The Hormonal Profile of Elite Super League Players During and After Competition

By
Julian D. Haigh

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Master of Philosophy.

May 2015

Abstract

It has been suggested that steroid hormones, particularly testosterone and cortisol, may play an important role in skeletal muscle growth, repair and even motivation to train, which have obvious implications for elite rugby. However, the hormonal profile of elite Super League players during and after competition is currently poorly understood. To gain insight into hormonal physiology in this athletic population, eighteen professional Super League Rugby League players were recruited for this project to profile the diurnal variation in salivary testosterone and cortisol levels (at rest and across matches). All players were familiarised with the saliva collection techniques, and samples were collected over 4 separate fixtures during the 2012-13 season. Salivary testosterone and cortisol levels were monitored on the nearest rest day to the game at 3 key time points corresponding to game day, just after waking (09.30), pre-game (14.00) and post-game (17.30). In order to examine the effects of elite rugby league match play, a second set of samples were collected at the same time points on game day during 4 fixtures and then analysed using a competitive ELISA.

Initial findings confirmed excellent reliability for the repeated assessment of salivary testosterone and cortisol levels in the study population (CV's = 1.1 – 3.3%). Diurnal variation in salivary testosterone and cortisol was also identified at rest, with both hormones decreasing across the day at each time point cortisol 09.30 (0.275 ± 0.050μG/DL), 14.00 (0.087 ± 0.014μG/DL) and 17.30 (0.079 ± 0.027μG/DL) and testosterone 09.30 (141.9 ± 18.6pg.dl), 14.00 (110.3± 17.4pg.dl) and 17.30 (101.3± 9.5pg.dl), except the testosterone measurement between 14.00 and 17.30 ($P > 0.05$). As a pooled dataset, salivary testosterone was elevated on game day, compared to resting data, 09.30 (162.4 ± 18.0pg.dl), 14.00 (134.5± 13.0pg.dl) and 17.30 (169.1± 12.8pg.dl). There was also a significant decrease between the game-day salivary testosterone from AM to pre (-16%), before increasing from pre to post game (28%). Salivary cortisol levels increased to 09.30 (0.337 ± 0.105μG/DL), 14.00 (0.362 ± 0.042μG/DL) and 17.30 (1.101 ± 0.219μG/DL) compared to resting data. There was also a significant game-day increase in cortisol between AM and pre game (249%) and from pre to post game (219%).

Individual match analysis further revealed some different hormonal patterns, possibly due to the nature of the individual games played and the opposition.

In summary, this thesis has provided novel findings on professional rugby league players with circadian variation in salivary testosterone and cortisol levels identified on a resting day (decreasing from morning to afternoon), as well as match-day changes (increases) that can be attributed to the playing of professional rugby league matches. As a further benefit, this project has validated the utility of saliva-based measurements within the elite sporting environment. Research has suggested that steroid hormones such as testosterone can influence athlete motivation to perform; therefore, future research could investigate factors that affect game-to-game variations in these hormones and assess if such factors may affect game-day performance.

Contents

Acknowledgements

Firstly, I would like to thank my director of studies Dr Graeme Close for providing me with this opportunity to work with you and for the opportunity in applied sport that has ultimately led to my current job. Without your help and guidance this project would not have been possible, despite my best endeavours to make this your longest running M.Phil project. I look forward to continuing working with you in the future.

Secondly, Dr Blair Crewther, without your expert knowledge I would not have completed this thesis. Your feedback and direction have been essential to the success of this project and without I doubt I would have ever finished it.

Thirdly, Clive Brewer, Head of Strength and Conditioning at Widnes Vikings Rugby League. Thank you for your continued mentorship, help, guidance and advice both during this project and in my career. Thank you also for the opportunity to work with you and helping into my first job, your friendship and guidance have been invaluable.

Thanks also to Dr James Morton and Dr Warren Gregson for forming my supervisory team. Your continued help and guidance both academically and professionally has always been appreciated and I hope to continue working with you both in the future.

I'd also like to thank all of the staff and players at Widnes Vikings RLFC. I've thoroughly enjoyed my time at the club up until now and continue to do so. I look forward to continuing the clubs progress and working to make it a success.

Dedication

I would like to dedicate this thesis to my family. Without the support of my grandparents Dan and Helena Kohn, and Donald and Joan Haigh, this would never have been possible. However my biggest thank you has to be to my parents Linda and Roger and brother Nicky. For your words of encouragement and continued help, love and support throughout I will be eternally grateful. I hope that this thesis will have done you proud.

List of Figures

List of Tables

List of Abbreviations

ACTH	Adrenocorticotropic Hormone
CRH	Corticotropin Releasing Hormone
CWI	Cold Water Immersion
ESL	European Super League
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
GPS	Global Positioning System
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
LH	Luteinizing Hormone
NRL	National Rugby League
PVN	Paraventricular Nucleus

CHAPTER 1.0

GENERAL INTRODUCTION

This chapter contains a general introduction to the area together with the aims and objectives of this thesis.

1.1 General Introduction

Rugby league is a contact sport involving frequent bouts of high intensity work (sprinting, tackling and running) interspersed by low intensity efforts (Gabbett et al., 2008a). In order to be competitive elite rugby players require highly developed speed, agility, muscular strength and power and high levels of maximal aerobic power (Gabbett, 2005). Teams are divided up into 4 positional groups, middle unit forwards (props and loose forward), wide running forwards (second rows), pivots (hookers and half backs) and outside backs (centres, wingers and full backs) (Evans et al., 2015). Each positional group is defined by separate roles and dominant physical characteristics.

One factor currently poorly understood in elite rugby league is the role and response of steroid hormones prior to and during elite rugby league match play. Based on previous research, it has been suggested that fluctuations in hormone levels have a role to play in moderating athlete performance, possibly due to changes in motivational behaviours (Cook and Beaven, 2013). However, before performance variables can be considered, it is necessary to reliably and accurately quantify hormone dynamics at rest and across exercise. This thesis will aim to establish the normal diurnal variation and reliability of salivary testosterone and cortisol in ESL rugby league players, before quantifying any change that may occur prior to and post ESL match play. This is an important first step, as no previous literature has attempted to profile the changes in testosterone and cortisol from at rest to pre and post rugby league competition.

Traditionally, testosterone and cortisol have been associated with skeletal muscle anabolism and catabolism (Kraemer and Ratamess, 2005). However, recent research by West and Phillips (2012) has shown that acute physiological changes in steroid hormones such as testosterone and cortisol have no effect on muscular hypertrophy following resistance exercise. Recent literature is now suggesting an effect of increased levels of steroid hormones in motivation to perform and displaying dominance (Cook and Beaven, 2013). Interventional studies looking at the effects of video type and the effects of different types of feedback have reported concomitant increases in testosterone concentrations

and subsequent athletic performance (Cook and Crewther, 2012a, Cook and Crewther, 2012b), such as increased strength and positive actions during sport. Hormonal linkage to behaviour has been reported in other research domains, for example, higher testosterone concentrations have been associated with taking more risks in the financial industry in both males and females (Coates et al., 2010, Coates and Herbert, 2008, Stanton et al., 2011a).

Cortisol is thought to be a key physiological marker of stress and often associated with a reduction in performance and overtraining (Gleeson, 2002); however, the metabolic effects of cortisol may in fact be essential to working capacity and optimal functioning (Viru et al., 2001). As with testosterone, cortisol has been linked to the expression of competitive behaviours and performance (Salvador, 2005). Indirectly, cortisol may also influence the effect of testosterone on behaviour (e.g. motivation) via interactions with androgen receptors and/or testosterone production (Liening, 2010). Therefore, it would be prudent to monitor both hormones concurrently to better understand any potential interactions during elite match play.

Based upon recent studies there is growing interest in the role of testosterone and cortisol in moderating athlete performance. Previous research in rugby league in the southern hemispheres' NRL competition has shown that salivary testosterone levels decrease from 24h pre-game until 24hours post game (McLellan et al., 2010). Salivary cortisol however increased from 30 minutes pre-game peaking 30 minutes post game continuing to rise until 48h post-game. The increase in cortisol was attributed to the psychological stress of collisions and a large adrenal response caused by high-intensity intermittent exercise (McLellan et al., 2010). The cited study did fail to account for diurnal variation in hormone secretion (Hayes et al., 2010) when comparing 24 hours pre-game (15.30-16.30) relative to 24 hours post (19.00). To the author's knowledge, these hormonal profiles (resting and match-day) have never been examined within the ESL competition.

Therefore, in order to allow greater understanding of the potential role of testosterone and cortisol on match day performance, the overall aim of this

project was to examine the testosterone and cortisol responses (pre to post game and rest to game day changes) in professional rugby league players. To test this we developed 3 specific objectives:

1. To assess the reliability of salivary hormone testing with CV's <10%
2. To examine the circadian rhythms of testosterone and cortisol in elite rugby league players on resting days
3. To compare resting and match-day hormones to quantify the actual changes occurring due to rugby league competition.

The following hypotheses were formulated:

1. The repeated measurement of salivary testosterone and cortisol levels on resting days would be reliable.
2. Monitoring salivary testosterone and cortisol levels across the resting day (3 time points) would reveal a typical diurnal rhythm, being higher in the morning than the afternoon.
3. Game-day salivary testosterone and cortisol levels would be significantly increased compared to at rest.

CHAPTER 2.0

LITERATURE REVIEW

The following chapter will examine current literature investigating the physiological demands of rugby, steroid hormone secretion and assessment, steroid hormones as markers of stress, the effects of match play on steroid hormones and finally the effects of steroid hormone level on performance.

2.1 Physiological demands of rugby league

Rugby League is a high-intensity intermittent team sport interspersed with heavy collisions and repeated sprints (Gabbett, 2005, Meir et al., 2001). Played over two 40-minute halves separated by a 10-minute half time, teams consist of 17 players: 13 starters and 4 non-starters to be used throughout the game as part of the teams' 10 interchanges (Gabbett et al., 2011). Each team has 6 tackles with the ball and once completed the ball is handed over to the other team (Gissane et al., 2003). A tackle is completed when the attacking player is brought to a stop by the defenders. The objective is to advance downfield and score a try in the opposition 'in goal' area. The ball must be passed backwards and, in order to progress forward, it can be carried or kicked towards the opposition in-goal area. Rugby league is played worldwide with one main competition in each hemisphere. The European Super League (ESL) is currently the top league for elite rugby league players in the northern hemisphere (Twist and Highton, 2013) and the National Rugby League (NRL) competition is the elite league in the southern hemisphere.

Teams have traditionally been categorized into two (forwards and backs) or three positional groups; hit-up forwards (props, second rows and loose forward) pivots (hooker, scrum half and stand off) and outside backs (center, wingers and full backs) (Gabbett et al., 2008a). More recently Gabbett et al. (2012): Evans et al. (2015) have suggested that, based on the physiological demands of rugby league, positional groups should be classified into four 4 groups. Gabbett et al. (2012) proposed dividing the playing groups up as: middle units (props), wide running forwards (second rows and loose forwards), pivots (hooker, scrum half, stand off and fullback) and outside backs (center and wingers). (Evans et al., 2015) proposed that groups should be divided based on time played +/- 2SD's, due to the influence of recent rule changes increasing the number of interchanges. Based on this groups were divided into; middle units (props and loose forwards), wide running forwards (second rows), pivots (hooker, scrum half and stand off) and outside backs (centers, wingers and full back).

Hit-up / middle unit forwards position themselves in the middle of the field, and are primarily responsible for carrying the ball directly into contact near the play of the ball. In defence, they tend to be involved in the most collisions by stopping the forward progression of the opposing team through tackling, (Meir et al., 2001). Outside backs generally follow the play on the fringes with their main involvement coming during kick chases, returns, chasing down opposing players or making a line break (Meir et al., 2001). The pivots main role is to distribute and control the play taking the ball to the defence line to react and evade defensive players, therefore spending a majority of time following the movement of the ball (Gabbett, 2005, Meir et al., 2001). However, the role of the pivots change in defence, with the hookers tending to defend in the middle in case of a quick turn over and the scrumhalves and stand offs defending towards the edges (Gabbett, 2005). Elite rugby league players require highly developed speed, agility, muscular strength and power, and maximal aerobic power (Gabbett, 2005).Thus, the physiological demands of each role can be quantified using quantitative on-field GPS data and sport-specific performance testing and qualitative stats through the use of video and OPTA. OPTA is an independent analysis service provided through contract with the Rugby Football League (the governing body for the sport in the UK).

2.1.1 Anthropometrics

Rugby league players body mass is the only physical characteristic that successfully predicts selection into first grade rugby league (Gabbett, 2002a), or as a forward or back (Gabbett, 2002b). However as Gabbett et al. (2011) has since shown the difference between positional groups has decreased as the game has become more physical. A larger body mass increases momentum (mass x velocity) during tackling, ball play and other collisions. However, it's important to consider body composition as well. Excess body fat has been shown to negatively influence performance (e.g. power to body mass ratio, thermoregulation and aerobic capacity) (Meir et al., 2001). As shown in table 1 body fat levels are significantly higher in forwards than they are in backs. Forwards spend significantly more time involved in tackles and physical collisions than backs Evans *et al.,* (2015), so the higher body fat of the forwards

assists in the development of greater impact forces associated with these events. It has also been suggested that the increased body fat acts as protection from impact injuries (Meir, 1993); however, there is currently no scientific evidence supporting this (Gabbett et al., 2007).

Table 2.1 Rugby League players' anthropometrics

Author	Competition	Time of Season	Positional Group	N =	Age (Years)	Body Mass (kg)	Σ skinfolds (Sum 4, 7*)	Height (cm)
(Baker, 2002)	NRL	End of preseason	Whole	22	24.3 ± 3.7	93.4 ± 96	NR	181.7 ± 6.9
(Brewer, 1994)		NR	Forwards	NR	NR	92.1 ± 10.4	NR	184.0 ± 7.0
		NR	Backs	NR	NR	79.8 ± 8.0	NR	178.0 ± 8.0
Evans *et al.,* (2015)	SL	Whole season	Whole	33	24.0 ± 4.0	96.0 ± 10.0	NR	183.0 ± 6.0
(Gabbett et al., 2012)	NRL	Mid-competition	Forwards	11	25.1 ± 2.1	91.9 ± 6.6	NR	NR
			Backs	9	23.4 ± 2.5	88.6 ± 4.2	NR	NR
(Gabbett et al., 2011)	NRL	End of preseason	Starters	NR	24.6 ± 3.9	95.6 ± 8.0	52.4 ± 12.4*	184.6 ± 5.3
			Non-starter	NR	23.3 ± 3.9	98.9 ± 12.4	56.5 ± 12.0*	182.4 ± 5.7
			Non-select	NR	22.2 ± 3.5	95.6 ± 10.8	72.0 ± 22.9*	183.1 ± 6.5
(Meir et al., 2001)	NRL + SL	End of preseason	Forwards	74^1, 58^2	NR	97.5 ± 8.7^1	54.0 ± 20.5^2	NR
			Backs	71^1, 59^2	NR	85.2 ± 6.7^1	39.6 ± 9.1^2	NR
			Forwards	57^1, 44^2	NR	100.8 ± 7.1^1	57.3 ± 21.3^2	NR
			Distributors	20^1, 18^2	NR	81.0 ± 6.4^1	43.1 ± 10.9^2	NR
			Adjustable	20^1, 15^2	NR	89.8 ± 5.8^1	43.7 ± 12.8^2	NR
			Out Back	48^1, 40^2	NR	86.2 ± 6.7^1	37.9 ± 8.4^2	NR

Key: NRL = National Rugby League, SL = Super League, NR = Not Reported

2.1.2 Physical Demands

Forwards often have greater body mass and fat (vs. backs) in order to increase their momentum and forces on impact (e.g. tackling). Brewer and Davis (1995) stated that collisions and tackling are widely acknowledged as the most demanding aspect of rugby league. Similarly, Gabbett et al. (2012) reported that, in the NRL, the most significant differences amongst playing positions were the high-intensity collisions and repeated effort demands of match play. This is supported by Evans *et al.,* (2015) who reported that, during an average super league game, players experience between 20 and 36 collision-based actions; Middle units = 34.5, wide running = 35.6, pivots = 22.6 and outside backs = 19.5 (OPTA). GPS data from the same study shows that players can experience impacts up to 15G (Table 2), with middle unit (0.71) and wide running forwards (0.46) experiencing significantly more collisions per minute that outside backs (0.22) and pivots (0.31). Middle unit (0.63) and wide running (0.41) forwards also had greater exposure to impacts between 9-15G per minute than outside backs (0.27) and pivots (0.32). These data highlight the physical nature of this sport and positional group differences.

