptible to the effects of the Overtraining Syndrome when the normal process of acute localised inflammation become chronic and pathologic (Robson, 2003). Understanding the recovery timelines of SAA in athletes, helps practitioners to fully understand how their athletes respond to competitive match play, as well as the post-match recovery timelines of this protein as a measure of the acute-phase inflammatory response (Sack Jr, 2018). Data presented in the current research reported competitive Gaelic football match play resulted in a negligible decrease (~2%) in SAA concentrations for pooled samples from 2h pre- to 0h post-match (see Figure 4.4). Mean pooled SAA concentrations increased from 0h to 48h post-match (~4%), before a marginal reduction was reported at 72h post-match (0.003%). Interestingly, the increase (~4%) in SAA concentrations described from 46h to 2h pre-match, is the same increase reported from 0h to 48h post-match (~4%). Considering there was 34-48h between both samples, and that both the 46h pre-match and 0h post-match samples were taken immediately post-exercise, it may suggest that both the 2h pre-match and 48h post-match samples represent the acute-phase response protein activity to both training and match-play, respectively.

The magnitude of change across the timepoints for SAA concentrations did not appear to match the increases (~100%) in CRP that were reported by Cunniffe *et al.* (2010), or the 1.5 fold increase reported 24h after a 5 km race by Drenth *et al.* (1998). Despite the 1.5 fold increase reported by Drenth *et al.* (1998), these increases were not significant and reported similar mean concentrations when compared to the current study (~100ng.mL^{-1}), immediately before as well as immediately and 3h after the race. Drenth *et al.* (1998) reported a mean increase (~100%) 24h post-match with concentrations

increasing to ~200ng.mL^{-1} after an intensive 5km race, SAA concentrations remained elevated 48h post-match, despite a lower magnitude (mean; ~150ng.mL^{-1}). Nonetheless, the activity of acute-phase response markers have previously been described as peaking 48h post-exercise, with CRP peak concentrations reported at 38h post-match in elite rugby union players (Cunniffe *et al.,* 2010), which was similar to the current research.

Individualised analysis on a single game (see Figure 4.8) reported a significant effect for time in SAA concentrations, despite post-hoc analysis revealing no significant differences between timepoints. SAA concentrations increased (~10%) from 0h to 48h post-match, before decreasing (~1%) between 48h and 72h post-match. The significance of these findings may have been inflated due to the low sample size of *n* = 4 and should be interpreted with caution as a result. Nonetheless, peak SAA concentrations were reported in the current research study 48h post-match which is in line with previous research for CRP concentrations which were reported to peak 38h post-match (Cunniffe *et al.,* 2010). IL-6 concentrations have been reported to stimulate the increase in CRP concentrations at the onset of inflammation (Steensberg *et al.,* 2003), in addition to being a precursor for APR proteins such as SAA (Sack Jr, 2018). The increases in both CRP and SAA have been reported as similar, which would support the findings whereby a delayed increase is reported post-match in the current research for SAA (Malle *et al.,* 1996), and CRP in rugby union (Cunniffe *et al.,* 2010).

To the authors knowledge, this is the first time SAA has been used to explore the acute-phase response in elite team sport athletes, and certainly elite Gaelic football players. While understanding how elite Gaelic footballers respond to competitive match-play, better informs coaches of the demands of competition, in addition to the recovery timelines of athletes, it is interesting to note that the reported responses differ when compared to the existing research in ultramarathon runners (Margeli *et al.,* 2005). It must be noted however, that an ultramarathon consists of a 246km race that lasted 36h, which is significantly longer than a 70-minute GAA or 80-minute rugby match, inducing greater muscle damage as a result. Firstly, a 108-fold increase occurs in SAA concentrations from immediately pre- to immediately post-race (mean ± SD; 3.2 ± 1.9 *vs.* 340.8 ± 206.4 mg.L^{-1}, respectively), rather than the decrease reported in the current research. Secondly, the athletes in the current research were not within the normative ranges for healthy males which was previous reported by (Wilkins *et al.,* 1994) as 0.7-26.4 mg.L^{-1} (mean; 3.7 mg.L^{-1}) or 700-26400ng.mL^{-1} (mean; 37000ng.mL^{-1}) in the units utilised by the current research. The pre-race SAA concentrations in the ultramarathon runners were just below mean concentrations reported by Wilkins *et al.* (1994). The range for SAA concentrations in the

current research was 74.6-184.5ng.mL^{-1} or 0.07-0.18 mg.L^{-1}, which would appear to be extremely low in comparison to baseline concentrations previously reported in 105 healthy adults (Wilkins *et al.,* 1994). While there is no current research that compares the SAA concentrations of well-trained athletes to controls, there is research that reported significantly lower CRP concentrations in well trained athletes when compared to controls (Dufaux *et al.,* 1984). Dufaux *et al.* (1984) reported that distance runners have median CRP concentrations of 315ng.mL^{-1}, while controls had median concentrations of 502ng.mL^{-1}. However, these concentrations are significantly lower than those reported by Cunniffe *et al.* (2010), whereby baseline CRP concentrations of ~1000ng.mL^{-1} were reported. In both CRP and SAA, it is apparent that there are inconsistent baselines reported between papers, despite both being used as markers of the APR (Yoo and Desiderio, 2003). Nonetheless, SAA concentrations in the current paper in elite Gaelic footballers, are comparable to the SAA concentrations reported in a paper conducted in 10 endurance trained athletes who ran 20-40km per week and regularly competed in running events (Drenth *et al.,* 1998). These findings may suggest that athletes may experience lower resting SAA concentrations than their healthy counterparts. Moreover, the demands of elite Gaelic football competitive match-play, do not appear to elicit significant increases in SAA concentrations in either Gaelic football players across multiple games or endurance trained runner in a 5k race (Drenth *et al.,* 1998).

SAA concentrations in athletes competing in a 5km race, reported by Drenth *et al.* (1998), were similar to those reported in the current research with mean SAA concentrations collected immediately before and after, as well as 3h post-competition, ~100ng.mL^{-1} with very large variance also reported. Unfortunately, exact concentrations were not reported so direct comparison is difficult. The study conducted by Drenth *et al.* (1998) used a 5km race as a measure of physiological stress rather than the 250km ultramarathon used in the research by Margeli *et al.* (2005), as 5km was deemed as more "physiologically regarded as a part of the regular human exercise pattern" (Drenth *et al.,* 1998). It is possible that the ultramarathon runners included in the research above may have demonstrated higher resting SAA concentrations than both competitive runners who ran 20-40km per week, and Gaelic football players who would typically cover similar distances across a week of training as observed in the specific group utilised in the current research. There is limited research on the quantification of elite GAA training loads across teams, due to the limited number of elite teams and their unwillingness to share data. However, future research should aim to establish in-season training demands in elite GAA players, similar to the research conducted by Bradley *et al.* (2015) in rugby league. Understanding how training demands fluctuate across the season may guide future immunoendocrine research by highlighting problem times in the season where player management is

paramount, similar to the research conducted by (Tiernan *et al.,* 2019), rather than pooling data across multiple games resulting in no significant changes being reported across time.