2.1.3. Strength and Power Requirements for Professional Rugby League Players

The ability to rapidly generate high levels of muscular force is a key characteristic of a successful rugby league player (Gabbett et al., 2008a, Gabbett, 2005, King, 2009). In order for players to dominate and ‘win’ the collisions in both attack and defence they must be physically strong and powerful. In attack players are required to have high levels of muscular strength and power in order to provide fast play-the-ball speed and facilitate leg drive in a tackles (Gabbett et al., 2008a). In defence players are required to effectively tackle, lift, push and pull opponents during a match (Meir et al., 2001).

The strength and power profiles of rugby league players has been well documented (see table 3) with many studies showing a significant difference between forwards and backs (Baker, 2002), thereby reflecting different

positional and training demands within rugby league. This work also stresses the need for a high level of strength and conditioning work to be done with the players to allow them to compete successfully at the top level. The outcomes of which can often have a large bearing on performance and factors affecting performance as shown in chapter 2.3.

Table 2.2 AThe amount of impacts and collision per position in the average super league game (Evans *et al.,* 2015)

Key: OB = Outside Backs, P = Pivots, MU = Middle Unit, WR = Wide Receiver, P/min = Per minute

	Collision		Absolute	Averages			P/Min	Average	
Collisions	Zone (G)	OB	P	MU	WR	OB	P	MU	WR
Defensive	(Tackles)	9.8 ± 2.5	17.9 ± 2.9	24.5 ± 5.4	25.1 ± 4.7	0.1	0.3	0.5	0.3
Attacking	(Carries)	9.8 ± 1.6	4.6 ± 1.6	10.3 ± 2.0	10.5 ± 2.4	0.1	0.1	0.2	0.4
Total		19.5 ± 3.4	22.6 ± 3.2	35.6 ± 6.6	35.6 ± 5.1	0.2	0.3	0.7	0.5
Impacts	3-5	2231.8 ± 115.1	2228.5 ± 349.1	1465.1 ± 241.1	2108.1 ± 265.9	25.7	31.1	31.2	27.6
	5-7	520.0 ± 72.7	711.2 ± 134.1	521.7 ± 128.5	552. 6 ± 95.5	6.0	9.8	10.8	7.3
	7-9	134.7 ± 40.7	199.7 ± 66.7	165.6 ± 70.0	177.4 ± 48.1	1.6	2.8	3.9	2.3
	9-11	17.5 ± 3.1	18.3 ± 3.9	23.0 ± 8.1	24.0 ± 7.6	0.2	0.3	0.7	0.3
	11-13	5.6 ± 1.9	4.6 ± 1.9	6.6 ± 2.1	6.3 ± 2.8	0.1	0.1	0.5	0.1
	13-15	0.7 ± 0.4	0.3 ± 0.4	0.5 ± 0.2	0.6 ± 0.5	0.0	0.0	0.1	0.0

2.1.4 Speed and Agility in rugby league

Speed is important for rugby league players to quickly position themselves in both attack and defence (Meir et al., 2001). Previous literature has shown that players are not often required to sprint further than 40m at a time, with forwards rarely sprinting further than 10m (Gabbett et al., 2008b). This suggests that acceleration is of greater importance than actual top speed attained. Whilst there have been no significant differences between backs and forwards over 10m, backs are significantly faster over 40m (see table 3). In game data taken over a super league season by Evans *et al.,* (2015) reported that outside backs (421 ± 89) cover significantly more distance (m) at a sprint (5.5 > m/s^{-1}) than both middle unit forwards (185 ± 58) and wide running forwards (296 ± 82) but not pivots (310 ± 108). Outside backs also complete a significant higher number of sprints (25 ± 4) and have a greater max sprint distance per game (52 ± 11) than middle unit forwards (11 ± 4) and (38 ± 4), wide running forwards (19 ± 4) and (41 ± 7) and pivots (18 ± 6) and (42 ± 9).

Rugby league players also require the ability to rapidly accelerate, decelerate and change direction (Gabbett et al., 2008b). In attack, this is important in order to take on and beat defenders using skills or footwork. In defence, players need to be able to react to sudden changes of direction of the both the attacker and the bouncing ball, and to manoeuvre one's self to find supporting players. Gabbett et al. (2008b) reported that higher and lower skilled players could not be discriminated between a series of pre-planned change of direction tests, however on the reactive agility test higher skilled players scored much better without accuracy being compromised. This could be explained by the higher skilled players ability to read postural cues. In the literature different tests have been used to assess running agility making it difficult to compare across studies as seen in table 4. Again it is important to address these demands in the players every day training taking into account factors that could affect performance.

Table 2.3. Strength, speed and power in rugby league players.

Study	Comp	Position	N=	Squat (kg) (1RM*, 3RM)	Bench Pr (kg) (1RM*, 3RM)	Vert. Jump (cm)	10m (s)	40m (s)
(Baker, 2001)	NRL	Whole	22	NR	134.8 ± 15.2	NR	NR	NR
(Gabbett et al., 2012)	NRL	Starters	NR	NR	NR	63.9 ± 6.0	1.71 ± 0.07	5.19 ± 0.19
		Non-Starters	NR	NR	NR	61.5 ± 4.6	1.77 ± 0.06	5.37 ± 0.13
(Gabbett et al., 2011)	NRL	Forwards	11	NR	NR	48.7 ± 6.6	2.05 ± 0.08	5.86 ± 0.10
		Backs	9	NR	NR	50.9 ± 3.4	1.98 ± 0.05	5.69 ± 0.11
		Backs		168*	113*	NR	NR	NR
(Meir et al., 2001)	NRL +	Forwards	63[1],52[2]	NR	123 ± 11.76[1]	NR	NR	5.27 ± 0.19
	SL	Backs	55[1], 50[2]	NR	114 ± 17.03[1]	NR	NR	5.08 ± 0.20
		Forwards	38	NR	NR	NR	NR	5.30 ± 0.20
		Distributors	16	NR	NR	NR	NR	5.21 ± 0.18
		Adjustable	16	NR	NR	NR	NR	5.20 ± 0.14
		Outside Back	32	NR	NR	NR	NR	4.98 ± 0.16

Key: NRL = National Rugby League, SL = Super League, NR = Not Reported, [1] = Study group 1, [2] = Study 2, Bench Pr = Bench Press, Vert. Jump = Vertical Jump, RM = Repetition Max, N = number, cm = Centimeter, S = second

Table 2.4. Agility in Rugby League players.

Study	Comp	Position	N=	Test	Results
(Gabbett, 2002b)	NRL (1st grade)	Forwards	11	Illinois Agility Test	17.2 ± 0.6
		Backs	9		17.4 ± 0.7
(Gabbett et al., 2011)	NRL (1st grade)	Starters	NR	5-0-5 Test	2.20 ± 0.21
		Non-Starters	NR		2.29 ± 0.20
		Non-Selected	NR		2.32 ± 0.17
(Meir, 1993)	(1st grade)	Forwards	NR	L-Agility Test	5.46 ± 0.21
		Backs	NR		5.37 ± 0.22
(O'Connor, 1996)		Backs	NR	Glycolytic Agility Test	45.3 ± 3.33
		Halves	NR		45.89 ± 3.03
		Back Row	NR		46.55 ± 2.73
		Props	NR		46.71 ± 2.89
		Hookers	NR		46.20 ± 2.84

Key: NR = Not Reported,

2.1.5 Metabolic Demands of rugby league

Rugby league players' strength and speed has less value for a team if they are unable to match the maximal aerobic demands of the sport. Due to the high intensity, intermittent nature of rugby league, repeated sprint ability is very important for players (Gabbett et al., 2008a). For example, a player may be required to move quickly off the defensive line, make a cover defending tackle, and then chase from the play the ball, requires the ability to generate high levels power, and then recover quickly in order to produce more high intensity efforts (Gabbett et al., 2008a). In fact, the physical outputs (e.g. distance covered, maximal accelerations and repeat high intensity effort performance) of winning teams are often significantly higher than the losing team (Gabbett, 2013).

The above findings suggest that the competitive advantage of successful NRL teams is their ability to maintain a higher playing intensity than their opponents. As supporting evidence, winning sides in professional rugby league were found to cover greater total distance per minute of match play including greater distances at low intensity (Gabbett, 2013). Evans *et al.,* (2015) reported that the average distance covered by the bottom team during a super league game is 7246 ± 333 by outside backs, 6549 ± 853 by pivots, 4318 ± 570 by middle unit forwards and 6408 ± 629 by wide running forwards. Specifically the pace (meters per minute) they cover these distances at was 84 ± 3 by outside backs, 90 ± 3 by pivots, 91 ± 2 by middle unit forwards and 83 ± 2 by wide running forwards. These high intensity efforts are reflected in the percentage of time players spend above 80% of HR max: outside backs (26.86%), pivots (27.85%), middle unit forwards (23.44%) and wide running forwards (20.09%). This places a high demand on players' ability to maintain high levels of anaerobic power and recover quickly.

All of these factors can be influenced and increased by athletes' preparation during the week leading up to fixtures. As summarized by Gabbett (2013), the competitive advantage of the most successful teams is closely linked to their ability to maintain a greater physical intensity for longer periods of time. This places considerable emphasis on gaining every advantage possible to achieve

the physiological edge and maintain a higher intensity over opponents, including the ability to control behavioural and mental focus and preparation. One current area of research that is not fully understood is the role of steroid hormones and their effects and role in modifying performance.

2.2 Steroid Hormone Secretion

All steroid hormones originate from the lipophilic low-density lipoprotein compound cholesterol in the mitochondria via steroidogenesis (Miller, 2013, Miller and Bose, 2011, Stanfield, 2012). Once synthesised they are secreted in the blood stream where they bind with either albumin or specific protein carriers, allowing for them to be transported to their target cells (Stanfield, 2012). Due to the cells lipophilic properties they pass straight through the cell membrane to bind with the specific receptor in the cell cytoplasm and begin their effects on the target cell (Stanfield, 2012).

2.2.1. Secretory Control

Testosterone secretion control

Testosterone secretion is controlled via a positive and negative feedback loop via the HPG axis. Upon stress and homeostatic-related information reaching the anterior hypothalamus and pre-optic area of the brain, the peptide gonadotropin-releasing hormone (GnRH) is synthesized and released, which is the primary regulator of the pituitary gonadotrophs (Stanfield, 2012). Once GnRH reaches the anterior pituitary, it stimulates the gonadotropic cells to release luteinizing hormone (LH) and follicle stimulating hormone (FSH) into general circulation (Stanfield, 2012). These hormones then bind to receptors in the testes and ovaries to stimulate testosterone production (See fig 2.1)

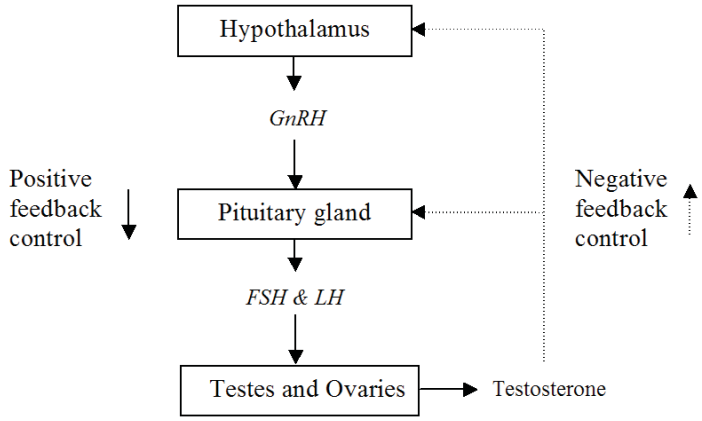

Figure 2.1 Simplified schematic of the hypothalamic-pituitary-gonadal axis demonstrating the process of Testosterone release via the positive and negative feedback control

Cortisol secretion control (The hypothalamic-pituitary-adrenal (HPA) axis)

Cortisol secretion is regulated by the positive and negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis (Viau, 2002). The HPA axis begins in the hypothalamus at the paraventricular nucleus (PVN). More specifically, afferents conveying stress and homeostatic-related information in the brain ultimately end at the medial parvocellular neurons of the PVN (Viau, 2002). As a result of these signals, the medial parvocellular neurons of the PVN release several peptides into pituitary portal circulation of the hypophyseal stalk (Mastorakos et al., 2006). Foremost of these peptides are corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP). The medial parvocellular neurones of the PVN represent the neuroendocrine arm of the HPA axis, compromising the pathway through which the brain drives the anterior pituitary corticotropins via stimulation from the release of CRH to synthesize and release adrenocorticotropic hormone (ACTH) (Tsigos and Chrousos, 2002, Viau, 2002). ACTH is then transported to the adrenal cortex of the adrenal glands to stimulate cortisol production and secretion (Miller, 2013). (See fig 6). Sustained ACTH release during acute stress, or an elevated ACTH response during chronic stress, is thought to be mediated by the potentiating effects of AVP on ACTH release, otherwise inhibited by the

negative feedback actions of elevated plasma glucocorticoid concentrations (Miller, 2013).

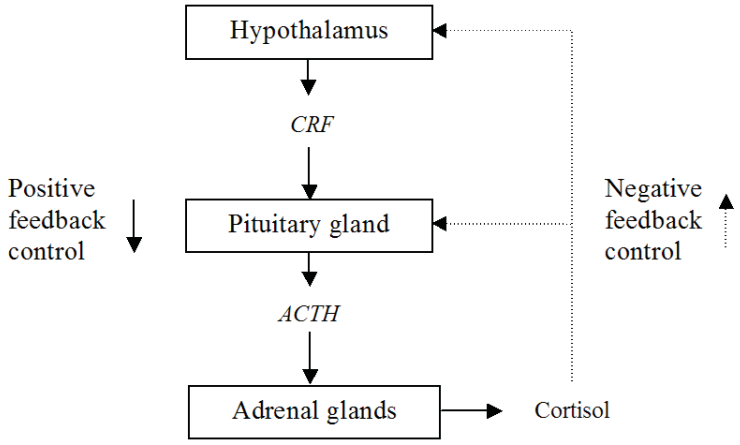

Figure 2.2 Simplified schematic of the hypothalamic-pituitary-adrenal axis demonstrating the process of Cortisol release via the positive and negative feedback control

In non-stressful situations, both CRH and AVP are secreted in a circadian, pulsatile fashion with a frequency of 2 or 3 secretions per hour. Under resting conditions, the amplitude of the CRH and AVP pulses increase in the early morning, resulting in ACTH and cortisol secretory bursts in general circulation (Hayes et al., 2010). These diurnal variations are perturbed by changes in lighting, feeding schedules, activity and acute stress. During acute stress, the amplitude and synchronization of the CRH and AVP pulsations in the hypophyseal portal system increases, thereby increasing ACTH and cortisol secretion.

Two main processes regulate the secretion of pituitary and pituitary-dependent hormones; the circadian signal generated by the endogenous pacemaker in the suprachiasmatic nucleus within the anterior hypothalamus (Hayes et al., 2010)and the alternant of wakefulness and sleep (Dickmeis et al., 2013, Sensi et al., 1993). In general, the circadian profiling of testosterone and cortisol exhibit a consistent pattern, with the highest levels reported early in the morning, followed by decreases across the waking day and a return to morning levels

during sleep (Hayes et al., 2010). This circadian pattern highlights the need to collect control data to quantify any hormonal change associated with any intervention against normal biological changes.

It is important to note that steroid hormones are essentially chemical signals that support or regulate tissue and cell responses to stress to maintain homeostasis. Living organisms survive by maintaining an immensely complex dynamic state known as homeostasis, which is constantly challenged by intrinsic or extrinsic disturbing forces or stressors (Magiakou et al., 1997). Steroid hormones of the adrenal gland are an integral component of the stress response and regulate many physiological processes, including metabolism and immune response (Dickmeis et al., 2013). Therefore, it is important to consider whether these changes are occurring in preparation for or as a result of a perceived challenge or threat to maintaining homeostasis..

Despite the importance of the circadian rhythm, little research has profiled testosterone and cortisol secretion across a resting day in the elite sporting environment to allow effectiveness comparisons with the collection of training and competition data. To the authors' knowledge, this has never been looked at in professional rugby league, specifically a direct comparison between data at rest and on a match day in this population of athletes.

2.2.2 Assessment of Hormone Status

Some of the constraints of biological sampling on elite athletes (e.g. poor compliance, limited time) present difficulties when undertaking hormonal research in the sporting environment. Saliva can provide a useful, non-invasive alternative to the collection of serum and plasma, which can be collected rapidly frequently and without stress (Papacosta and Nassis, 2011). Salivary analysis requires little medical training and can be carried out on a sports field. Due to the pulsatile release of steroid hormones and the circadian effect, it is important to be able to sample quickly and effectively, both of which are achievable via saliva sampling (Brown et al., 2008).

In order to collect meaningful data, the measures need to be both valid and reliable. Salivary steroids such as testosterone and cortisol show a correlation with their free-form serum concentrations in the blood (Gatti and De Palo, 2011). However, the absolute concentrations of salivary steroids may differ from serum as the salivary glands have the ability to metabolize steroids (Gatti and De Palo, 2011). Crewther et al. (2010) validated the use of salivary cortisol and salivary testosterone measures in response to a short, high-intensity cycle bout. They also found that the hormonal changes to exercise are greater in saliva than corresponding blood measurements, similar to other work (Gozansky et al., 2005). Thus, salivary hormones may provide a more sensitive measure for tracking exercise-induced change than corresponding blood markers, possibly reflecting the more dynamic free hormone. Cadore et al. (2008) also reported that salivary cortisol and serum are significantly correlated before ($r = 0.52$, $P < 0.005$) and after ($r = 0.62$, $P < 0.001$) resistance training in both non-competitive strength-trained and untrained individuals.

The importance of reliability is often overlooked with regards to hormonal analysis, irrespective of the biological or tissue media used. Without sufficient reliability, it is impossible to assess the accuracy of any measurement tool and thus, how meaningful any changes (or lack thereof) actually are. Furthermore, when using multiple kits it is important to make sure all kits are reliable (i.e. by comparing kit controls). To address any variation that may exist between assay kits, it is suggested that the samples from the same individuals be tested within the same assay plate. Dabbs (1990) also reported some stability in salivary testosterone measurements (same samples) taken over 2 days (r=0.62) and after 7-8 weeks (r = 0.52), but in practice all batch samples should be tested on the same day.

2.3 Steroid Hormones and Performance

The endocrine system, especially the steroid hormones, is thought to play a key role in mediating adaptive physiology with training (Crewther et al., 2011). Training evokes both acute and chronic changes in steroid hormone levels, which help to signal neuromuscular changes or responses linked to muscle

growth and/or neuromuscular function (Crewther et al., 2011). Due to the perceived role of anabolic hormones (e.g. testosterone) and catabolic hormones (e.g. cortisol), it has been proposed that the acute response to resistance exercise contributes to the remodelling of skeletal muscle and thus, hypertrophy (Kraemer and Ratamess, 2005). Although this may be the case when our exposure to circulating hormones increases dramatically (e.g. during puberty, supplementation with exogenous hormones), recent data indicates that physiological changes in testosterone levels and other anabolic hormones play little or no role in the regulation of muscular protein synthesis (West et al., 2013b, West et al., 2009, West and Phillips, 2012).

There is growing evidence for an additional effect of testosterone (on athlete performance and training outcomes) that is mediated by competitive behaviours and motivation to perform. Several models provide a theoretical explanation for this linkage. According to the challenge hypothesis, testosterone levels in males (birds) rise to facilitate mating competition (Wingfield (1990), and this model has been extended to include human behaviours and competition (Archer, 2006). It has also been suggested that testosterone has a positive influence on dominance striving, or power motivation, so high testosterone levels may predispose some individuals towards sports participation (Schultheiss et al., 2003, Schultheiss et al., 2005). This model is however overly simplistic and assumes that testosterone is the key factor underpinning performance.

Recent work on elite athletes has highlighted a possible role for testosterone in moderating motivational behaviours (Cook and Beaven, 2013). Cook and Beaven (2013) found a strong relationship between greater self-selected loads and higher levels of testosterone in female netballers. In a related study, Cook and Crewther (2012a) demonstrated that watching short video clips with different content can modify testosterone levels and subsequently 3RM squat performance can be improved. Testosterone increased following the presentation of erotic, aggressive, humorous and training clips, resulting in an improved 3RM performance after the erotic, aggressive and training videos. Similarly, a pre-game motivational intervention that involved a positive video

clip with positive coach feedback led to increase in testosterone levels and performance in elite rugby union players (Cook and Crewther, 2012b). These studies highlight a potential role of testosterone in improved performance, and also the potential role of motivational interventions to improve this.

The influence of testosterone on motivational behaviours has been demonstrated in other domains. For example, males and females with higher testosterone concentrations have been shown to take greater risks in a financial domain (Coates et al., 2010, Coates and Herbert, 2008, Stanton et al., 2011a) and low testosterone has been linked to risk aversion in a financial domain, although this relationship appears to be non-linear (Sapienza et al., 2009, Stanton et al., 2011b). Impulsivity (Flegr et al., 2012) and risk tolerance associated with power posing (Carney et al., 2010) are other related outcomes linked to adult testosterone levels in a physiological range.

Like testosterone, cortisol has been linked to competitive behaviours and performance in sport (Salvador, 2005) and the behavioural responses (and effects) of testosterone might in fact be influenced by the cortisol effects on androgen receptors in threatening situations (Liening, 2010), which can be likened to elite sports competition (Crewther et al., 2013). To complicate matters, basal testosterone may in turn be used to predict changes in cortisol level and subsequent behaviours after a defeat or victory (Mehta et al., 2008). The literature in this area is somewhat unclear and reflects issues in the research such as one off sampling and complexity of simulations as supposed to longitudinal research. A lot of the work in this area (competitive behaviours) has also been carried out in non-athletic populations and in a lab-based simulation; and therefore, may have little relevance to an elite sporting environment.

There is a growing body of evidence that steroid hormones, specifically normal day-to-day variations in testosterone, may provide additional information regarding training motivation and readiness to perform in elite athletic populations (Cook and Beaven, 2013, Cook and Crewther, 2012a). So specific hormonal increases and decreases on an individual level (and within normal

circadian variation) may provide a novel indicator of an athletes' recovery state and/or readiness to perform. As shown in the previous research, rugby league is an extreme physical sport with large neuromuscular demands that may be enhanced by an increase in pre game testosterone, or it may show how well an athlete has recovered for future competition (Crewther et al., 2013).

2.3.1 Steroid Hormones and Game-day patterns

Testosterone secretion

The response of salivary testosterone levels in elite rugby league players prior to and post game at multiple time points in the ESL has yet to be established. To date, only one study has examined the effects of elite rugby league match play on steroid hormones. McLellan et al. (2010) reported a significant decrease in salivary testosterone (-47%) from 24 hours pre-game to 30 minutes pre-game. However this study was only conducted during one fixture with unknown circumstances. Previous literature has shown the effects of opposition (Gabbett, 2013), venue (Carre, 2009) and motivation (Cook and Beaven, 2013) to influence salivary testosterone levels, therefore by only looking at one time point in one fixture these findings may be a unique occurrence.

Findings in rugby union have shown a significant difference between game day and rest day salivary testosterone concentrations. Elloumi et al. (2008) reported no circadian rhythm across 5 time points on 2 separate occasions. During fixture one of no relative importance there was a significant decreases immediately post game -16% and 2 hours post game -28% of salivary testosterone levels. However, in game 2, which determined qualification to the African cup of nations, salivary testosterone levels were significantly increased on the morning of 7% and post game by 22%. So the importance of the match played would seem influence testosterone dynamics.

Previous literature has shown an effect for elite rugby league match play on salivary testosterone levels with McLellan et al. (2010) reporting a 14% increase from pre to post game. However, testosterone levels in this study were still significantly reduced post game relative to 24 hours pre-game. This result

fails to take into account the potential effects of the circadian rhythm present in testosterone secretion (Hayes et al., 2010). This small increase in salivary testosterone is in agreement with those of Elloumi et al. (2008) who found post game testosterone to be increased relative to pre game.

Elloumi et al. (2003) proposed that the overall decrease found by McLellan et al. (2010) decrease in testosterone from pre to post game occurred at a cellular level due to its known protective effect against proteolytic pathways (opposing cortisol's effects) and a sparing effect on glycogen stores (Guezennec et al., 1986). The decrease in testosterone limits these protective effects and allows the energy supply to be enhanced from these cellular substrates. Elloumi et al. (2003) went on to suggest that the post-game changes in testosterone may be due to the coticotrophic axis inhibiting the gonadotropic axis at the hypothalamic level by a direct effect (or an increase of beta endorphin) of CRH on GnRH secretion (Barbarino et al., 1989, Cumming et al., 1983). This may explain the decline in testosterone after the game when cortisol levels were high. This is in agreement with (but does not explain) the testosterone rise during the recovery phase when cortisol is low. Elloumi et al. (2003) goes on to suggest that it would have been of interest to measure CRH, ACTH, GnRH and LH as currently there is explanation for these post testosterone and cortisol changes and no proof they are linked.

Cortisol secretion

In the McLellan et al. (2010) report on rugby league, salivary cortisol levels increased from 24h pre game to 30mins pre game (28%) and this thought to be due to anticipatory stress response. Similar findings in elite rugby union have initially shown a clear circadian rhythm occurring across 5 separate time point (08.00, 11.00, 16.00, 18.00 and 20.00) on 2 separate rest day with no significant differences (Elloumi et al., 2008). Elloumi et al. (2008) then goes on to report significant increase in game day cortisol levels pre (24%), post (124%) and 2 hours post (99%) during game one of no significance. However critically during game 2 of greater importance there was a significantly greater increase in cortisol level at the pre (49%), post (216%), 2 hours post (167%) and in the

evening following (48%), again showing the potential effects of match importance on hormone levels. Interestingly, there was no significant increase in game-day cortisol levels in the AM samples just after waking at 08.00. This may be explained by the natural early morning rise in cortisol.

McLellan et al. (2010) goes on to report that cortisol increased by 68% peaking post game, with levels still significantly increased at 24 hours post game (+36%) before returning to baseline at 48 hours. These findings are in agreement with other high intensity, aggressive, intermittent sports such as rugby union. West et al. (2013a) shows that following professional rugby union match play cortisol levels were elevated at 12 hours (56 ± 49%) and 36 hours (59 ± 64%) before returning to baseline at 60 hours. These findings support initial research by Elloumi et al. (2003) who also reported the same pattern of change following a rugby union match.

Elloumi et al. (2003) suggested that rises in pre-competition cortisol levels are often higher due to cognitive anticipation and anxiety (Passelergue et al., 1995, Salvador, 2005, Salvador et al., 2003) . This may not evident in the Elloumi et al. (2003) work with the AM samples taken 6 hours before the match started. Alternatively, the rise in cortisol shortly after waking may have masked this response (Hayes et al., 2010). As shown in previous literature cortisol rises significantly during competition. In Elloumi et al. (2003) cortisol levels increased 2.5 times compared to at rest. They suggested this rise in cortisol occurs in types of exercise with intensities above 60% max power and longer than 30 minutes (Kirschbaum and Hellhammer, 1989, Snegovskaya and Viru, 1993). This large rise can also be attributed to the demands of rugby league, as covered in chapter one, given that the cortisol response increases with exercise intensity and duration (Lac and Berthon, 2000). The adrenal response is also stronger during intermittent anaerobic exercise, compared to continuous aerobic exercises (Jensen et al., 1991), and competition can further magnify this stress (Passelergue et al., 1995).

It is evident from the limited studies to date that hormone status is disrupted by the effects and demands of elite collision sports, which may impact upon a

team’s preparation and recovery for the up and coming weeks training and fixtures. However, the previous rugby league data was based on a single game (McLellan et al., 2010), so the results may be limited to that specific game, the opposition faced and the outcome. Other teams with different tactical approaches may produce different physiological and hormonal responses. Furthermore, data were only collected at one time of day, leaving the question is the diurnal variation of these hormones displaced during a competitive rugby league match? The baseline sample was collected on the day before the game, during which time hormone levels could feasibly increase as a response to be mental and physical game preparation. Had this been taken on a rest on day mid week, it may have given a different result.

Table 2.5 Summary of steroid hormones on game day

Author	N=	Competition	Significant Hormone Change
(Elloumi et al., 2003)	20	Tunisian Rugby Union	*Testosterone:* pre – post (-16%), Increase 8am Mon, Wed, Sat *Cortisol*: pre – post (+148%), Suppressed Mon – Thur
(Elloumi et al., 2008)	20	Tunisian Rugby Union	*Testosterone*: (-16%) from pre – post game 1 and (-28%) at 2 hours post game, (+7%) on the morning of game 2, (+22%) pre – post game *Cortisol:* Pre game cortisol (+24% and 49%), pre – post (+148% and +216%).
(McLellan et al., 2010)	17	NRL, Rugby League	*Testosterone:* (-47%) from 24h pre – 30mins pre, (-34%) post game, returning to baseline at 24h post. *Cortisol:* (68%) pre – post.
(Passelergue et al., 1995)	15	National & International Wrestlers	*Testosterone:* increased post competition and remained elevated during the recovery period *Cortisol:* increased two and half fold from pre to post game. Returned to baseline figures within 1.5h
(West et al., 2013a)	14	Professional Rugby Union	*Testosterone:* (-26%±-34) at 12h post game, (-15% ± -34%) at 30h post game & (–8% ± -15%) at 60 h post game. *Cortisol:* (56% ± 49%) 12 h post game, (59% ± 64%) at 30 h post returning to baseline at 60h post game.

2.3.2 Steroid Hormones as stress biomarkers

It has been suggested that acute and transient changes in testosterone levels could facilitate the learning of neural pathways important to future behaviours and performance (Edwards, 2006). This information could be used to predict emotional state and competitive readiness for forthcoming events. Potentially, exposure to psychological stress may influence the neuroendocrine signalling of future stress and/or behaviour responses, possibly as an adaptive response to establish and maintain dominance (Mehta and Josephs, 2011).

Recent works have reported associations between higher testosterone responses to a mid-week stressor with winning in rugby league (Crewther et al., 2013) and better game-ranked outcomes in rugby union (Crewther and Cook, 2012), but there was no correlation with distance covered in professional rugby league (McLellan et al., 2010). Crewther et al. (2013) found that following mid-week gym workouts that preceded victories (n=3) in elite rugby league there was a significant increase in pooled and relative change in testosterone levels. However, there were no testosterone increases prior to losses (n=2). Thus, a mid-week testosterone response to a workout may provide some indication of readiness to perform in elite rugby league. However, no game-day hormone data were collected. In related work, Crewther and Cook (2012) repeated 2 post-game feedback sessions, 2 with positive feedback and 2 with negative feedback. Following positive feedback, testosterone increased as did subsequent game performance (days later) and these results were superior to the negative feedback treatment. Whether or not these increases caused the match improvement, and simply reflect other mid-week factors relating to better performance, is still unclear.

Winning in sport could elicit higher testosterone levels and a rise in status and mood state, which in turn could reinforce assertive or aggressive behaviours to aid performance in future competitions (Mazur, 1985, Booth et al., 1989). However, the hormonal responses to competition is not a direct consequence of winning or losing, but rather mediated by complex psychological processors (Salvador, 2005). Indeed, hormonal associations with any behaviour likely to

influence individual or team performance might depend upon previous competitions, the opposition played and the venue, corresponding game plans, team dynamics and culture (Gaviglio et al., 2013).

2.4 Summary

Previous literature has shown rugby players to require highly developed speed, agility, muscular strength and power, and maximal aerobic power (Gabbett, 2005). These factors are enhanced by the endocrine system, especially the steroid hormones, which are thought to play a key role in mediating adaptive physiology with training (Crewther et al., 2011). Training evokes both acute and chronic changes in steroid levels, which help to signal neuromuscular changes in order to improve performance, now suggested to be through increased motivation and positive behaviours (Cook and Beaven, 2013). To the author's knowledge, the effect of rugby league on the circadian rhythm of steroid hormone levels has never been previously examined, and henceforth their role in mediating performance is still not fully understood. Therefore, one of the aims of this study was to examine the effect of professional rugby league games on salivary hormone levels on a rest day and game day. Hormone levels exhibit a degree of individual variation and thus, it was important that testing was done via separate assessments for each player (Papacosta and Nassis, 2011).