5.1.3 Significance of immunoendocrine responses to competitive match-play in elite Gaelic footballers

While the data in current research, reported similar salivary hormonal activity in team sport athletes, it is important to question whether the observed change is meaningful or not. While no significant effect for time was reported for all immunoendocrine concentrations aside from C in the pooled samples, analysis of the partial eta squared (Cohen 1973) values gives an insight into the effect sizes observed. Analysis of partial eta squared values has previously been implemented in research, to explore the effect sizes for changes in acute biochemical markers in rugby union (Lindsay *et al.,* 2015). Effect sizes for the pooled samples were examined with C concentrations demonstrating the largest effect size ($\eta 2 = 0.509$), which emulated previous results reported by Lindsay *et al.* (2015) where a large partial eta squared ($\eta 2 = 0.583$) was described for 24h pre-game to immediately post-match in rugby union players. The authors also highlighted that C concentrations for all athletes increased from pre-post game, with observably different magnitudes. This point further highlights the interindividual variation in how athletes respond to competitive match-play. The T:C ratio demonstrated a moderate effect size ($\eta 2 = 0.065$) and T concentrations demonstrated a small-moderate effect size ($\eta 2 = 0.051$), which was a lesser effect size when compared to those reported by West *et al.* (2014). West *et al.* (2014) reported a large effect size for both T concentrations ($\eta 2 = 0.246$) and the T:C ratio ($\eta 2 = 0.466$), however their effect size was lower than that of the current research ($\eta 2 = 0.296$). The ranges reported by Lindsay *et al.* (2015) for differences between baseline and 36h post-match for C concentrations (~300ng.mL^{-1}) and T concentrations (~0.06ng.mL^{-1}), were larger and smaller, respectively, than those in the current research, possibly explaining the larger effect size for T concentrations, and lower effect size for C concentrations. SAA concentrations displayed the lowest partial eta squared values with a small effect size reported ($\eta 2 = 0.027$). Despite the lack of significance reported in this study, the fluctuations in immunoendocrine responses appear to be largely affected by competitive match-play in elite Gaelic footballers. However, these findings may also be influenced by the inter-individual variation as there were some outliers that may have skewed the data. Furthermore, the additional external stressors like coach-athlete relationships and family relationships, as outlined by Sarkar and Fletcher (2014), may have contributed to psychosocial stress, subsequently affecting the athletes T and C concentrations across timepoints.

Despite the similar effect sizes to previous research (West *et al.,* 2014; Lindsay *et al.,* 2015) and comparable timelines of recovery to previous research in team sports (Elloumi *et al.,* 2003; Coutts *et al.,* 2007; West *et al.,* 2014), it is unclear as to whether there is any biological significance to the changes reported in these immunoendocrine markers. The immunoendocrine activity reported in this research, specifically salivary hormones, do not compare to the critical difference described by Hayes *et al.* (2014). The critical difference before and after exercise for both salivary T and C concentrations were discussed by the authors who determined salivary T as having a critical difference of 90% and salivary C concentrations of 148% when determining if a biologically meaningful change has been reported when using ELISAs to measure the salivary T and C concentrations. None of the observed mean pre- to post-game salivary hormonal concentrations surpassed this critical difference, which was described by the authors as being more important than significance (Hayes *et al.,* 2014).

Four out of the 18 samples increased T concentrations from 2h pre- to 0h post-match, all four of which surpassed the critical difference of 90% (range; 143-2137%). Individual timelines are outlined below in Figure 4.9. Individual data for these players can be found in Appendix F. It should also be noted that the samples for player 5 came from game 3, while the remaining players came from game 4.

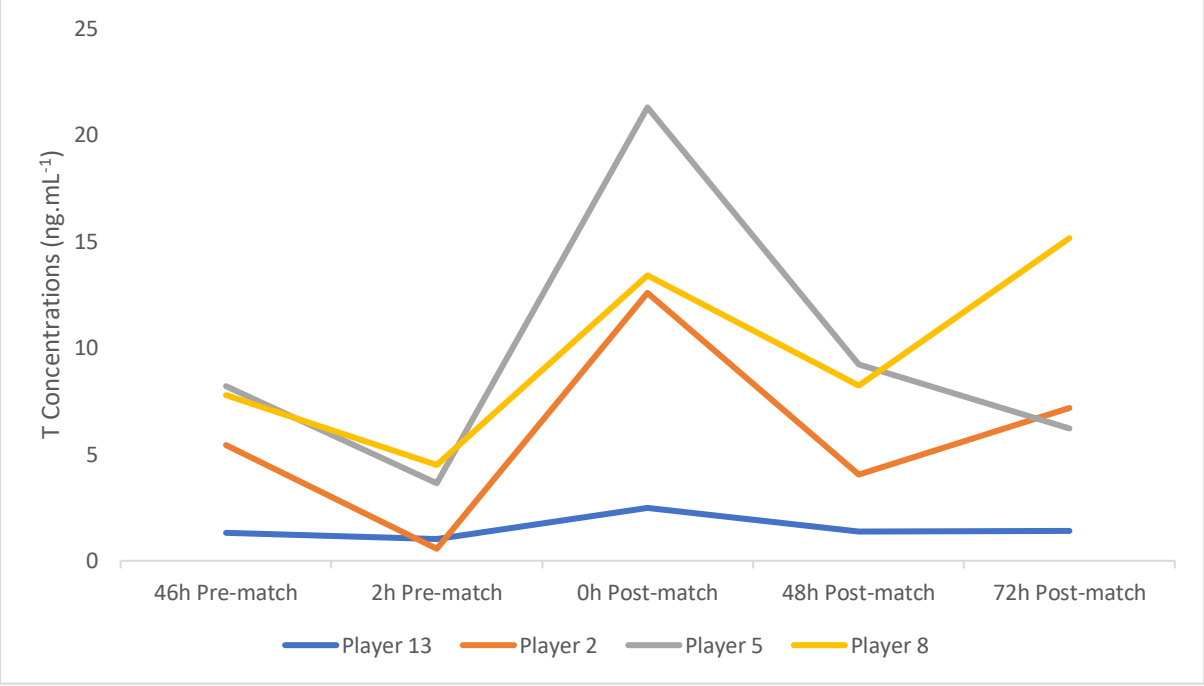

Figure 4.9 – Timelines for T concentrations for players who met the critical difference requirements from 2h pre- to 0h post-match (Hayes *et al.,* 2014)

Moreover, 4 players surpassed the critical difference of 148% in C concentrations (range; 157-196%). These players timelines are outlined below in Figure 4.10. Individual data can be found in Appendix G. Player 1 (blue) provided the samples in game 2, while the same player (grey) provided samples in game 4. Player 5 provided samples in game 3, while player 8 provided the samples in game 4. It is interesting to note that the players who met the critical difference criteria for T concentrations all increased, which would oppose previous findings in rugby union, where players T concentrations are reported to decrease from pre-post-match (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2010; Lindsay *et al.,* 2015). Players who met the critical difference criteria for C concentrations, as outlined by Hayes *et al.* (2014), all followed the trend previously reported in rugby union (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015), whereby post-match C concentrations increased from baseline concentrations. Interestingly, increases in salivary T concentrations have previously been associated with winning after a competitive, but pre-determined computer task, with increases reported in winners (mean ± SEM; 3.4 ± 3.1%) from pre- to post-competition, despite no significant interaction between time and outcome. No pre-competition differences was recorded in either T or C concentrations for either winners or losers (Zilioli and Watson, 2013).

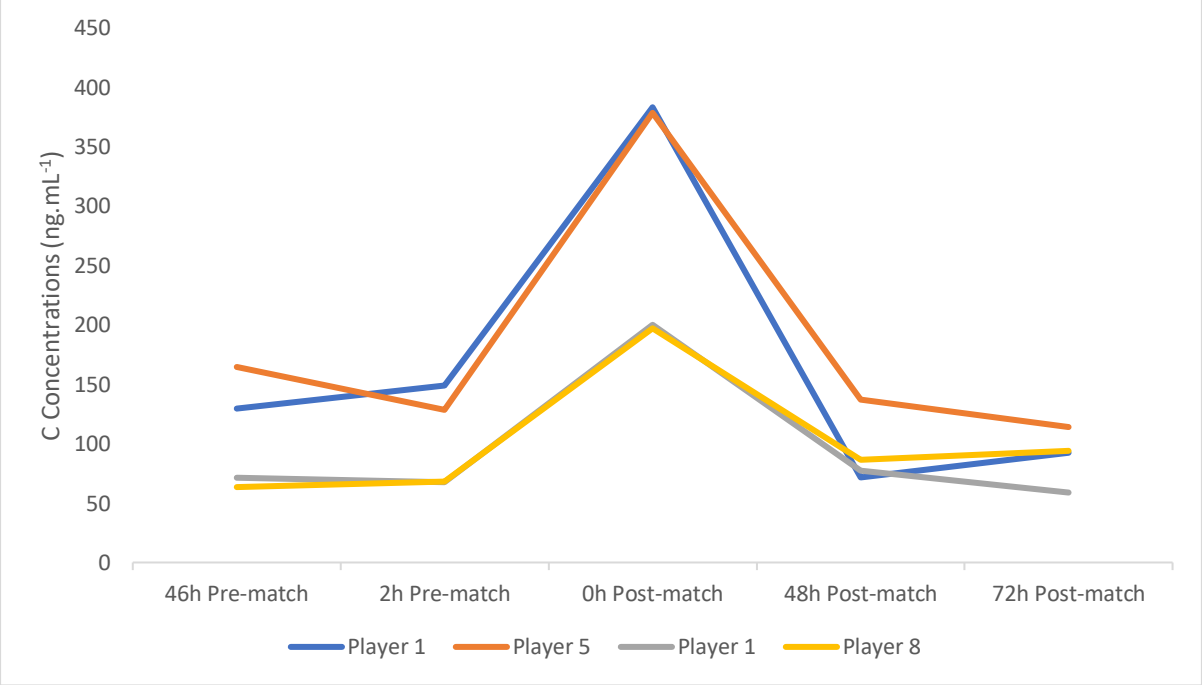

Figure 4.10 – Timelines for C concentrations for players who met the critical difference requirements from 2h pre- to 0h post-match (Hayes *et al,*. 2014)

Perhaps future research should measure the critical differences of both T and C concentrations in addition to reporting the mean ± SEM to fully describe the responses of athletes to competitive match-play. This inclusion of critical difference could be coupled with an assessment of mood or motivation to explore the associations that pre-match psychological state has on the reported hormonal concentrations post-match, similar to that of Zilioli and Watson (2013). While the current findings educate practitioners on how elite Gaelic footballers respond to competitive match-play, exploring the factors that contribute to changes larger than the critical difference may be warranted in future research. Athletes who experiences changes surpassing the critical differences may be of interest as these players could potentially be subject to experiencing chronic elevations in salivary T and C concentrations which are associated with Overreaching Syndrome (Coutts *et al.,* 2007). While it may be difficult to determine the cause of these individual players exceeding the critical difference for T and C concentrations as outlined by Hayes *et al.* (2014), it does emphasise the individual nature of salivary hormonal responses.

5.1.4 Use of Δ immunoendocrine concentrations to explore changes in response to competitive match-play

Concentration Δ were utilised to explore the variance between specific sampling timepoints, in addition to the repeated measures analysis of variance to provide context to the changes as previously recommended by Hayes *et al.* (2016). The first Δ concentration (Δ46-0) analysed the end of the training week (46h pre-match) and 2h pre-match to determine the extent of player recovery between the end of the final session and pre-match. The purpose of the Δ concentration (Δ2-0) from samples collected 2h pre-match to the sample collected immediately post-match (0h post-match), demonstrates the acute effects that match-play has on immunoendocrine concentrations of elite Gaelic football players. The Δ concentration from 0h post-match and both 48- and 72h post-match were utilised to determine the extent of recovery of immunoendocrine concentrations at 48- and 72h window post-match (Δ0-48 and Δ0-72, respectively).

The team utilised in this study typically played on a Saturday evening and did not recommence training until Monday evening when they completed a team gym session with their Strength and Conditioning coach. The purpose of this research was to understand whether this 48h window allows players sufficient time to recover post-match, prior to training again. While salivary hormone concentrations

returned to baseline at 48h post-match, it is interesting to note that serum inflammation markers remain disturbed at 72h post-match. The fluctuations reported in SAA concentrations, would not appear to be detrimental to performance as they so not appear to increase to the same extent after competitive Gaelic football match-play, when compared to an ultra-marathon (Margeli *et al.,* 2005b). As a result, recommendations to practitioners in Gaelic football would simply be to ensure players are monitored on an ongoing basis post-match to ensure they are given sufficient recovery time, particularly in the 48- to 72h window. Similarly, recovery may be negatively affected in an amateur team environment, where there may be external pressure on players to play for their clubs after playing for their respective inter-county team on the weekend. Conversely, when an athlete plays on a Saturday evening, and is given 48h to recover, they appear to be able to tolerate the load without significant increases in the circulation of inflammatory proteins which would indicate they have not fully recovered.

5.1.5 The relationships between GPS metrics and the reported variance in immunoendocrine concentrations

For the purpose of comparing GPS metrics to the fluctuations observed in the immunoendocrine markers in the current study, the Δ concentrations were utilised. Making comparisons between the Δ concentrations allows practitioners to understand which GPS metrics effect the magnitude of change the most. By understanding which metrics impact the acute changes and subsequent recovery timelines of immunoendocrine markers, athletes can be monitored more appropriately around competitive match-play. Little is known about whether the volume or intensity of the distances covered in the game, directly affect the magnitude of change in these immunoendocrine markers. Pearson's product-moment correlation results as well as both linear and multiple regression results were analysed to explore the relationships between GPS metrics and Δ immunoendocrine concentrations, as well as to understand which metrics best explain the changes in the Δ concentrations.

An overview of the Pearson's product-moment correlation analysis (see Table 4.6), revealed no significant correlations for any GPS metrics in the pooled samples. This was possibly due to the fact that in an intermittent team-sport like Gaelic Football, there are numerous contextual factors, such as tactics, which can alter the demands placed on a player (Mangan *et al.,* 2017). While there are

moderate relationships reported, the lack of statistical significance would suggest that GPS metrics do not possess the ability to determine pre- to post-match changes in immunoendocrine responses in elite Gaelic footballers.

Analysis of the correlations between Δ immunoendocrine concentrations and GPS metrics for the individual game samples, reported only three significant correlations (see Table 4.6). The first significant correlation was between TΔ0-48 and HSR% (r = 0.83, P=0.020). These findings suggest that players who endure greater HSR% during the game, will experience a greater decrease in salivary T concentrations from 0- to 48h post-match in elite Gaelic footballers. The other two significant strong negative correlations were described between SAAΔ2-0 and both TD (r = -0.97; P=0.035) and relative TD (r = -0.97; P=0.035). They are both identical in correlation and significance as all players played the same number of minutes, and so, when TD was significantly correlated to SAAΔ2-0, relative TD was naturally going to replicate the findings as it is relative to the players minutes, which were all identical. These findings suggest that players who covered greater TD during the game, experienced a greater reduction in SAA concentrations from 2h pre- to 0h post-match. Similarly, players who covered greater TD relative to minutes played, also experienced a greater reduction in SAA concentrations from 2h pre- to 0h post-match. All other correlations for individual game samples were not significant.