Chapter 3.0

General Methods

This chapter will provide an overview of the techniques and procedures used during this study.

3.1 Participants

Eighteen male participants (mean age 24 ± 4 years, body mass 96 ± 10 kg, height 183 ± 6 cm) were recruited. All participants were professional rugby league players from the same team and competing in the Stobart Super League. Each player was considered healthy and injury free at the time of this study, with no medical problems that would influence the study outcomes. Participants provided written and informed consent after having the protocols and procedures of the study fully explained in lay terms, which was approved by the ethics committee of Liverpool John Moores University (12/SPS/027).

3.2 Experimental Design

Prior to their participation in this study, the subjects completed a substantial familiarization process to ensure consistent saliva sample collection. The process was explained by the lead investigator and shown to subjects before testing commenced. Six samples were collected per fixture, three on a rest day 2-3 days prior to the game and three on game day. Samples were provided at three different time points: in the morning (09:30), 1 hour before the scheduled kick off (14.00) and 30 minutes post final whistle (17.30). This was repeated across four fixtures to provide four sets of data, as detailed in figure 3.2. Occasion 1 took place on the rest day nearest to game day, so data would not be affected by training. Sampling was carried out at the same time of day, on both occasions (rest day and game day), to minimize the effects of the circadian rhythm (see figure 3.1).

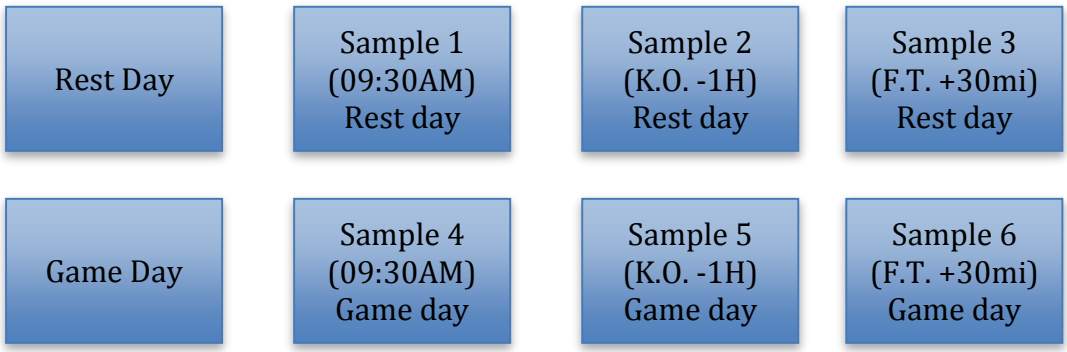

Figure 3.1 Schematic of experimental design showing sampling time points.

3.3 Training Schedule

Data were collected over a 4-month period at the start of the 2012-13 season. An overall training schedule can be seen in figure 3.2. Full training days ran from 10.00 till 13:00 and consisted of weights sessions, skills sessions, video sessions, field rugby sessions and cold-water immersion (CWI) recovery sessions. Gym workouts were performed 2 - 4 days after fixtures so to allow for full recovery of hormonal and neuromuscular systems (McLellan et al., 2010) and 48 hours prior to any further fixture. Before any training started players completed simple power tests (counter-movement jumps), physiotherapy screening and well-being questionnaires (therugbysquad.com) to clear the players of injuries and to allow staff the time to make any alterations to training type and load deemed suitable. Gym programs consisted of individualized exercise selections and loading protocols. Weekly adjustments to the schedule were also made depending on the fixture list. Due to the hectic fixture schedule at the time of testing, low-volume high-intensity sessions were often employed to allow for maximum recovery. To aid recovery, ice bath sessions (lasting 4 minutes) were carried out using a Cryospa ice bath. Video sessions were carried out post game to analyse performance and during game preparation days (team run on figure 3.2) to analyse the opposition and tactics. The type of feedback given was variable depending on the coaches' aims, but not sufficient to influence the study outcomes (Cook and Crewther, 2012b).

23/03	24/03	25/03	26/03	27/03	28/03	29/03
Game Vs. Wigan (A)	Recovery Day Team Swim 20 min Stretch 20 min	Gym 60 min Field 90 min Video 30min CWI 4 min	Rest and Sampling Day Samples (1, 2, 3)	Field 90 min CWI 4 min	Captains Run 45 min Video 30 min CWI 4min	Game Vs. Warrington (H) 15:00 W 38-22 (Samples 4, 5, 6)

Figure 3.2 Schedule of Week 1 of Testing

30/03	31/04	01/04	02/04	03/04	04/04	05/04	06/04
Team Swim 20 min Stretch 20 min	Team Run 45 min Video 60 min CWI 4 min	Game Vs. Hudd (A) L 62-6	Rest Day	Video Edits 60 min Rugby 60 min CWI 4 min	Rest And Sample Day (Sample 1, 2, 3)	Team Run 45 min Video 60 min CWI 4 min	Game Vs. Salford (H) 15:00 W 58-24 (Sample 4, 5, 6)

Figure 3.3 Schedule of Week 2 Testing

08/04	09/04	10/04	11/04	12/04	13/04
Recovery Day Team Swim 20 min Stretch 20 min	Video 60 min Rugby 60 min Gym 60 min CWI 4min	Rest and Samples Day (Sample 1, 2, 3,)	Rugby 90 min Gym 60 min	Video 60 min Rugby 45 min CWI 4 min	Game Vs. Castleford (A) 14:15 L 28-26 (Samples 4, 5, 6)

Figure 3.4 Schedule Week 3 of Testing

24/06	25/06	26/06	27/06	28/06	29/06	30/06
Recovery Day Team Swim 20 min Stretch 20 min	Video 60 min Rugby 60 min Gym 60 min CWI 4min	Rest Day	Video 60 min Rugby 60 min Gym 60 min CWI 4min	Rest and Samples Day (Sample 1, 2, 3,)	Video 60 min Rugby 45 min CWI 4 min	Game Vs. Leeds (H) 15:00 L 36-52 (Samples 4, 5, 6)

Figure 3.5 Schedule Week 4 of Testing

09:00	13:30	14:00	14:35	15:00	17:00
Sample 4	Meet and Game Prep	Sample 5	Warm Up	Kick Off	Sample 6

Figure 3.6 Game day schedules

3.3 Saliva Collection

On each day of testing the players were instructed to; if possible, not eat within 2 hours of submitting a sample (however if this was not always possible to leave a minimum gap of one hour) and to rinse their mouth out with water and not consume any drinks or brush their teeth within 15 minutes of sampling (Papacosta and Nassis, 2011). Following this, the players were instructed to place the oral swab (Salimetrics Oral Swab (SOS), Salimetrics, Suffolk) under their tongue for 2 minutes. The swab was placed into cryovials (Salimetrics Collection Device, Salimetrics, Suffolk) and these were frozen (in a -20°C freezer) immediately after collection. The samples remained frozen until the time of analysis. Due to the nature of the sampling rest day samples were conducted at the homes of the participants, whilst game day samples were provided at the club.

3.4 Saliva Analysis

Once all samples had been collected, they were thawed and centrifuged at 1500RCF for 15 minutes in a temperature-controlled (4°C) centrifuge (Eppendorf Centrifuge 5810R, Hamburg, Germany). Samples were analysed in duplicate for testosterone and cortisol concentrations using commercial kits (Salimetrics Cortisol Kit, Salimetrics, Suffolk), according to the manufacturers guidelines to reduce any matrix effects. The samples were tested using a competitive ELISA. The samples, standards and controls are all introduced to a microtiter plate coated in monoclonal antibodies where they then compete with cortisol/ testosterone linked HRP for antibody binding sites. 200µl of conjugate solution is then added to the wells, and the plate is then shaken for

3 minutes before being incubated at 18-23°C for 57 minutes. The plate is then washed 4 times with wash buffer to remove unbound components. The bound conjugate reacts with 200µl of TMB turning from blue to yellow after being shaken for 5 minutes and left to incubate in the dark for 25 minutes. Sulphuric acid stop solution is then added to stop the reaction turning the plate yellow. The optical density on the plate reader is then set to 450nm-620nm to take the reading, providing an inversely proportional concentration. A standard curve then converts the optical density to concentration. The Salimetrics kit inserts reported strong correlations between serum and salivary testosterone ($r^2 = 0.96$, $P<0.001$), and between salivary and serum cortisol measurements ($r^2 = 0.91$, $P = 0.0001$). The testosterone assays had a range of 6.1 – 600 pg/ml and cortisol assays had a range of 0.012 – 3.0 µg/dl.

3.5 Statistical Analysis

All data were analysed using the statistical package for Social Sciences (Version 21.0 for Windows, SPSS Inc, Chicago, IL). The hormonal results were examined using the Generalized Estimating Equation, which allows for the testing of repeated measurements with missing data points by replacing them with the generalized average of the population data. Post Hoc analysis was carried out using pairwise comparisons. Statistical significance was set at an alpha level of $P \leq 0.05$. Sample sizes were calculated using powerandsamplesize.com. Using an α value of 5% and a β value of 0.8, based upon a meaningful effect size of 50 pg.dl with a SD of 40pg.dl (Crewther et al., 2013) n=8 was deemed sufficient subject numbers for Testosterone. Similarly for cortisol a meaningful effect size of 0.3µG.dl with a SD of 0.2 µG.dl was used to calculate subject numbers (Crewther et al., 2013). This suggest a that n=8 sufficient to detect change.

Chapter 4.0

Evidence of a reliable salivary hormone test and diurnal rhythm in professional rugby league players

Before we are able to examine game day changes in salivary testosterone and cortisol we must first establish the reliability and rhythm in which these hormones are secreted. Therefore the following chapter will look to assess the reliability and circadian variation of salivary testosterone and cortisol in ESL rugby league players at rest.

4.1 Introduction

Salivary steroid measurements are widely used in behavioural research where participants are often reluctant and unable to give blood samples (Dabbs, 1990). The use of saliva as a tool for assessing biochemical markers (in particular testosterone and cortisol) in elite sport is developing rapidly, due to ease in sample collection and frequency, and without stress (Papacosta and Nassis, 2011). For example, this sampling technique has been used in professional rugby league to show hormonal changes across and following a game, and also training effects (McLellan et al., 2010, Crewther et al., 2013). Similarly, studies in rugby union (Crewther and Cook, 2012, Gaviglio et al., 2013, West et al., 2013a) have all looked at recovery patterns in salivary hormonal status following match play.

Testosterone and cortisol are both known to display a diurnal rhythm (in saliva and blood), with peak concentrations noted in the morning and reduced concentrations in the evening and overnight (Hayes et al., 2010, Touitou and Haus, 2000). Previous work by Crewther et al. (2013) and McLellan et al. (2010) monitored testosterone and cortisol changes in rugby league players, but they did not collect any resting-day samples to assess diurnal variation and better assess the actual changes occurring due to exercise. A study on rugby union players, by Elloumi et al. (2008), has shown a clear diurnal rhythm at 5 time points across the day (generally decreasing across the waking day) on two separate occasions; however, data outlining diurnal hormonal changes in elite rugby league players is limited. Reports of reliability during circadian testing in elite athlete populations are also scarce.

The monitoring of circadian hormone variation is clearly important when interpreting physiological data. When administering a test of physical performance, one may expect some physiological responses to exercise and this rhythm might “mask” what is the true effect of the stimulus itself (Drust et al., 2005). For instance, an early morning rise in cortisol could be viewed as stimulus-induced increase, whereas the afternoon decline could be seen as a depression in hormone secretion. In contact sports, it is highly likely that

matches themselves will produce changes in the hormone milieu (McLellan et al., 2010), as well as pre-game changes due to alterations in anxiety levels, motivation, mood and stress perception. This highlights the need to assess athletes in a rested state in order to quantify the true basal response, in order to better characterize any dynamic change occurring within sport.

The usefulness of salivary hormone measurements also depends on their reliability (Dabbs, 1990). Dabbs (1990) conducted 4 studies on 270 males and 175 females with samples collected between 30 minutes to 8 weeks apart. The study participants provided saliva after waking, mid-morning, mid-afternoon and evening. Mean reliability across 2 days was r=0.62 and across 7-8 weeks was r=0.52, indicating only moderate reliability. However, improvements in assay methods, sample preparation and collection techniques have produced more reliable measurements. Salivary hormone analysis can be further improved by using polyester swabs (cortisol recovery = 99.8/102.3, testosterone recovery = 96.3/96.8, volume recovery = 98±1ml) (Groschl and Rauh, 2006). Salivary testosterone and cortisol levels have also been validated (against blood measurements) during dynamic exercise involving Wingate sprints (Crewther et al., 2010).

In order to examine any potential change to salivary hormone levels on a match day we must first establish the reliability of our tests. Also, there is little data to describe the circadian variation in these markers in this athletic population, which may differ from non-athletes due to nature of the training environment and associated stressors. Therefore, the aims of this study are to:

1. Establish the reliability of salivary testosterone and cortisol concentration testing methods.
2. Profile the diurnal rhythms of salivary testosterone and cortisol concentrations across a resting day

4.2 Methods

4.2.1 Participants

Eighteen professional rugby league players from one super league club were recruited for this study. Subject characteristics can be seen in chapter 3.1.

4.2.2 Experimental Design

Prior to taking part in this study all participants completed a substantial familiarization process, as detailed in chapter 3.2. During this initial study, salivary hormone data was collected at 3 time points (09.30, 14.00 and 17.30) to establish a normal circadian rhythm on the nearest rest day to competition. Data was collected over 4 fixtures to assess results were reliable and repeatable. Training and fixture schedule can be seen in figures 3.2, 3.3, 3.4 and 3.5.

4.2.3 Salivary collection and Analysis

Saliva was collected via the use of oral swabs (Salimetrics Oral Swab (SOS), Salimetrics, Suffolk) and players were instructed not to eat in the 2 hours prior to testing or drink or brush their teeth in the 15 minutes prior to sample collection. Samples were then stored at (-30°C) up until the time of analysis. Analysis was carried out in duplicate using commercially available salivary hormone testing kits (Salimetrics Cortisol/ Testosterone Kits, Salimetrics, Suffolk) and administered using the manufactures guidelines detailed in chapter 3.4. Kit reliability is detailed in Chapter 3.4. The samples for each athlete were tested in the same assay to reduce inter-assay variance.

All rest day data across all fixtures was initially pooled in order to examine the over all trend (figure 4.1 and 4.3) before being pooled as a match day squad to provide analysis between weeks (figure 4.2 and 4.4). Missing data points were included in the statistical analysis as detailed in 4.2.4.

4.2.4 Statistical Analysis

All data were analysed using the statistical package for Social Sciences (Version 21.0 for Windows, SPSS Inc, Chicago, IL). The hormonal results were examined using the Generalized Estimating Equation, which allows for the testing of repeated measurements with missing data points by replacing them with the generalized average of the population data. Post Hoc analysis was carried out using pairwise comparisons. Statistical significance was set at an alpha level of $P \leq 0.05$

4.3 Results

4.3.1 Reliability

Testosterone had an intra-assay CV's of 7.7% (high control) and 8.8% (low control). Cortisol had an intra-assay CV's of 5.6% (high control) and 5.1% (low control). The repeated testing of 10 samples (3 repeats each) by the same technician revealed excellent (CV's = 1.1-3.3%) reliability. There was an inter-assay cortisol CV of 7% and inter-assay testosterone CV of 5.1%.

4.3.2 Cortisol

There was a significant decrease in main effect for time ($P \leq 0.0005$). Post hoc analysis reveals reductions between 09.30 – 14.00 ($P \leq 0.0005$) and 09.30 – 17.30 ($P \leq 0.0005$). There was also a significant reduction at 14.00 – 17.30 ($P < 0.05$).

Significant week-to-week variation in salivary cortisol was also identified ($P \leq 0.0005$). Post hoc analysis revealed differences between weeks 1 – 2,3,4 and weeks 2 - 3 ($P \leq 0.0005$) and also between weeks 2 and 4 ($P < 0.05$). However, there were no differences between week 3 and 4 ($P > 0.05$). There was a significant interaction between time and week ($P \leq 0.0005$). Post hoc analysis can be seen below in table 4.1.