GPS metrics have previously been correlated with post-match changes in serum immunoendocrine concentrations in elite soccer players (Romagnoli *et al.,* 2016). Romagnoli *et al.* (2016) described significant moderate positive relationships between post-match serum C concentrations and TD (r = 0.502, P=0.034), as well as significant moderate positive relationships between pre- to post-match Δ IL-6 concentrations and TD (r = 0.521, P=0.027). Pre- to 48h post-match Δ C concentrations reported a significant positive moderate correlation with TD (r = 0.515, P=0.029), such that the players who experience the greatest decreases in C concentrations from pre-match to 48h post-match covered the greatest total distances in the non-official elite soccer match (Romagnoli *et al.,* 2016). Interestingly, Romagnoli *et al.* (2016) recorded a significant decrease in C concentrations 24h post-match when compared to pre-match concentrations (mean ± SD; 145.78 ± 33.93 *vs.* 219.23 ± 51.68µg.L^{-1}, respectively).

5.1.6 Individual GPS metrics and their ability to explain the magnitude of variance in immunoendocrine concentrations

Understanding the relationships between GPS metrics and Δ immunoendocrine responses is beneficial in explaining which metrics are related to pre- to post-game changes and post-game changes in the 48h and 72h recovery windows. However, regression analysis is more appropriate in identifying the best metrics for explaining the Δ changes. Linear regression analysis was conducted to determine which individual metric best explained the Δ changes in immunoendocrine markers. Multiple regression analysis was conducted to determine which combination of GPS metrics best explained the Δ changes between timepoints. Volume metrics were combined to determine if the combination of TD and HSR would best explain the variance, or whether a combination of TD or HSR, combined with minutes played would best explain the variance. When combining the volume metric with minutes played, it ultimately created a relative metric as it is either TD or HSR relative to minutes played. HSR and HSR% were combined to explore whether combining the volume of intense running metres, plus the way in which they were accumulated, would best explain the Δ changes. Finally, a combination of TD covered per minute, as described by relative TD combined with the density to which HSR was accumulated, as defined by HSR%, were combined to explore whether a combination of intensity metrics measuring the accumulation of TD and HSR, would best explain the Δ changes.

5.1.6.1 Variance Explained in Δ Immunoendocrine Concentrations in Pooled Game Samples

No single GPS metric was able to explain 50% or more of the Δ concentrations in either T, C or SAA concentrations, nor the T:C ratio (Table 4.3). The highest percentage variance explained was by all GPS metrics and TΔ46-0, whereby 56% of the variance was explained. Similarly, all GPS metrics explained 50% of the variance in SAAΔ0-48 concentrations. Similar low coefficient of determination values were reported when metrics were combined using multiple regressions to explore combinations of volume and intensity metrics (Table 4.4). The highest percentage of variance reported was 40% by HSR and Mins in TΔ2-0.

It's interesting to note that no individual GPS metrics or combinations of volume and intensity metrics reported significant correlations with Δ C concentrations, despite them being the only hormonal marker that reported a significant effect for time in pooled samples. These findings further support the previous points highlighted, whereby elevations in C concentrations post-match appear to related

more to protein catabolism for gluconeogenesis (Gleeson *et al.,* 2004), in addition the anti-inflammatory effect of C after intense exercise (Papacosta and Gleeson, 2013). The results in the current research study would indicate that despite the positional differences in running demands evident in elite Gaelic football (Malone *et al.,* 2016), it would appear that athletes ultimately respond the same way in terms of salivary C increases, independent of the external workload endured as quantified by GPS.

In the linear regression analysis (Table 4.3), all GPS metrics explained 50% of the variance in SAAΔ0-48, which may suggest that the 48h recovery window may be dictated by game volume and intensity, with players who have higher game volume and intensity metrics, experiencing a greater increase in SAA concentrations from 0h post-match to 48h post-match. Research by Jones *et al.* (2014) previously reported no differences in CK concentrations between rugby forwards and backs pre-match, with backs having significantly higher CK concentrations when compared to forwards at 16h post-match but not 40h post-match. When GPS metrics were reported for both positional groups, backs played significantly longer and covered greater relative and absolute total distances. Furthermore, backs performed more sprints and covered more sprint and high-speed running distance than forwards (Jones *et al.,* 2014). As CK is utilised as a marker of NMF and is dependent on sarcomeric damage (Brancaccio *et al.,* 2007), it could be inferred that similar to the research conducted by Jones *et al.* (2014), the athletes in the current study who were exposed to greater volumes and intensities, experienced greater disturbances in immune marker concentrations from 0- to 48h post-match, in this case SAA concentrations. The increased number of accelerations associated with increases in sprint number, may also have been effected by the increased eccentric load associated with accelerations and decelerations (Stauber, 2004).

5.1.6.2 Variance Explained in Δ Immunoendocrine Concentrations in Individual Game Samples

Individual game analysis (see Table 4.7) reported that all GPS metrics and individual game metrics explained a much higher percentage of the variance than pooled samples. All GPS metrics explained 93% of the variance reported in TΔ46-0 concentrations, and 84% of the variance in TΔ0-48. However, no metrics explained 50% or more of the variance in TΔ46-0. HSR explained more than 50% of the variance in TΔ0-48, with 52% of the variance explained, while HSR% explained 72%. Fifty three percent of the variance in TΔ0-72 was explained by minutes played. Only all GPS metrics explained more than 50% of the variance in Δ C concentrations for individual game samples with 56% of the variance

explained in CΔ46-0. The anti-inflammatory role of C through the regulation of host defence processes as previously discussed by Buckingham (2006) may explain the reason all GPS metrics explain over half of the variance in CΔ46-0 as players with increased external workload as quantified by GPS, experience greater neuromuscular fatigue post-match (Duffield *et al.,* 2012). Furthermore, in TCΔ46-0 only all GPS metrics explained more than 50% of the variance with 84% of the variance explained. Due to the absence of a significant effect for time for any salivary hormone concentrations, it is difficult to provide too much context to these findings, especially when considering the low sample size. It is interesting however, that all GPS metrics which ultimately represented the total of the external load endured by players, explained variance from baseline at 46h pre-match to 0h post-match, but not in either the 48- or 72h recovery windows post-match. These findings do not corroborate previously reported data whereby a greater reduction in C concentrations was described in soccer players who covered more distance (Romagnoli *et al.,* 2016), especially considering the authors only reported these findings for C concentrations, and not T concentrations unlike the current findings in this research for HSR and TΔ0-48. Moreover, the lack of significance in the correlation analysis would suggest that the regression analysis findings should be interpreted with caution as the large percentage variances explained, may in fact be false positives, as a result of the small samples size and number of levels included in the regression.

While Δ SAA concentrations appeared to be largely explained by individual GPS metrics, it must be note that only four players were included in the linear regression analysis. While it appeared that game volume, as measured by TD and HSR, explained a large portion of the variance from bot 46- and 2h pre-match to 0h post-match, there was a very limited sample size. Results must be interpreted with caution as a result. While these findings would echo those of Romagnoli *et al.* (2016), as IL-6 was reported to be related to TD covered in soccer players, the results were based on a much larger sample size of twenty players. Similarly, when considering the previous research by Brownstein *et al.* (2017) that reported a significant decrease in maximal voluntary contraction after a 90min soccer match, which recovered at 48h post-match. These findings are like those reported in rugby union whereby a reduction in PPO was reported for 48h post-match (West *et al.,* 2014). While the sample size is low, research has reported increased neuromuscular fatigue and increased inflammation post-match which would suggest these results could be replicated in an increased sample size. Future research should attempt to increase the sample size and explore similar timelines to fully understand how elite Gaelic footballers respond to competitive match-play, as there is limited strength in the results presented in the current research.