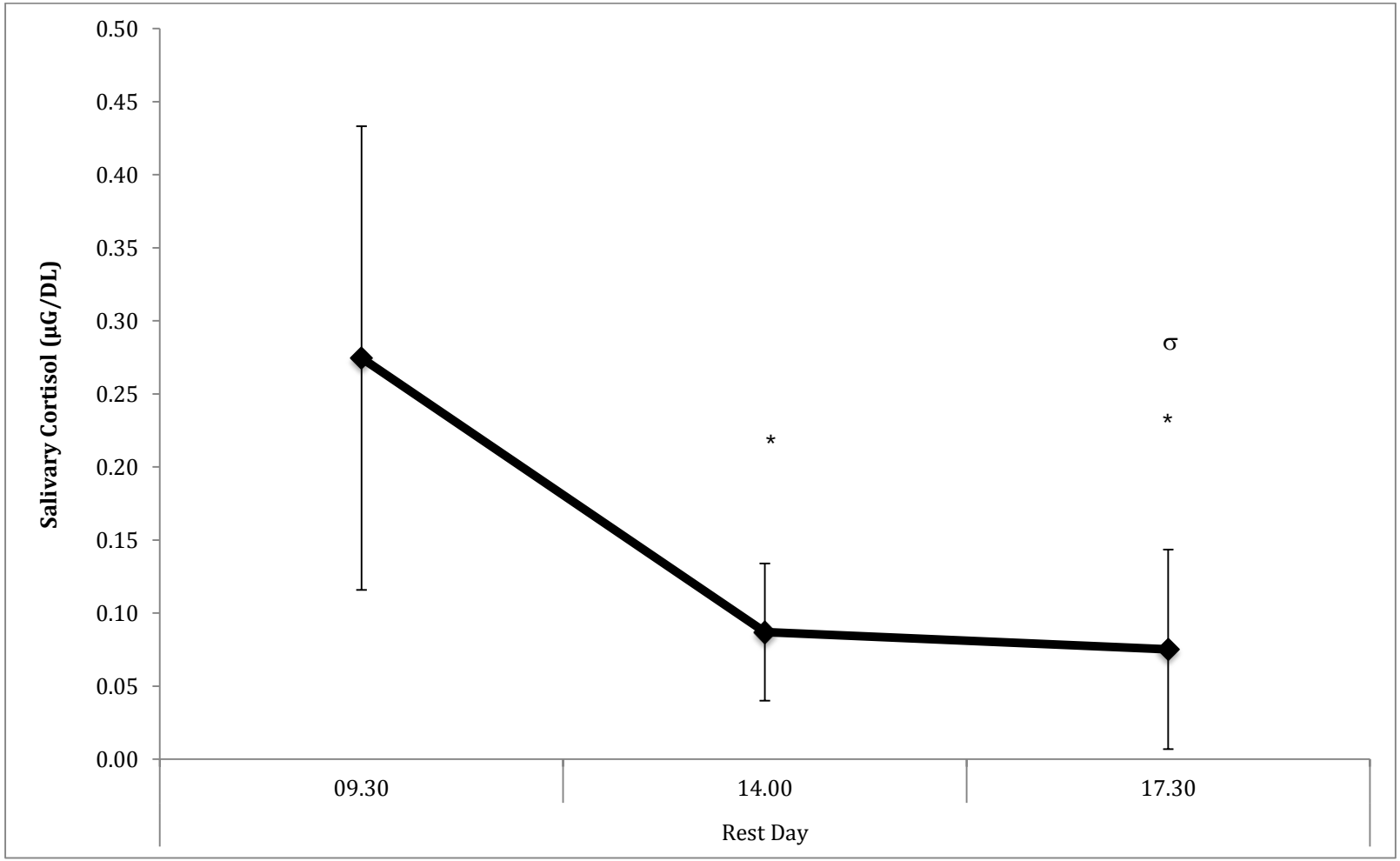

Figure 4.1 Mean (±SD) team pooled cortisol results for time of day across 4 separate rest days. * Illustrates a significant difference from 09.30, σ illustrates a significant difference from 14.00.

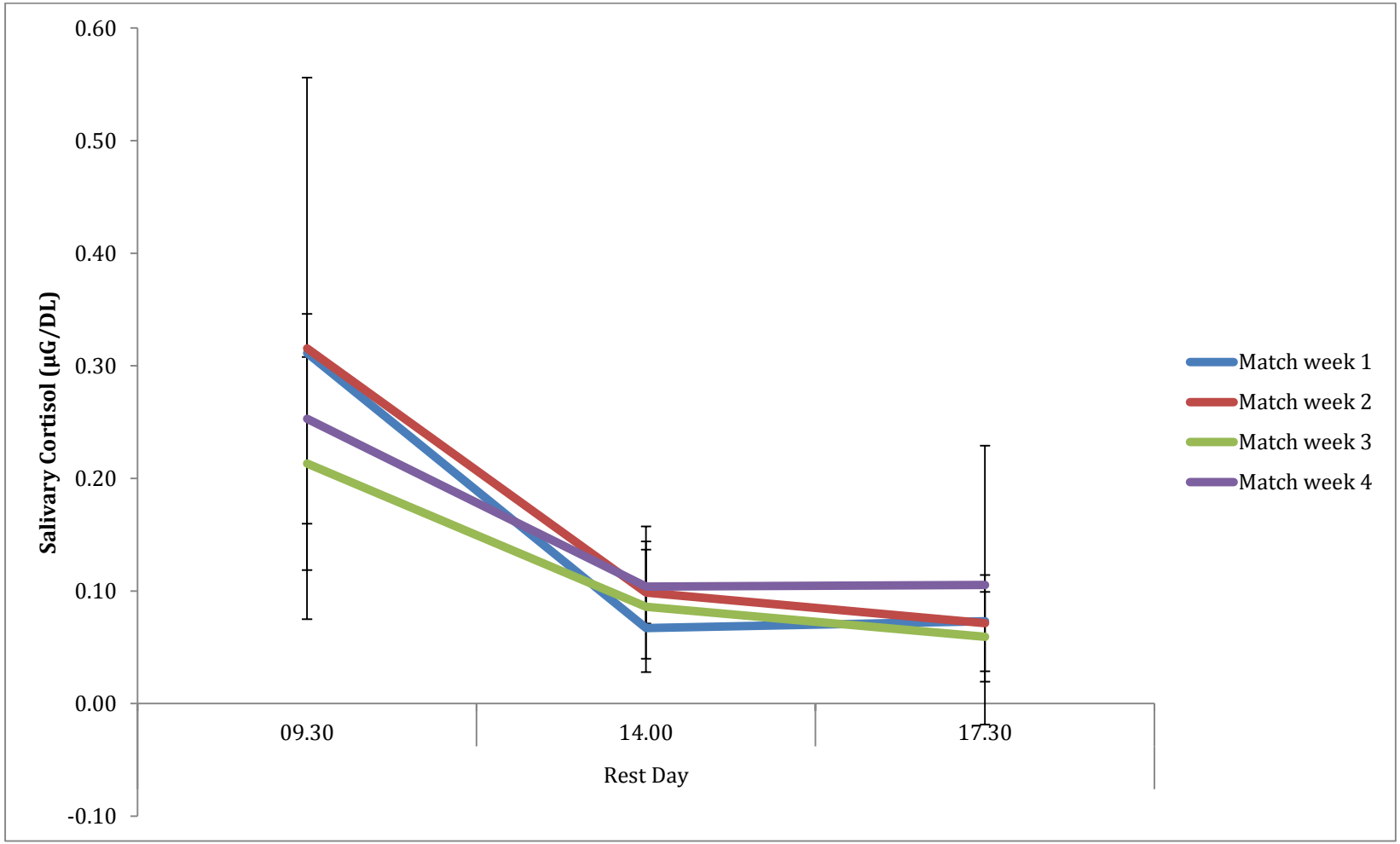

Figure 4.2 Mean (±SD) pooled cortisol results of the game day squads during 4 separate fixtures, showing a similar decreasing trend across the day.

4.3.3 Testosterone

For testosterone, there was a significant decrease across the day ($P \leq 0.0005$). Post hoc analysis revealed a significant reduction in salivary testosterone between 09.30 – 14.00 ($P \leq 0.0005$) and 09.30 – 17.30 ($P \leq 0.0005$). However, there was no significant reduction at 14.00 – 17.30 ($P > 0.05$), as shown in figure 4.3.

There was a significant week-to-week variation in salivary testosterone ($P < 0.05$). Post hoc analysis reveals a significant difference between weeks 1 – 2 ($P < 0.05$). However, there were no significant differences between weeks 1 – 3 ($P > 0.05$), weeks 1 – 4 ($P > 0.05$), weeks 2 – 3 ($P > 0.05$), weeks 2 – 4 ($P > 0.05$) and weeks 3 – 4 ($P > 0.05$).

There was also a significant main effect between time and week ($P \leq 0.0005$). These are shown in table 4.2

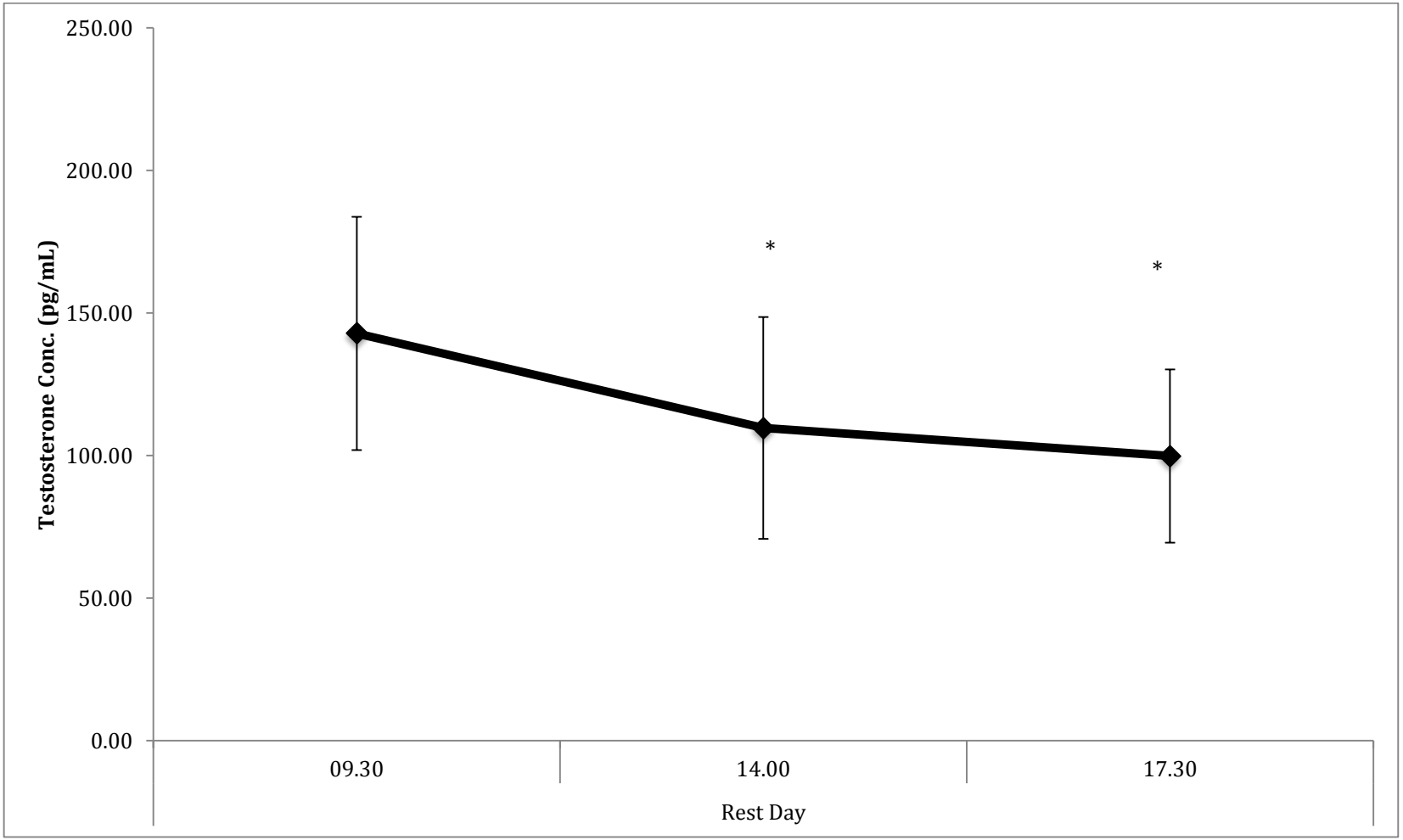

Figure 4.3 Mean (±SD) team pooled testosterone results for time of day across 4 separate rest days. * Illustrates a significant difference from 09.30.

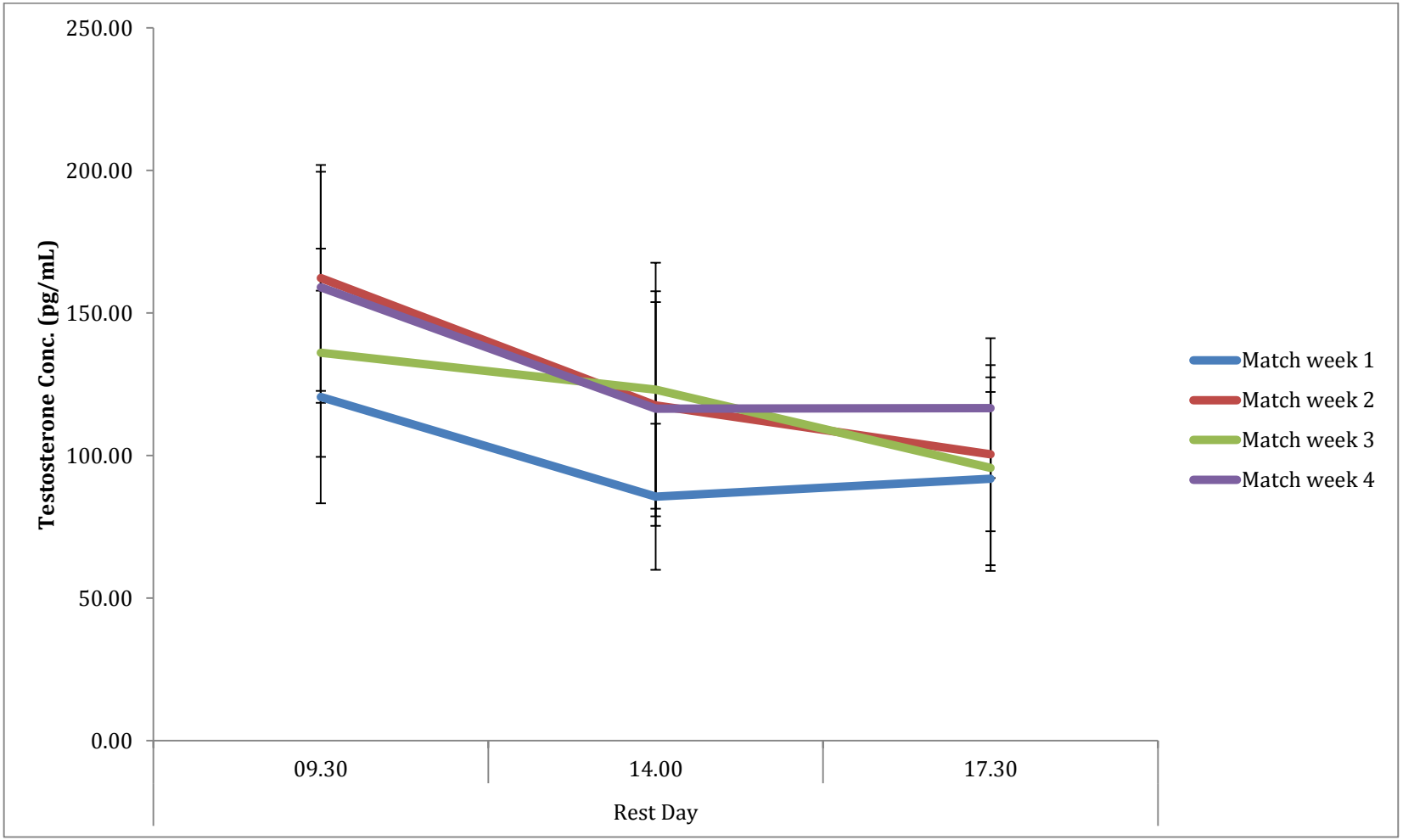

Figure 4.4 Mean (±SD) pooled testosterone results of the game day squads during 4 separate fixtures, showing a similar decreasing trend across the day

4.4 Discussion

The aims of the present study were to; (1) assess the reliability of the salivary hormone measurements in elite athletes within the rugby league environment and, (2) establish the circadian profile of testosterone and cortisol in this athletic group. A low CV (<9% inter-assay, <4% on sample repeats) in both the testosterone and cortisol measures allow us to confidently monitor the circadian rhythm, which was characterised by a typical morning to afternoon decline in both hormones.