Similar considerations must be remembered when interpreting the multiple regression analysis in individual game samples. While the large coefficient of determination values appear to report large trends for certain metrics in explaining Δ immunoendocrine concentrations, the fact that no significant effects for time were reported for any salivary hormones, must be considered. As has already been discussed in the previous sections, there are a multitude of factors that can affect salivary hormonal responses and serum immune protein responses, that the lack of significance in the ANOVA outputs would confirm. While the regression analysis was included to explore an alternative avenue in determining the ability of GPS metrics to explain the magnitude of variance in immunoendocrine responses, the inconsistent results would suggest that they may be more random than initially anticipated. Ultimately, the lack of a significant effect for time in all metrics except for C in the pooled samples and SAA in the individual samples, bearing in mind the small sample size in the latter, coupled with the lack of significant correlations, shows that GPS metrics may not be the most applicable tool to measure the magnitude of change in immunoendocrine concentrations. That being said, the research explored a novel topic in the area of elite Gaelic football, and must be built on in future research, before practitioners can fully understand how players respond to competitive match-play. These findings do, however, further substantiate the observations made by Halson (2014), whereby the implementation of a combination of internal and external load monitoring measures was recommended. The fact that GPS metrics can not dictate the magnitude of change, would suggest that both internal measures of fatigue and the quantification of external load, play uniquely important roles in load monitoring.

5.2 Limitations

This research does not come without its' limitations. An extensive review was completed by Hayes *et al.* (2015) which outlined several factors which researchers are advised to control in order to minimise methodological errors in sampling. While the current research attempted to adhere to the considerations outlined, some areas proved more difficult than others to monitor due to the amateur nature of the team sport athletes in the current study.

As outlined above, all samples were collected after 12pm, minimising the effect of the circadian rhythm. Athletes in the current research participated in training sessions which occurred between the hours of 6 and 9pm for the duration of the study. On match days, athletes were usually required to arrive 2-3h prior to the start of the game and were asked to provide saliva samples upon arrival. Three

out of the 4 games (games 1-3) included in this research took place at 7pm in the evening which coincided with the sampling times around training, while one game took place at 2.30pm. Athletes were instructed to maintain their habitual dietary intake which was guided by a team nutritionist during the season. Players were also asked to refrain from eating or drinking for at least 30min prior to providing saliva samples to avoid contaminating the saliva sample, with the exception of water which could be consumed up to 10min prior to providing a sample to reduce biological influences as previously described by Hayes *et al.* (2016). However, as this was a free-living experiment, and due to the limited control the authors had over the amateur athletes included in this study, ensuring that the athletes adhered to the time constraints the research placed on the consumption of food and drink was difficult.

Furthermore, Hayes *et al.* (2016) emphasised that caffeine should be avoided 24h before sample collected due to its effects on both salivary T and C concentrations. High levels of salivary caffeine have been observed post-match in Super Rugby players (Dunican *et al.,* 2018) who compete at similar times to Gaelic football players (~7pm), and caffeine supplementation pre-game has been linked with numerous performance benefits (Del Coso *et al.,* 2013). While it has many associated performance benefits, Hayes *et al.* (2016) highlighted its direct impact on both salivary T and C concentrations, as well as its' indirect effect which negatively impacts sleep post-game (Dunican *et al.,* 2018), which subsequently impacts salivary T and C concentrations. Players in the current study were requested to refrain from caffeine where possible. However, due to the nature of an 'observe and report' study, as well as attempting to minimise the external stress the research may cause, it was difficult for the researchers to ask athletes to deviate from their normal pre- and intra-match routines, which commonly include the ingestion of caffeine supplementation. In addition to the effect caffeine can have on both T and C concentrations, anticipation has also been reported to affect their concentrations in athletes (Eubank *et al.,* 1997; Salvador *et al.,* 2003). In the current research, the authors were unable to differentiate between saliva hormones that were affected by the physical stress of the game, or the somatic stress associated with either the anticipation of training or competition, or their own personal lives. Future research should include markers of internal load in the form of BAMS questionnaires to assess psychological stress and mood. Another limitation of the current research study is the lack of a true baseline measure for all immunoendocrine markers. As testing began in early January when the team began their competitive season, athletes had already been training since December for the pre-season. Access to players prior to this pre-season was not possible. As a result, pre-game comparisons in this study are either affected by post-training neuromuscular stress, or pre-game anticipatory stress. Future studies should aim to take baseline

samples with no training for at least 48h prior. However, as discussed by Cunniffe *et al.* (2010), researchers must ensure that the baseline samples are not taken after an intensified period of training as even 48h may not be sufficient time to recover and athletes may still present in a fatigued state.

Moreover, ingestion of a 6% carbohydrate drink, both before and during a marathon have shown to significantly reduce C concentration increases from pre- to post-race when compared to a placebo group (Nieman *et al.,* 2001). As outlined in the methods, this was a free-living experiment and the athletes included in this study had access to exogenous carbohydrate supplements both before, during, and after matches, which may have altered the reported responses. Future research could monitor the carbohydrate intake of athletes to better understand their 2h pre- to 0h post-match C responses, as well as the subsequent recovery timelines of C concentrations.

Furthermore, due to the elite nature of the Gaelic football team included in this study, athletes were required to adhere to a strict training process. While players were typical diligent in limiting their training to squad sessions, or completing individuals after squad sessions, players may have completed individual sessions on non-training days which may have influenced their immunoendocrine markers. As the players included were amateurs, they were never in their training facility for consecutive days, like the camps which have been previously analysed (Cunniffe *et al.,* 2011; Serpell *et al.,* 2018). Future research should attempt to further standardise the training stimulus during the sampling week by utilising a training camp if possible. Finally, in the current study, analysis could not be conducted in the same group of players across multiple games, partly due to the methodological limitations outlined above which resulted in poor quality samples, but also due to the weekly changes to the training teams due to competition for places. This meant the researchers were limited to analysing an individual game, in addition to pooling samples to increase the sample size for analysis of trends and allow comparison of results. The current research is the first study to report the immunoendocrine responses of elite Gaelic footballers to competitive match-play. Unfortunately, it is difficult to make inferences about the chronic responses, despite for games being sampled across a four-month period. While four matches were included, each match included samples from different players who have inherently individual responses to match-play. Future research should aim to establish a core playing group for sampling across multiple games which would allow further analysis to be done between individuals and between games to analyse the chronic effects of competitive match-play on the immunoendocrine status of elite Gaelic football players. Furthermore, this would allow comparison

between competitions to see how immunoendocrine responses change as the pressure increases within the knock-out stages of the season.

5.3 Conclusion

Elite Gaelic football match-play appears to elicit significant increases in salivary C concentrations from pre- to post-match, only. While the timelines of change in T concentrations, T:C ratios, and SAA concentrations appear to follow the trends outlined in previous research (Elloumi *et al.,* 2003; Cunniffe *et al.,* 2010; West *et al.,* 2014; Lindsay *et al.,* 2015), the lack of significant effects for time, indicates that individual variation is just too great to outline a significant trend across games. The lack of significance and exploration of the individual responses has previously been reported (Moreira *et al.,* 2009) and future research should continue to build on the body of research whilst accounting for methodological considerations (Hayes *et al.,* 2016). That being said, there was still a clear trend for significant increases in salivary C concentrations from 46- and 2h pre-match to 0h post-match before they recovered at 48h post-match. While these are mostly as a result of protein catabolism for gluconeogenesis and C anti-inflammatory effects (Gleeson *et al.,* 2004; Buckingham, 2006), identifying that C concentrations recover at 48h post-match is an important finding for practitioners when monitoring their players around competition (*Djaoui et al.,* 2017; Papacosta and Nassis, 2011b; Halson and Jeukendrup, 2004). Moreover, identifying responders and non-responders, which as shown in this research can be masked by mean data, may allow better management of players in the pre-match window to optimise performance (Eubank *et al.,* 1997; Salvador *et al.,* 2003).