The current findings confirm a hormonal diurnal rhythm (decreasing across the waking day), present in both the testosterone and cortisol samples of professional rugby league players. Cortisol demonstrated a stronger rhythm, with levels significantly decreasing at every time point across the day. Both testosterone and cortisol exhibited a significant difference between 09.30 AM and both pre and post-game samples. However, testosterone did not differ significantly between 14.00 and 17.30. No previous studies have reported the presence of a diurnal rhythm in professional rugby league players. The findings of the present study support those of Elloumi et al. (2008) in rugby union, where cortisol was found to significantly decrease across the day. In the same study, no diurnal rhythm was present in salivary testosterone, similar to the current findings between 14.00 and 17.30. The slight decrease in testosterone was supported by data taken from the general population by Dabbs (1990), showing a decline across the waking day on multiple occasions.

Previous literature has shown that highest levels of naturally occurring testosterone and cortisol occur shortly after waking (Cortisol 7.00am, testosterone 8.00am) subject to individual sleep and wake cycles (Hayes et al., 2010). This cortisol response is believed to be part of the body's early morning reaction to prepare for the demands of the day increasing metabolism, stimulating gluconeogenesis and proteolytic activities (Touitou and Haus, 2000). The rise in testosterone is then seen as a response from the body to counteract the stimulatory influence of cortisol on skeletal muscle degradation (Hayes et al., 2010). As shown in the present study, cortisol decreased in parallel with

testosterone later in the day, in line with Dabbs (1990), who reported a 50% fall in testosterone levels from morning to evening.

The lack of a change in testosterone levels (between 14.00 and 17.30) could be due to the lower activation of the HPG-axis across the day more so in the afternoon, relative to the HPA-axis, and the short amount of time between the samples collected. Alternatively, the lack of a significant testosterone response may be due to the higher trained status and greater activity levels of the subject population, such that higher afternoon testosterone levels might represent an adaptive outcome to aid recovery in the days after training and competition. Research by Dabbs (1990) offered a further explanation stating the largest decrease in testosterone occurs during the morning, therefore there may be a less obvious change in the afternoon. We recognize that due to the limited number of sampling time points collected across the day, the temporal patterns of hormone secretion in the study population and the elite sporting environment are somewhat cursory, although they do fit general trends reported in literature.

Some weekly differences in testosterone and cortisol levels were identified, but as seen in figures 4.2 and 4.4, the overall trend remained the same. Overall, resting testosterone levels were somewhat consistent across weeks, whereas cortisol levels exhibited much greater variation week-by-week. It is important to first take into account changes in playing personnel, due to tactics or injuries, which can influence the observed profiles. The large variety of stressors athletes deal with both professionally and away from public knowledge, and how they cope individually, can add to this variability (Hayes et al., 2010). Recovery from previous fixtures should also be considered. McLellan et al. (2010) showed that following professional rugby league in the National Rugby League hormonal homeostasis returned after 48 hours. West et al. (2013a) also reported similar findings in professional rugby union players. They have reported that, at 60 hours post game, salivary testosterone and cortisol had recovered to levels comparable to baseline. However, in the present study, the samples were taken as a baseline marker for the upcoming fixture, not as a measure of recovery. Perhaps in future samples taken in the off-season may confirm these findings.

This study confirmed that the salivary testosterone and cortisol measures provide reliable assessment tools, and their application in professional rugby league players identified a circadian rhythm (decreasing from AM to PM) on a resting day. Some weekly hormonal variation was noted, and possibly due to the weekly stimuli of fixtures, home stress and/or training variation that week. These data highlight the need to collect multiple samples (across the day, across multiple weeks) to better understand hormone dynamics at rest, which is essential to allow interpretation of changes in testosterone and cortisol during a training week. Future research should now look at the effects of elite rugby league match play on salivary hormone concentrations.

Chapter 5.0

Changes in hormonal rhythms during elite rugby league match play

Having established the reliability and circadian rhythm of testosterone and cortisol, the present study assessed how these rhythms on game day. It was found that both salivary testosterone and cortisol were significantly increased at every time point, except game day cortisol AM sample. These data suggest a potential route for game day interventions to maximise hormonal profile.

5.1 Introduction

Having established the reliability of the hormonal measurements and the circadian patterns of testosterone and cortisol secretion within the study population (chapter 4), we are now able to examine and identify any changes that may occur on a match day. The effects of the circadian rhythm on performance in sport are well documented (Drust et al., 2005), with a general peak around mid-afternoon. Salivary hormones however peak in the morning shortly after waking, as shown in study 4 and previous literature (Hayes et al., 2010). This therefore leads to the question; can the demands of match play modify the circadian profiles of these steroid hormones?

The role of hormones in moderating athlete performance and the manner in which they are secreted has been debated in the literature, with initial studies suggesting testosterone and cortisol play a key role in skeletal muscle protein synthesis (Kraemer et al., 1990). More recent research has shown no role for endogenously increased testosterone in muscular protein synthesis, instead suggesting protein availability to be the key factor in anabolic hypertrophy (West et al., 2013b, West et al., 2009, West and Phillips, 2012, West et al., 2010). Recent literature has suggested that increased testosterone concentrations may actually enhance performance through effects on an athlete behaviour and motivation to perform (Cook and Beaven, 2013). Even with this new direction of the literature there is little known about how an athlete's hormone levels change from at rest to game day. Conversely, cortisol is known to play a key role in controlling the bodies metabolism and response when put under stress (Hellhammer et al., 2009).

To the authors knowledge there have been no studies investigating the effects of rugby league match play on the cortisol and testosterone circadian rhythms. McLellan et al. (2010) looked at the effects of match play on steroid hormone concentrations and reported that testosterone concentration was significantly depressed from 30 minutes pre-game till 24 hours post when it returned to normal. Cortisol however was significantly increased from 30minutes pre game until 48hours post in comparison to 24 hours pre-game. This increase in cortisol

could result from a pre-game anticipatory response, similar to other findings in elite collision sport (Elloumi et al., 2008, Elloumi et al., 2003, West et al., 2013a). This was only conducted over one fixture, set of conditions and game preparations all of which may influence the results. This study was also performed in the National Rugby League competition in the southern hemisphere, and no corresponding research has been conducted in Super League, in the elite northern competition.

Other studies in professional rugby league have shown that mid-week changes in testosterone can act as a predictor of outcomes in professional rugby league (Crewther et al., 2013). In this work, an elevated salivary testosterone response to a training session was seen before matches won, whereas no hormonal changes occurred prior to losses. These findings highlight the potential use of dynamic hormonal measurements as a training tool, although the analysis of circadian rhythms would have been useful to determine the real magnitude of these changes and how they may differ on a weekly basis.

Studies in rugby union also show evidence of hormonal displacement around games and during recovery. West et al. (2013a) found that testosterone was depressed at both 12 and 36 hours post game when compared to pre-game before returning to homeostasis at 60 hours post-game. Elloumi et al. (2008) found an effect for the importance of fixture on hormonal homeostasis. In a typical match they found no differences between rest and pre game testosterone, whereas testosterone was elevated in a match that determined qualification for the African cup. Also, the post-game changes (typical match) were similar to the findings of McLellan et al. (2010) with testosterone decreased immediately post game and 2 hours post. However, the qualification match resulted in higher levels immediately after and 2 hours post game.

Cortisol levels were found by West et al. (2013a) to be elevated at 12 and 36 hours post game before returning to baseline at 60 hours post-game. Elloumi et al. (2008) also found cortisol levels to be elevated pre-game, post-game and 2 hours post similar to the findings of McLellan et al. (2010) in rugby league. These previous findings (on rugby union and rugby league players) suggest an

effect of match play in contact sport at the elite level, but comparisons with resting data taken across the day are limited. Therefore, the aim of this study was to look at possible disruptions in the circadian hormonal rhythm on game day in elite rugby league, relative to resting data. Based on previous research we are able to hypothesize that:

i. There is significant effect on salivary testosterone between a rest day and game day.
ii. There will be a significant difference in salivary cortisol levels between a rest day and game day.

5.1 Methods

5.2.1 Participants

Eighteen professional rugby league players from one super league club were recruited for this study. Subject characteristics can be seen in chapter 3.1.

5.2.2 Experimental Design

Salivary hormone data was collected at 3 time points (09.30, 14.00 and 17.30) to establish a normal circadian rhythm on the nearest rest day to competition. A second set of samples was then taken on game day at the same time points. Data was collected over 4 fixtures to identify any trends. Training and fixture schedule can be seen in figures 3.2, 3.3, 3.4 and 3.5.

5.2.3 Salivary collection and Analysis

A total of 252 samples were collected over the 4 professional rugby league fixtures in the super league competition. Saliva was collected via the use of oral swabs (Salimetrics Oral Swab (SOS), Salimetrics, Suffolk). Participants were instructed not to eat in the 2 hours prior to testing or drink or brush their teeth in the 15 minutes prior to sample collection. Samples were then stored at (-30°C) up until the time of analysis. Analysis was carried out in duplicate using commercially available salivary hormone testing kits (Salimetrics Cortisol/ Testosterone Kits, Salimetrics, Suffolk) and administered using the manufactures guidelines detailed in chapter 3.4. Kit reliability is detailed in Chapter 3.4.

All rest day data across all fixtures was initially pooled in order to examine the overall trend (Mean (±SD)) (figure 5.1 and 5.3) before being pooled as a match day squad to provide analysis between weeks (figure 5.2 and 5.4). Missing data points were included in the statistical analysis as detailed in 5.2.4.

5.2.4 Statistical Analysis

Data was analyzed using the statistical package for Social Sciences (Version 21.0 for Windows, SPSS Inc, Chicago, IL). Results were then analyzed via general linear modelling, General Estimated Equation. Significances were reported to ($P \leq 0.05$). Post Hoc analysis was carried out using the pairwise comparison.

5.3 Results

Cortisol

There was a significant difference between days ($P \leq 0.0005$). There was also a significant main effect for time ($P \leq 0.0005$) and day*time ($P \leq 0.0005$).

Rest Day AM (09.30)

Post Hoc analysis shows a significant difference between the rest day AM samples and pre, post and game day AM and post samples ($P \leq 0.0005$). However there was no significant difference between rest day AM and game day pre ($P > 0.05$) samples.

Rest Day Pre (14.00)

There were significant differences between rest day pre samples and, game day AM, pre and post ($P \leq 0.0005$) samples. However there was no significant difference with rest day post ($P > 0.05$) samples.

Rest Day Post (17.30)

There are significant differences between rest day post and game day AM, pre and post ($P \leq 0.0005$) samples.

Game Day AM (09.30)

There was significant difference between game day AM samples and game day post samples ($P \leq 0.0005$). There was no significant difference between game day AM and game day pre ($P > 0.05$).

Game Day Pre (14.00)

There was a significant difference between game day pre and game day post ($P \leq 0.0005$).

.

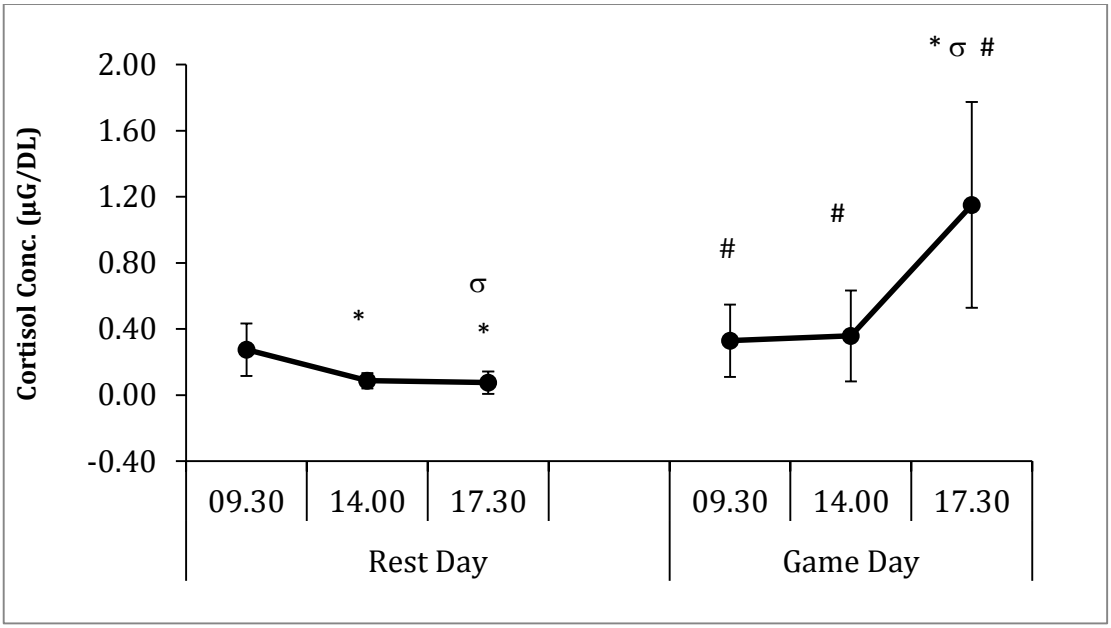

Figure 5.1 Pooled Cortisol Concentrations. * illustrates a significant difference from 09.30am same day, σ illustrates a significant difference from 14.30pm the same day and # illustrates a difference between the same time on the rest day

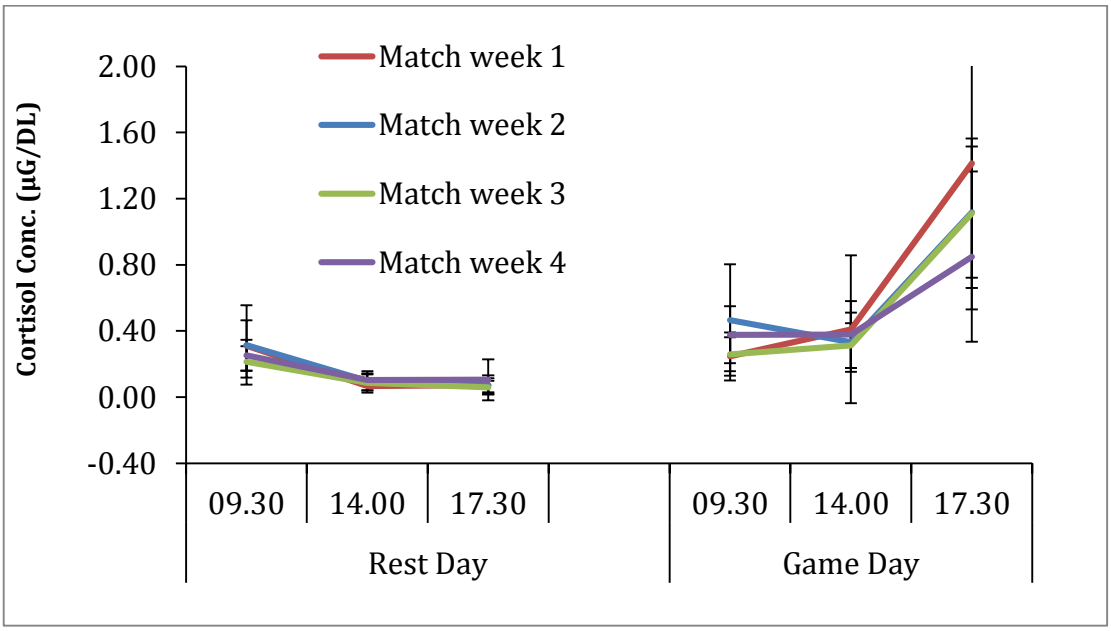

Figure 5.2 Mean (±SD) pooled cortisol concentrations of the match day squads during 4 separate fixtures

Testosterone

There was a significant difference between rest day and game days ($P \leq 0.0005$). There was also a significant difference for time of day ($P \leq 0.0005$) and day*time ($P \leq 0.0005$).