Analysis of the relationships between GPS metrics and Δ immunoendocrine responses reported inconsistent results for both pooled samples and individual game samples. While this research intended to identify which GPS metrics best dictate the magnitude of change in immunoendocrine markers, it appeared that there were too many factors influencing the changes in immunoendocrine concentrations in response to competition. With no significant relationships reported in pooled samples, and only three significant relationships reported in the individual game samples, with such a low sample size, future research would be required to better understand these relationships. While HSR% reported a significant relationship with TΔ0-48 and both absolute and relative TD reported a significant relationship with SAAΔ2-0, the small sample size was undeniable. Future research should aim to explore the relationships between GPS metrics and immunoendocrine responses with a larger sample size of individuals across multiple games. This would ensure better education of practitioners on player monitoring around competitive match-play in elite Gaelic football.

Practitioners should ensure 48h recovery post-match to ensure C concentrations return to baseline, avoiding chronic elevations which may lead to signs and symptoms of OTS (Meeusen *et al.,* 2013). Despite the research showing limited evidence for the ability of GPS metrics to dictate the magnitude of change in immunoendocrine responses of elite Gaelic footballers, the benefits of monitoring both internal and external load should not be forgotten (Halson, 2014).

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Appendix

Appendix A – T and C Research Overview

Author	Year	Participants & Sport	Timeline	Hormone	Results overview	Hormonal change	Recovery duration
Lindsay *et al.,*	2015	11 Elite Amateur Rugby Union Players	One competitive match; 24h pre-game, immediately post-game, 17, 25, 28, 62, 86h post-game	Salivary Cortisol	increase 24h pre-game to post-game	Mean ± SD (15.2 ± 7.2 to 60.5 ± 24.6 µmol.L-1)	17hrs post-game
West *et al.,*	2014	14 Professional Rugby Union Players	One competitive match; 36h pre-game, 12, 36 and 48h post-game	Salivary Cortisol	increase 36h pre-game to 12h post-game	CI (0.11 - 0.29 µg.dL-1)	60hrs post-game
				Salivary Testosterone	decrease 36h pre-game to 12h post-game	CI (-28.07 – -97.62 ph.ml-1)	60hrs post-game
				T:C ratio	decrease 36h pre-game to 12h post-game	CI (-182 – -389)	60hrs post-game
Cunniffe *et al.,*	2011	8 Welsh International Rugby Union Players	3 week International competition; immediately pre-game, immediately post-game, 38, 60h post-game	Serum Cortisol	significant increase from pre-post game in both games	Mean ± SEM (Game 1 = 348 ± 22 to 523 ± 58; Game 2 = 313 ± 10 to 533 ± 42 nmol.L-1)	38hrs post-game
				Serum Testosterone	significant decrease from pre-post game in both games	Mean ± SEM (Game 1 = 18.6 ± 2.1 to 11.4 ± 0.8; Game 2 = 21.3 ± 2.0 to 13.6 ± 2.0nmol.L-1)	38hrs post-game
				T:C ratio	significant decrease from pre-post game in both games		38hrs post-game
Moreira *et al.,*	2009	22 French Professional Soccer Players	Internal training match; 10min before warm-up, 10min post-game	Salivary Cortisol	increase pre-post game	Team A (7.6 ± 4.4 to 12.1 ± 6.0ng.ml-1) Team B (8.8 ± 3.0 to 10.3 ± 6.2ng.mL-1)	
Elloumi *et al.,*	2003	20 Tunisian International Rugby Union Players	One competitive match; match-day = 8am, 4pm (post-match), 6pm, 8pm. Follow up = 8am and 8pm (Monday to Saturday/6 days post-game)	Salivary Cortisol	significant increase post game vs 4pm on rest day	148% increase	4hrs post-game
				Salivary Testosterone	significant decrease post game vs 4pm on rest day	16% decrease	4hrs post-game
				T:C ratio	significant decrease post game vs 4pm on rest day	62% decrease	
Filaire *et al,*	2003	Soccer	Full competitive soccer season	Cortisol	increase from start of pre-season to end of in-season (September-April)	Mean ± SE (14.5 ± 1.2 to 18.4 ± 0.7nmol.L-1)	
				Testosterone	remained unchanged throughout season (September-September)	Range (274.4 ± 21.3 to 343.5 ± 35.0 pmol.L-1)	
Salvador *et al,*	2003	17 Judo competitors	8 resting sessions and 1 competition; 1 resting session every fortnight (corresponding sampling times), 1h and 30min pre-competition	Cortisol	significantly higher in competition *vs.* resting		
				Testosterone	no significant difference		
Eubank *et al.,*	1997	10 Elite Male Marathon Canoeists	One competitive event; 24h, 2h and 1h before	Plasma Cortisol	Significant increase from 2h to 1h pre-race for debilitatory group *vs.* consistently lower in facilitatory group across time	Mean ± SD (debilitatory group = 466 ± 52.7 to 642 ± 77.3nmol.L-1)	
				Plasma Testosterone	24h pre-race - significantly higher for debilitatory group *vs.* 1h pre-race - significantly higher for facilitatory group	Mean ± SD (24h pre-race = 19.3 ± 1.4 *vs.* 15.6 ± 3.4nmol.L-1) (1h pre-race = 20.9 ± 3.4 *vs.* 16.7 ± 1.6nmol.L-1)	
Banfi *et al.,*	1993	8 Elite Italian Speed Skaters (5 Male, 3 Female)	During an 8 month season; 29th June, 20th August, 1st September, 12th September, 6th January, 8th January	Serum Free Cortisol	significantly decreased at timepoints 3 and 5 for all athletes	Males (0.57 ± 0.10 *vs.* 0.28 ± 0.08 and 0.19 ± 0.01) Females (0.63 ± 0.08 *vs.* 0.41 ± 0.06 and 0.20 ± 0.06)	
				Serum Free Testosterone	significantly increased at timepoint 3 in males only	19.3 ± 4.7 *vs.* 29.5 ± 9.5	
				Serum Free T:C ratio	increase at timepoint 3 and 5 when compared to 1 for males	Males (0.67 ± 0.18 *vs.* 2.2 ± 7.9 and 2.6 ± 0.76)	

SD - Standard Deviation *CI* - Confidence Interval *SEM* - Standard Error of the Mean *SE* - Standard Error

Appendix B – Participant Information Leaflet

Institiúid Teicneolaíochta Cheatharlach
INSTITUTE of TECHNOLOGY CARLOW
At the Heart of South Leinster

PARTICIPANT INFORMATION LEAFLET
Department of Science and Health, IT Carlow. This project is being undertaken as part of MSc by research at IT Carlow.

1. Title of Study: The neuromuscular, endocrine, hormonal and physiological responses of Gaelic games players to competitive match-play

2. Introduction: Hormones and inflammatory markers have been identified in recent research as excellent indicators of training load tolerance. It has been shown that there are varying magnitudes of change in response to intense exercise, particularly competitive match-play. When we play a competitive match, our hormone levels are disrupted. By examining the time it takes for these hormones to return to a 'normal' level can indicate a players' ability to tolerate the training load. Overtime, we would expect to see an adaptation to training load, and a more efficient recovery of these hormones. This would hopefully provide more information on reducing the risk of injury and illness.
The time points at which you will provide samples, are based off of previous research in Rugby (Cunniffe *et al.,* 2010, 2011 and West *et al.,* 2014). Samples will be collected in various location depending on the location of training and matches. All samples will be analysed in the laboratories in IT Carlow.

3. Procedure:

Test	**Details**
Saliva Sample	• Passive drool sample • Analysed for Testosterone and Cortisol concentration • Pre-game (40 h) • Morning of the game • Post-game (immediately, ~18h, ~54h)
Blood Sample	• Analysed for Serum Amyloid-A • Pre-game (40 hours) • Morning of the game • Post-game (immediately, 18h, 54h)
Global Positioning System (GPS)	• Collects external volume and intensity metrics • Worn for duration of the warmup and game

You will be required to provide samples at 5 time points during the week of a game. Saliva samples will be analysed for Testosterone and Cortisol, and will be collected for 6 weeks during the year. Blood samples will be taken at the same 5 time points during the week but will be analysed for the inflammatory marker serum amyloid-A. Samples will be provide for 3 weeks during the season. Saliva sampling will be non-invasive. Blood samples will be conducted to provide minimal disruption to participants. Samples will be collected from the front of the arm through a cannula.

All other data collection will be taken as part of your normal training routine. Additional data collection includes GPS units.

4. Benefits: Training data will be provided in a session by session GPS report. As we collect hormone data, we will hopefully be able to provide more detailed and personalised recovery information to players.

5. Risks: Blood samples may result in some short-term minor bruising, tenderness or discomfort in this area. However, by following the advice of the investigator you should be able to resume full training within 48 hours of completion of each trial.

6. Exclusion from participation: Any person presenting any health abnormalities, respiratory difficulties or symptoms of a cold on the trial day. Anyone with an injury affecting their daily training will be excluded. Any persons presenting with epilepsy, hypertension, low blood pressure or metabolic disorders will be excluded. Any adverse health finding will be brought to the attention of your nominated general practitioner, with your consent.

7. Confidentiality: Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the study group. All data will be stored safe and secure within the department for a period of five years. Consent forms will be stored in a separate secure location in the department. Blood samples collected during the study will not be used for any purpose other than those related to the variables of interest in this study.

8. Compensation: The medical investigator involved in this study is covered by standard medical malpractice insurance. This study is covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights

9. Voluntary Participation: You have volunteered to participate in this study. You may withdraw participation at any time. If you decide not to participate, or if you withdraw, you will not be penalised and will not give up any benefits that you had before entering the study.

10. Stopping the study: You understand that the investigators may stop your participation in the study at any time without your consent.

11. Permission: This project has received approval from the Faculty Research Ethics Group.

12. Further Information: You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Cian Gormley (0879549982, Cian.Gormley@itcarlow.ie). If the study team learns of any important new information that might affect your desire to remain in the study, you will be informed at once.

Appendix C – Consent Form

Consent Form

Level 9 – Masters by Research

Lead Investigator – Cian Gormley

Supervisors – Dr. Colin J. Coyle, Mr. Declan Browne, Dr. Rosemary O'Hara

BACKGROUND:

The proposed research will examine the time-course of recovery and magnitude of change in biomarkers in elite Gaelic football players during training and competition, and will explore the training load tolerance of these players, which, to date, remains unreported. Furthermore, it will correlate the data from these biomarkers with other internal and external load monitoring data. This will allow support staff to understand the training load tolerance of their players using practical measures.

The objectives for the study are as follows…

1) Develop and understand of the time-course of hormonal responses to competitive match-play
2) Compare the hormonal responses of the various in-season competitions at different time points in the season
3) Correlate the biomarkers with internal and external load monitoring data

PROCEDURES:

	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Saliva Samples			1.		2. **GAME** 3.	4.	5.

TESTING:

- Saliva Samples (6 games)
    - Passive drool into sample tube
        1. Last training pre-game
        2. Morning of game
        3. Immediately after game
        4. Monday morning
        5. Tuesday evening

- GPS data (training and games)

PARTICIPATION:
Participation in this study is completely voluntary. All data will be kept confidential and you have the right to access your data at any time. You have the right to walk away from the study at any time. Testing procedures are outlined below and are designed to cause minimal disruption to performance. If any adverse events occur during testing, or medical abnormalities are discovered during medical screening, I consent for my GP to be advised of these events / abnormalities.

DECLARATION:
This study and this consent form have been explained to me. The investigator(s) has/have answered all my questions to my satisfaction. I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.

I understand I may withdraw from the study at any time. I understand that any data obtained as a result of my participating in this study will be held in a secure location for a period of 5 years and not subsequently used for any further studies without my due consent. I also understand that should any data from this study be published, my anonymity and confidentiality shall remain intact.

PARTICIPANT'S NAME: _____

CONTACT DETAILS _____

PARTICIPANT'S SIGNATURE: _____

Date: _____

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

INVESTIGATOR'S SIGNATURE: _____

Date: _____

(Keep the original of this form in the investigator's file, give one copy to the participant, and send one copy to the sponsor (if there is a sponsor).

Player Name	Height (cm)	Weight (kg)	Age (yr)	Competition	Game
Player 1	196	95.2	25	League 1	Kildare
Player 10	180	88.9	31	League 1	Kildare
Player 12	180	84.8	29	League 1	Kildare
Player 2	185	89.2	21	League 1	Kildare
Player 3	178	79.5	22	League 1	Kildare
Player 5	180	87.1	24	League 1	Kildare
Player 8	191	88.1	28	League 1	Kildare
Player 9	183	88.4	25	League 1	Kildare
Player 1	196	95.2	25	League 2	Tyrone
Player 6	190	87.1	18	League 2	Tyrone
Player 7	182	85.0	24	League 2	Tyrone
Player 11	190	94.1	32	League 3	Donegal
Player 13	180	77.7	23	League 3	Donegal
Player 5	180	87.1	24	League 3	Donegal
Player 6	190	87.1	28	League 3	Donegal
Player 1	196	95.2	25	Leinster Championship 1	Wicklow
Player 13	180	77.7	23	Leinster Championship 1	Wicklow
Player 2	185	89.2	21	Leinster Championship 1	Wicklow
Player 4	180	87.2	22	Leinster Championship 1	Wicklow
Player 8	191	88.1	28	Leinster Championship 1	Wicklow

Appendix E – GPS Data

Player Name	Competition	Game	TD	HSR	Minutes	M.min-1	HSR%
Player 1	League 1	Kildare	9671.2	1003.5	74.5	129.9	15.5%
Player 10	League 1	Kildare	3169.8	488.5	27.3	116.0	17.2%
Player 12	League 1	Kildare	5098.3	374.5	74.5	68.5	14.4%
Player 2	League 1	Kildare	10419.9	1019.8	74.5	139.9	12.7%
Player 3	League 1	Kildare	8632.9	1203.7	74.5	115.9	12.5%
Player 5	League 1	Kildare	7583.3	899.1	74.5	101.8	11.0%
Player 8	League 1	Kildare	8222.9	829.9	74.5	110.4	15.4%
Player 9	League 1	Kildare	8723.3	632.7	74.5	117.1	14.7%
Player 1	League 2	Tyrone	9748.3	1091.5	73.6	132.5	5.7%
Player 6	League 2	Tyrone	9204.0	674.6	69.2	132.9	9.1%
Player 7	League 2	Tyrone	1044.9	69.6	7.4	141.8	13.3%
Player 11	League 3	Donegal	9368.3	1039.9	67.3	139.3	10.0%
Player 13	League 3	Donegal	9507.5	1118.3	74.4	127.8	7.3%
Player 5	League 3	Donegal	8157.3	718.4	79.5	102.7	14.1%
Player 6	League 3	Donegal	1522.0	90.6	11.1	136.9	11.0%
Player 1	Leinster Championship 1	Wicklow	9718.5	1441.2	75.0	129.6	6.5%
Player 13	Leinster Championship 1	Wicklow	9160.6	1267.5	75.0	122.2	5.0%
Player 2	Leinster Championship 1	Wicklow	9670.7	836.5	75.0	129.0	9.1%
Player 4	Leinster Championship 1	Wicklow	6845.8	811.9	51.4	133.2	7.3%
Player 8	Leinster Championship 1	Wicklow	8560.2	569.4	75.0	114.2	9.8%