Rest Day AM (09.30)

Pairwise analysis shows significant differences between rest day AM samples and rest day pre, rest day post, game day AM ($P < 0.05$) and game day post samples ($P \leq 0.0005$). However there were no significant differences between rest day AM and game day pre ($P > 0.05$)

Rest Day Pre (14.00)

There were significant differences between rest day pre samples and, game day AM, game day pre ($P < 0.05$) and game day post ($P \leq 0.0005$). However there was no significant difference with rest day post ($P > 0.05$).

Rest Day Post (17.30)

There are significant differences between rest day post and game day AM, pre and post ($P \leq 0.0005$).

Game Day AM (09.30)

There are significant differences between game day AM samples and game day pre samples ($P \leq 0.0005$). There is no significant difference between game day AM and game day post ($P > 0.05$).

Game Day Pre (14.00)

There was a significant difference between game day pre and game day post ($P \leq 0.0005$).

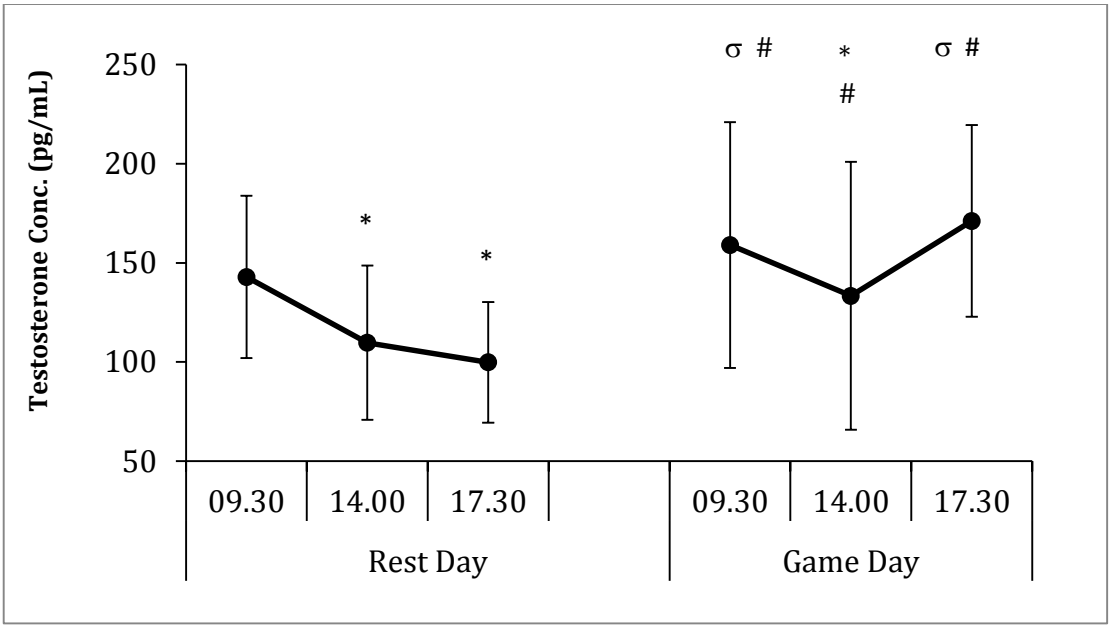

Figure 5.3 Pooled Testosterone Concentrations. * illustrates a significant difference from 09.30am same day, σ illustrates a significant difference from 14.30pm the same day and # illustrates a difference between the same time on the rest day

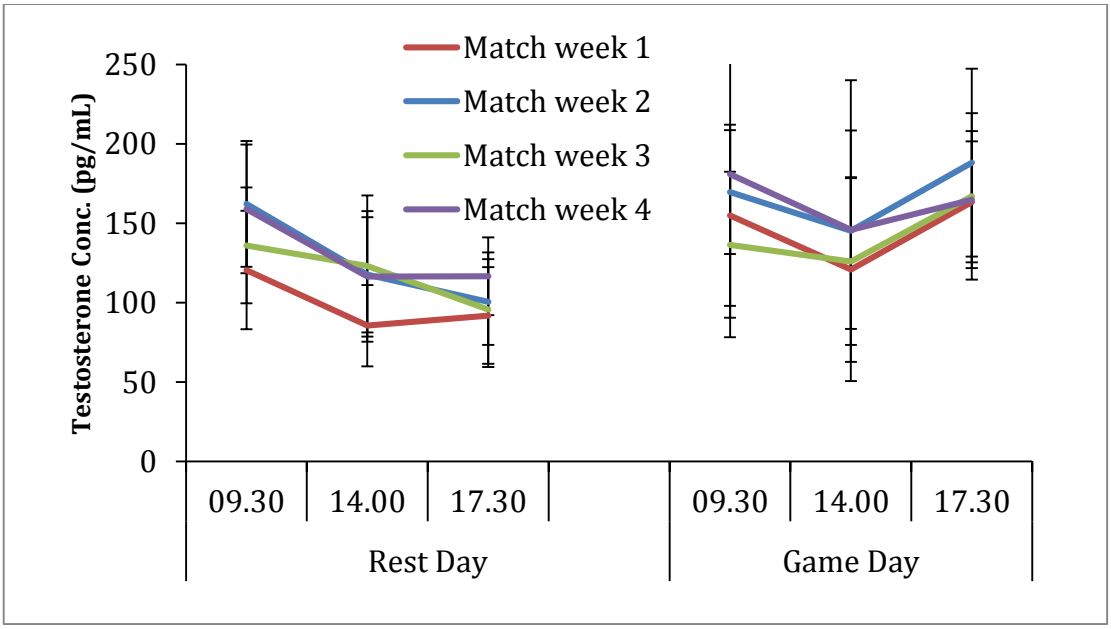

Figure 5.4 Mean (±SD) pooled testosterone concentration of match day squad during 4 separate fixtures

5.4 Discussion

The aim of the present study was to establish the game-day changes in salivary testosterone and cortisol concentrations to determine their potential implications in match-day performance, as well as investigating game-to-game variations in these hormones. This study confirmed and extended findings by McLellan et al. (2010) and Elloumi et al. (2008) in that testosterone and cortisol show a marked difference on game day compared to at rest in professional rugby league and union, respectively. We have also shown for the first time significant game-to-game variation of testosterone and cortisol. Given that testosterone and cortisol have been implicated in improving performance through motivation (Cook and Beaven, 2013), these data could suggest a potential route for game-day interventions to maximise hormonal profiles. Future research should address the use of practical strategies to manipulate hormone secretion to modify athlete performance, such as training interventions (Crewther et al., 2013), video sessions (Cook and Crewther, 2012a) and coach feedback (Cook and Crewther, 2012b).

Salivary testosterone increased on game day compared to at rest by 11% in the AM samples, 22% pre-game and 71% post-game. Such increases have the potential to enhance athlete performance, at least during training *(Cook and Beaven, 2013).* The AM increase is consistent with findings by Elloumi et al. (2008) in professional rugby union. To the authors' knowledge, no studies have compared the morning samples (rest day and match-day) in professional rugby league. Contrary to previous research in professional rugby league, the present study found pre-game salivary testosterone levels to be significantly increased compared to at rest, with McLellan et al. (2010) reporting a -47% decrease and Elloumi et al. (2008) reporting no change in professional rugby union. These increases prior to kick off (AM, PRE) on a game day, relative to rest day, have previously been linked with an athlete's motivation to perform via an anticipatory response to deal with upcoming physical competitive stresses (Papacosta et al., 2013, Salvador, 2005). These increases may also reflect changes in motivational status prior to a competitive challenge (Cook and Crewther, 2012b). Without this challenge (at rest), salivary testosterone concentrations decreased

between the AM and pre-game samples. Opposing this, Elloumi et al. (2008) found no significant difference between the game-day AM (08.00) concentrations and pre-game (11.00) concentrations. This however may be due to the shorter time frame between samples.

Post-game testosterone concentrations were also elevated compared to at rest. These findings are novel in rugby league and confirmed by Elloumi et al. (2008), where salivary testosterone levels increased by 22%. This post fixture increase in testosterone suggests activation of the HPG axis, resulting in the release of testosterone coming as a response to the demands of combative physical competition (Mehta et al., 2008, Salvador et al., 2003). Salivary testosterone levels also increase from pre to post game by 29%. This shows an effect for professional rugby league match play on salivary testosterone levels, and a disruption in normal circadian variation. These results confirm and extend the findings by McLellan et al. (2010) who found a 14% increase in salivary testosterone levels from pre to post game. However, McLellan et al. (2010) found both pre and post-game testosterone to be lower than 24 hours pre-game. The decreased post game sample may be partially explained by the effects of the circadian variation relative to the 24 hour pre-game time sample, although salivary testosterone did not return to pregame levels until 24 hours post game. Similarly West et al. (2013a) found salivary testosterone levels to be decreased up until 36 hours post game. The novel findings of this study, namely that salivary testosterone increased post game should therefore be researched further in order to establish any potential performance or recovery benefits in the subsequent recovery period. We decided not to relate the hormonal concentrations to the outcome given that this was the second season of the club in Super League and the match scores were not the only (or best) performance indicators. Future research should attempt to investigate the relevance of the match-day hormonal changes on key performance or recovery indicators.

This study also reports for the first time significant game-to-game variation in salivary cortisol and testosterone. Although game-day concentrations were significantly different, the overall trends remained the same (see fig. 5.3.4). Due

to the large number of uncontrollable factors associated with applied research and the current team situation, the decision was taken not to include performance variables. From previous research we are able to infer a potential effect of relative fixture importance, as every match was a competitive league game and testosterone increased similar to the Elloumi et al. (2008) results during a competitive fixture. The training session response may also play a role in explaining week-to-week variation (Crewther et al., 2013). Home and away advantage has also been shown to effect the week-to-week variations (Carre, 2009). Tactical influence on physiological demands (Gabbett, 2013) and also pre-game video and motivational strategies (Cook and Crewther, 2012a, Cook and Crewther, 2012b) could play a further role in dictating hormonal status in sport.

Salivary cortisol levels increased by 22% (non-significantly) in the morning, as well as pre-game by 300% and by 1338% post game compared to at rest. These findings both confirm and extend those of McLellan et al. (2010) who reported a significant difference (28%) between pre-game salivary cortisol levels on a game day and at rest. Our results are also consistent with Elloumi et al. (2008) who reported no significant increase in the AM cortisol samples, a pre-game increase of 49% and a post-game increase of 216% compared to at rest. Salivary cortisol peaks after waking, as part of the bodies response to prepare the days stresses (Hayes et al., 2010), and therefore may offer some explanation as to why there was no significant increase at this time point. The large pre-game increase in cortisol is a well-recognised anticipatory response, which serves to prepare the body for, and cope with, stress (Archer, 2006, McLellan et al., 2010, Schultheiss et al., 2005).

We also observed a pre to post game increase of 219% in cortisol. This finding is in agreement with McLellan et al. (2010) who reported an increase of 69%. This large increase in cortisol can be associated with several factors involved with elite rugby league match play. Rugby league is a high intensity, intermittent sport involving frequent collisions and high pressure to perform, thereby placing considerable stress on athletes and thus, the rise in cortisol levels. Lac and Berthon (2000) have shown that the cortisol response increases in line with

increases in exercise duration and intensity. Psychologically, Elloumi et al. (2008) noted a difference between salivary cortisol during a fixture of no importance and a much larger increase during a fixture of high importance. These large increases in game-day salivary cortisol levels highlight the need to determine the underlying sources of stress (e.g. physical, psychological) and to develop coping interventions in order to maximise potential performance (Crewther and Cook, 2012, Elloumi et al., 2008).

We are also able to report the novel findings of game-to-game hormonal variation in elite rugby league match play. As stated previously, there are several factors affecting salivary cortisol concentrations in professional rugby league. The first possible factor relates to team selections due to injury or tactical by coaching staff. Due to individual differences at rest, there will also be large differences when data are pooled across teams. As shown by Gabbett (2013), the tactics and standard of opposition play a key role in regulating the physiological demands of competition and the subsequent hormonal milieu. This is important when interpreting the present study results, because two top four teams and 2 bottom four teams were played.

The largest post-game increase in salivary cortisol came from match week 1 against a top 4 team considered to be a derby game and local rivalry, similar to game 2 in Elloumi et al. (2008). This again may go some way to explaining the large change in salivary cortisol, specifically due to added psychological pressures. The second largest change in salivary cortisol came following game week 3, which was played away from home, therefore suggesting a potential role for the effects of venue on performance. Despite all of these factors, the general trend remained the same with a significant pre and post-game increase compared to at rest, which indicates a significant disruption to normal circadian variation. Previous literature then goes on to show a return to pre-game levels between 24-48 hours post game (Elloumi et al., 2008, McLellan et al., 2010). Practitioners should be aware of these responses when planning sessions following games to allow for a return to hormonal homeostasis.

In summary, both salivary testosterone and cortisol levels increased from at rest to on and during elite rugby league game days, which indicates a real difference over and above normal circadian variation. Knowing how the hormonal measures are likely to change in this environment, future studies should look towards quantifying actual match performance and their correlation with these changes, as well as possible linkage with post-game performance recovery. Future research could also look at implementing practical interventions in order to maximise hormone status across a training week and/or across matches.

CHAPTER 6.0

Synthesis of Findings

The present chapter provides an analysis of the successful achievement of the aims and objectives of the present thesis. A synopsis of how the findings of the thesis link to one another and how they progress the field is provided.

6.0 Synthesis of Findings

The thesis aim was to examine and identify the circadian rhythm in salivary testosterone and cortisol levels in professional rugby league players, and to subsequently quantify the hormonal changes occurring on a rugby league match day. The novel findings of this study will be summarized in the following chapter in relation to the aims presented in chapter 1. Finally, the limitations of the thesis will then be discussed followed by practical recommendations and suggestions for future research.

6.1 Achievements of aims and objectives

OBJECTIVE 1: To confirm the reliability of salivary hormone testing.

Objective 1 was addressed in chapter 4. In order for this study to be valid it is important to establish reliability across the samples tested and in the testing kits used. If reliability was not achieved, any measured changes that may occur on game day, may not be accurate. The data presented indicates that there was a high level of reliability within the kits. The testosterone assays had intra-assay CV's of 7.7% (high control) and 8.8% (low control) with corresponding intra-assay CV's of 5.6% (high control) and 5.1% (low control) for the cortisol assays. The repeated testing of random samples (3 repeats each) by the same technician revealed excellent (CV's = 1.1-3.3%) reliability.

OBJECTIVE 2: To confirm the circadian rhythms of testosterone and cortisol in elite rugby league players on resting days.

Salivary testosterone and cortisol samples were assessed at 3 time points across the day. This demonstrated a circadian rhythm with salivary concentrations decreasing from the AM samples to the PM samples, on 4 separate occasions. The only non-significant decrease occurred in resting testosterone levels between 14.30 and 17.00. This may have been due to the short window of time across which these samples were collected. Although

there were some differences between the multiple rest days tested, for both testosterone and cortisol, the overall circadian trend remained the same.

OBJECTIVE 3: To compare resting and match-day hormones to quantify the actual changes occurring due to rugby league competition.

Objective 3 was assessed in chapter 5. The findings provide evidence for an effect of professional rugby league match play on the circadian rhythms exhibited by salivary testosterone and cortisol in professional rugby league players. The only time point not to exhibit a significant difference between game day and at rest was salivary cortisol in the morning shortly after waking. This may have been due to the already elevated cortisol during the morning samples, as part of the circadian rhythm.

In summary, all of the aims and objectives of this thesis have been met. Moreover, this novel research has extended the current knowledge regarding the effects of rugby league matches on salivary testosterone and cortisol levels, and how these patterns differ from normal biological (circadian) variation. This provides a stronger base from to advance the literature around hormone physiology in order to assess any potential effects or linkage to athlete performance and recovery.

6.2 Limitations

Whilst the present thesis has advanced the knowledge base with regards to the effects of match day on salivary hormone levels in elite rugby league players, it is not without its limitations. Many of the limitations come about due to carrying the work out in a professional sporting environment, with limited resources. The major limitations are outlined below.