Appendix F – Salivary T Data

Player Name	Competition	Game	T(-46)	T(-2)	T(+0)	T(+48)	T(+72)
Player 1	League 1	Kildare	5.2	10.1	0.6	5.9	8.0
Player 10	League 1	Kildare	5.6	4.4	0.5	7.4	12.1
Player 12	League 1	Kildare					
Player 2	League 1	Kildare	16.5	12.1	10.3	8.5	11.7
Player 3	League 1	Kildare	1.1	4.2	4.1	1.5	0.2
Player 5	League 1	Kildare	2.2	5.2	3.3	2.9	6.6
Player 8	League 1	Kildare	11.7	7.6	6.9	7.3	9.8
Player 9	League 1	Kildare	3.8	5.6	4.0	7.1	4.5
Player 1	League 2	Tyrone	2.8	6.3	5.3	4.5	2.6
Player 6	League 2	Tyrone	0.5	8.7	2.2	3.5	2.5
Player 7	League 2	Tyrone	4.6	6.7	3.7	5.1	3.5
Player 11	League 3	Donegal					
Player 13	League 3	Donegal					
Player 5	League 3	Donegal	8.2	3.6	21.3	9.2	6.2
Player 6	League 3	Donegal	3.4	3.9	1.8	4.2	4.7
Player 1	Leinster Championship 1	Wicklow	7.5	67.8	7.2	0.8	4.8
Player 13	Leinster Championship 1	Wicklow	1.3	1.0	2.5	1.4	1.4
Player 2	Leinster Championship 1	Wicklow	5.4	0.6	12.6	4.0	7.2
Player 4	Leinster Championship 1	Wicklow	2.6	5.4	0.8	6.1	2.2
Player 8	Leinster Championship 1	Wicklow	7.8	4.5	13.4	8.2	15.1

Appendix G – Salivary C Data

Player Name	Competition	Game	C(-46)	C(-2)	C(+0)	C(+48)	C(+72)
Player 1	League 1	Kildare	92.7	95.8	198.5	70.7	83.8
Player 10	League 1	Kildare	159.8	126.0	104.1	113.6	139.6
Player 12	League 1	Kildare					
Player 2	League 1	Kildare	108.1	121.6	116.6	80.2	90.3
Player 3	League 1	Kildare	104.1	130.0	133.3	109.2	96.1
Player 5	League 1	Kildare	75.1	117.7	278.9	95.5	93.1
Player 8	League 1	Kildare	118.8	125.2	173.0	119.8	131.3
Player 9	League 1	Kildare	75.9	115.3	75.6	106.0	114.0
Player 1	League 2	Tyrone	129.7	149.1	383.1	71.6	92.1
Player 6	League 2	Tyrone	91.3	133.3	229.6	110.8	101.4
Player 7	League 2	Tyrone	185.7	121.1	216.2	153.5	138.9
Player 11	League 3	Donegal					
Player 13	League 3	Donegal					
Player 5	League 3	Donegal	164.6	128.2	378.5	137.2	114.0
Player 6	League 3	Donegal	120.0	194.6	246.2	171.3	138.6
Player 1	Leinster Championship 1	Wicklow	71.3	67.7	200.1	77.0	58.9
Player 13	Leinster Championship 1	Wicklow	50.2	70.4	121.0	72.5	69.4
Player 2	Leinster Championship 1	Wicklow	87.5	78.5	155.6	106.2	85.7
Player 4	Leinster Championship 1	Wicklow	81.6	103.9	129.8	85.0	88.2
Player 8	Leinster Championship 1	Wicklow	63.5	68.0	197.1	86.7	93.9

Appendix H – T:C Ratio Data

Player Name	Competition	Game	T:C(-46)	T:C(-2)	T:C(+0)	T:C(+48)	T:C(+72)
Player 1	League 1	Kildare	0.06	0.11	0.00	0.08	0.10
Player 10	League 1	Kildare	0.04	0.04	0.00	0.07	0.09
Player 12	League 1	Kildare					
Player 2	League 1	Kildare	0.15	0.10	0.09	0.11	0.13
Player 3	League 1	Kildare	0.01	0.03	0.03	0.01	0.00
Player 5	League 1	Kildare	0.03	0.04	0.01	0.03	0.07
Player 8	League 1	Kildare	0.10	0.06	0.04	0.06	0.07
Player 9	League 1	Kildare	0.05	0.05	0.05	0.07	0.04
Player 1	League 2	Tyrone	0.02	0.04	0.01	0.06	0.03
Player 6	League 2	Tyrone	0.01	0.07	0.01	0.03	0.03
Player 7	League 2	Tyrone	0.02	0.06	0.02	0.03	0.02
Player 11	League 3	Donegal					
Player 13	League 3	Donegal					
Player 5	League 3	Donegal	0.05	0.03	0.06	0.07	0.05
Player 6	League 3	Donegal	0.03	0.02	0.01	0.02	0.03
Player 1	Leinster Championship 1	Wicklow	0.10	1.00	0.04	0.01	0.08
Player 13	Leinster Championship 1	Wicklow	0.03	0.01	0.02	0.02	0.02
Player 2	Leinster Championship 1	Wicklow	0.06	0.01	0.08	0.04	0.08
Player 4	Leinster Championship 1	Wicklow	0.03	0.05	0.01	0.07	0.02
Player 8	Leinster Championship 1	Wicklow	0.12	0.07	0.07	0.09	0.16

Appendix I – SAA Data

Player Name	Competition	Game	SAA(-46)	SAA(-2)	SAA(+0)	SAA(+48)	SAA(+72)
Player 1	League 1	Kildare	132.0	137.2	116.7	131.8	132.1
Player 10	League 1	Kildare					
Player 12	League 1	Kildare	154.6	144.1	145.6	151.9	147.7
Player 2	League 1	Kildare	142.7	150.6	119.5	128.3	137.4
Player 3	League 1	Kildare					
Player 5	League 1	Kildare					
Player 8	League 1	Kildare	139.1	133.6	123.1	141.6	128.6
Player 9	League 1	Kildare					
Player 1	League 2	Tyrone					
Player 6	League 2	Tyrone					
Player 7	League 2	Tyrone					
Player 11	League 3	Donegal	85.4	98.4	178.8	108.2	89.2
Player 13	League 3	Donegal	93.5	87.7	92.0	92.0	95.0
Player 5	League 3	Donegal	102.3	147.5	111.4	184.5	167.8
Player 6	League 3	Donegal	93.2	97.9	88.1	97.4	65.1
Player 1	Leinster Championship 1	Wicklow	123.0	122.9	119.8	129.1	130.0
Player 13	Leinster Championship 1	Wicklow	126.1	118.2	113.7	111.9	119.9
Player 2	Leinster Championship 1	Wicklow					
Player 4	Leinster Championship 1	Wicklow					
Player 8	Leinster Championship 1	Wicklow	123.9	129.2	130.4	119.2	121.9