The main limitation of this study was a lack of complete samples collected from players across each day, due to players either not being selected or missing out through injury. This reduced the statistical power of the study findings and possibly contributed to the many weak non-significant individual correlations.

However, this was not possible due to practical restraints of an elite sporting environment. Adding to this, only four fixtures were assessed and match data from only one super league team was collected during the 2012-13 season. It would have been useful to compare hormonal changes occurring during matches of different importance and using a variety of teams with different skill levels.

It is believed that nutritional intake plays a large role in steroid hormone level, particularly fat levels (Hayes et al., 2010). It has been shown by Goldin et al. (1994) that 40% has been linked to high testosterone levels when compared with a 20% dietary fat levels. Thus, the collection of dietary information may have been useful in the context of this study to determine what factors underlie individual differences in hormone levels and reactivity to match-day stressors.

Due to logistical constraints, the players were required to take their own samples on the rest days and store these before testing. Under these conditions, it is difficult to control for all possible confounding variables, although we mitigated these effects by prior training in both sample collection and storage, as well as providing written instructions for players.

The study was also limited by a lack of outcome variables. However in order to first establish any effect of hormones on outcomes and vice versa this study aimed to quantify the effects of game play. The outcome variables must also be carefully selected due to extraneous factors. To improve the study I would've liked to of added psychology mood questionnaire and counter movement to provide more information as to player state going into competition and help to draw conclusions as to the role of salivary hormones.

6.3 Practical implications

Despite the limitations of this study, the findings have raised a number of practical implications that should be noted by practitioners and elite athletes.

1. The collection of 2-3 samples at selected time points across the day appears to be sufficient for identifying a circadian pattern in hormone secretion. Likewise, the selected timing of pre and post samples (across a match) could be used to identify a hormonal change.
2. Having identified a significant difference between game day and rest day hormones, coaches and training staff should consider those variables influencing the match profiles whether they are linked to better or worse performance.
3. If a positive testosterone response to exercise can predict readiness to compete several days later (Crewther et al., 2013), then the large changes in post-game testosterone levels may offer similar benefits for predicting recovery or performance in subsequent matches.
4. The large change in salivary hormones post game, and the added week to week variation, should make practitioners aware of the fluctuating profiles of athletes and the need for recovery strategies to ensure hormonal restoration, as detailed in (McLellan et al., 2010, West et al., 2013a).
5. The individual hormonal difference noted between athletes also highlights the existence of different genetic profiles (even within a relatively homogenous athlete cohort) with implications for testing and recovery, as well as differences in how we each perceive, and respond to, stress.

6.4 Recommendations for future research

1. **Collect Key Performance Indicators to Correlate with the Changes in Hormone Level.** This would then start to allow us to look at the effects of the game day changes on performance, or the effects of performance on salivary hormone level.
2. **Introduce Measures of Training Load in order to maximise hormonal response.** By look at responses of hormone levels to training stimuli it may be possible to influence match day hormone in order to maximise performance
3. **Repeat the study at multiple super league clubs.** This will allow us to see if these responses are consistent across all rugby leagues, and not just at this club.
4. **Introduce more sensitive testing to examine player's state going into games.** By testing players psychological and physiological state prior to and post-game.
5. **Testing more time points throughout the day to establish a stronger diurnal variation.** In order to establish a clear diurnal variation testing every 3 hours would be more accurate.
6. **Examine more hormones.** To get a more accurate picture of what's going on you could measure multiple hormones

6.5 Conclusions

In summary, this thesis has provided novel data to show that a normal hormonal circadian rhythm deceasing across the day exists in professional rugby league players for testosterone and cortisol on a rest day. Moreover, we were also able to show the significant difference that occurs on an elite rugby league match day compared to at rest. Interestingly there was a significant difference between days at rest, suggesting a role of training during the week in influencing hormone levels. Similarly during fixtures the effects of location, team and league position may also play a role. It is important to note that within the hormone levels in this study there is a large degree of individual variance, however all results were significant on a group level allowing us to identify the key general hormonal trends of testosterone and cortisol in elite rugby league players during match play.

CHAPTER 7.0

References

This chapter provides an alphabetical reference list of all the materials cited in this thesis. Journal articles are cited as follows: Authors. (year). Article Title. Journal. Volume: page numbers.

ARCHER, J. 2006. Testosterone and human aggression: an evaluation of the challenge hypothesis. *Neurosci Biobehav Rev,* 30**,** 319-45.
BAKER, D. 2001. Comparison of upper-body strength and power between professional and college-aged rugby league players. *J Strength Cond Res,* 15**,** 30-5.
BAKER, D. 2002. Differences in strength and power among junior-high, senior-high, college-aged, and elite professional rugby league players. *J Strength Cond Res,* 16**,** 581-5.
BARBARINO, A., DE MARINIS, L., TOFANI, A., DELLA CASA, S., D'AMICO, C., MANCINI, A., CORSELLO, S. M., SCIUTO, R. & BARINI, A. 1989. Corticotropin-releasing hormone inhibition of gonadotropin release and the effect of opioid blockade. *J Clin Endocrinol Metab,* 68**,** 523-8.
BOOTH, A., SHELLEY, G., MAZUR, A., THARP, G. & KITTOK, R. 1989. Testosterone, and winning and losing in human competition. *Hormones and Behavior,* 23**,** 556-571.
BREWER, J. & DAVIS, J. 1995. Applied physiology of rugby league. *Sports Med,* 20**,** 129-35.
BREWER, J. D., J.; KEAR, J. 1994. A comparison of the physiological charateristics of rugby league forwards and backs (Abstract). *Journal of Sport Science,* 12**,** 1.
BROWN, G. L., MCGARVEY, E. L., SHIRTCLIFF, E. A., KELLER, A., GRANGER, D. A. & FLAVIN, K. 2008. Salivary cortisol, dehydroepiandrosterone, and testosterone interrelationships in healthy young males: a pilot study with implications for studies of aggressive behavior. *Psychiatry Res,* 159**,** 67-76.
CADORE, E., LHULLIER, F., BRENTANO, M., SILVA, E., AMBROSINI, M., SPINELLI, R., SILVA, R. & KRUEL, L. 2008. Correlations between serum and salivary hormonal concentrations in response to resistance exercise. *J Sports Sci,* 26**,** 1067-72.
CARNEY, D. R., CUDDY, A. J. & YAP, A. J. 2010. Power posing: brief nonverbal displays affect neuroendocrine levels and risk tolerance. *Psychological Science,* 21**,** 1363-1368.
CARRE, J. M. 2009. No place like home: testosterone responses to victory depend on game location. *Am J Hum Biol,* 21**,** 392-4.
COATES, J. M., GURNELL, M. & SARNYAI, Z. 2010. From molecule to market: steroid hormones and financial risk-taking. *Philosophical Transactions of the Royal Society of Biological Sciences,* 365**,** 1363-1368.
COATES, J. M. & HERBERT, J. 2008. Endogenous steroids and financial risk taking on a London trading floor. *Proceedings of the National Academy of Sciences of the United States of America,* 105**,** 6167-6172.
COOK, C. J. & BEAVEN, C. M. 2013. Salivary testosterone is related to self-selected training load in elite female athletes. *Physiol Behav,* 116-117**,** 8-12.
COOK, C. J. & CREWTHER, B. T. 2012a. Changes in salivary testosterone concentrations and subsequent voluntary squat performance following the presentation of short video clips. *Horm Behav,* 61**,** 17-22.
COOK, C. J. & CREWTHER, B. T. 2012b. The effects of different pre-game motivational interventions on athlete free hormonal state and subsequent performance in professional rugby union matches. *Physiol Behav,* 106**,** 683-8.

CREWTHER, B. T., COOK, C., CARDINALE, M., WEATHERBY, R. P. & LOWE, T. 2011. Two emerging concepts for elite athletes: the short-term effects of testosterone and cortisol on the neuromuscular system and the dose-response training role of these endogenous hormones. *Sports Med,* 41**,** 103-23.

CREWTHER, B. T. & COOK, C. J. 2012. Effects of different post-match recovery interventions on subsequent athlete hormonal state and game performance. *Physiol Behav,* 106**,** 471-5.

CREWTHER, B. T., LOWE, T. E., INGRAM, J. & WEATHERBY, R. P. 2010. Validating the salivary testosterone and cortisol concentration measures in response to short high-intensity exercise. *J Sports Med Phys Fitness,* 50**,** 85-92.

CREWTHER, B. T., SANCTUARY, C. E., KILDUFF, L. P., CARRUTHERS, J. S., GAVIGLIO, C. M. & COOK, C. J. 2013. The workout responses of salivary-free testosterone and cortisol concentrations and their association with the subsequent competition outcomes in professional rugby league. *J Strength Cond Res,* 27**,** 471-6.

CUMMING, D. C., QUIGLEY, M. E. & YEN, S. S. 1983. Acute suppression of circulating testosterone levels by cortisol in men. *J Clin Endocrinol Metab,* 57**,** 671-3.

DABBS, J. M., JR. 1990. Salivary testosterone measurements: reliability across hours, days, and weeks. *Physiol Behav,* 48**,** 83-6.

DICKMEIS, T., WEGER, B. D. & WEGER, M. 2013. The circadian clock and glucocorticoids--interactions across many time scales. *Mol Cell Endocrinol,* 380**,** 2-15.

DRUST, B., WATERHOUSE, J., ATKINSON, G., EDWARDS, B. & REILLY, T. 2005. Circadian rhythms in sports performance--an update. *Chronobiol Int,* 22**,** 21-44.

EDWARDS, D. A. 2006. Competition and testosterone. *Hormones and Behavior,* 50**,** 681-683.

ELLOUMI, M., BEN OUNIS, O., TABKA, Z., VAN PRAAGH, E., MICHAUX, O. & LAC, G. 2008. Psychoendocrine and physical performance responses in male Tunisian rugby players during an international competitive season. *Aggress Behav,* 34**,** 623-32.

ELLOUMI, M., MASO, F., MICHAUX, O., ROBERT, A. & LAC, G. 2003. Behaviour of saliva cortisol [C], testosterone [T] and the T/C ratio during a rugby match and during the post-competition recovery days. *Eur J Appl Physiol,* 90**,** 23-8.

EVANS, S. D., BREWER, C., HAIGH, J. D., LAKE, M., MORTON, J. P. & CLOSE, G. L. 2015. The physical demands of Super League rugby: Experiences of a newly promoted franchise. *Eur J Sport Sci***,** 1-9.

FLEGR, J., HAMPL, R., ČERNOCHOVÁ, D., PREISS, M., BIČÍKOVÁ, M., SIEGER, L., PŘÍPLATOVÁ, L., KAŇKOVÁ, Š. & KLOSE, J. 2012. The relation of cortisol and sex hormone levels to results of psychological, performance, IQ and memory tests in military men and women. *Neuro Endocrinology Letters,* 33**,** 224-235.

GABBETT, T., KELLY, J. & PEZET, T. 2007. Relationship between physical fitness and playing ability in rugby league players. *J Strength Cond Res,* 21**,** 1126-33.

GABBETT, T., KING, T. & JENKINS, D. 2008a. Applied physiology of rugby league. *Sports Med,* 38**,** 119-38.
GABBETT, T. J. 2002a. Influence of physiological characteristics on selection in a semi-professional first grade rugby league team: a case study. *J Sports Sci,* 20**,** 399-405.
GABBETT, T. J. 2002b. Physiological characteristics of junior and senior rugby league players. *Br J Sports Med,* 36**,** 334-9.
GABBETT, T. J. 2005. Science of rugby league football: a review. *J Sports Sci,* 23**,** 961-76.
GABBETT, T. J. 2013. Influence of the opposing team on the physical demands of elite rugby league match play. *J Strength Cond Res,* 27**,** 1629-35.
GABBETT, T. J., JENKINS, D. G. & ABERNETHY, B. 2011. Relative importance of physiological, anthropometric, and skill qualities to team selection in professional rugby league. *J Sports Sci,* 29**,** 1453-61.
GABBETT, T. J., JENKINS, D. G. & ABERNETHY, B. 2012. Physical demands of professional rugby league training and competition using microtechnology. *J Sci Med Sport,* 15**,** 80-6.
GABBETT, T. J., KELLY, J. N. & SHEPPARD, J. M. 2008b. Speed, change of direction speed, and reactive agility of rugby league players. *J Strength Cond Res,* 22**,** 174-81.
GATTI, R. & DE PALO, E. F. 2011. An update: salivary hormones and physical exercise. *Scand J Med Sci Sports,* 21**,** 157-69.
GAVIGLIO, C. M., CREWTHER, B. T., KILDUFF, L. P., STOKES, K. A. & COOK, C. J. 2013. Relationship Between Pre-Game Free Testosterone Concentrations and Outcome in Rugby Union. *Int J Sports Physiol Perform*.
GISSANE, C., JENNINGS, D., KERR, K. & WHITE, J. 2003. Injury rates in rugby league football: impact of change in playing season. *Am J Sports Med,* 31**,** 954-8.
GLEESON, M. 2002. Biochemical and immunological markers of over-training. *J Sports Sci Med,* 1**,** 31-41.
GOLDIN, B. R., WOODS, M. N., SPIEGELMAN, D. L., LONGCOPE, C., MORRILL-LABRODE, A., DWYER, J. T., GUALTIERI, L. J., HERTZMARK, E. & GORBACH, S. L. 1994. The effect of dietary fat and fiber on serum estrogen concentrations in premenopausal women under controlled dietary conditions. *Cancer,* 74**,** 1125-31.
GOZANSKY, W. S., LYNN, J. S., LAUDENSLAGER, M. L. & KOHRT, W. M. 2005. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic--pituitary--adrenal axis activity. *Clin Endocrinol (Oxf),* 63**,** 336-41.
GROSCHL, M. & RAUH, M. 2006. Influence of commercial collection devices for saliva on the reliability of salivary steroids analysis. *Steroids,* 71**,** 1097-100.
GUEZENNEC, Y., LEGER, L., LHOSTE, F., AYMONOD, M. & PESQUIES, P. C. 1986. Hormone and metabolite response to weight-lifting training sessions. *Int J Sports Med,* 7**,** 100-5.
HAYES, L. D., BICKERSTAFF, G. F. & BAKER, J. S. 2010. Interactions of cortisol, testosterone, and resistance training: influence of circadian rhythms. *Chronobiol Int,* 27**,** 675-705.

HELLHAMMER, D. H., WUST, S. & KUDIELKA, B. M. 2009. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology,* 34**,** 163-71.
JENSEN, J., OFTEBRO, H., BREIGAN, B., JOHNSSON, A., OHLIN, K., MEEN, H. D., STROMME, S. B. & DAHL, H. A. 1991. Comparison of changes in testosterone concentrations after strength and endurance exercise in well trained men. *Eur J Appl Physiol Occup Physiol,* 63**,** 467-71.
KING, D. H., P.; MILBURN, P.; GUTTENBEIL, D. 2009. A review of the physiological and anthropometrical characteristics of rugby league players. *South African Journal for Research in Sport, Physical Education and Recreation,* 31**,** 49-67.
KIRSCHBAUM, C. & HELLHAMMER, D. H. 1989. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology,* 22**,** 150-69.
KRAEMER, W. J., MARCHITELLI, L., GORDON, S. E., HARMAN, E., DZIADOS, J. E., MELLO, R., FRYKMAN, P., MCCURRY, D. & FLECK, S. J. 1990. Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol (1985),* 69**,** 1442-50.
KRAEMER, W. J. & RATAMESS, N. A. 2005. Hormonal responses and adaptations to resistance exercise and training. *Sports Med,* 35**,** 339-61.
LAC, G. & BERTHON, P. 2000. Changes in cortisol and testosterone levels and T/C ratio during an endurance competition and recovery. *J Sports Med Phys Fitness,* 40**,** 139-44.
LIENING, S. H. J., R. A. 2010. It Is Not Just About Testosterone: Physiological Mediators and Moderators of Testosterone's Behavioural Effects. *Social and Personality Psychology Compass,* 4**,** 982-994.
MAGIAKOU, M. A., MASTORAKOS, G., WEBSTER, E. & CHROUSOS, G. P. 1997. The hypothalamic-pituitary-adrenal axis and the female reproductive system. *Ann N Y Acad Sci,* 816**,** 42-56.
MASTORAKOS, G., PAVLATOU, M. G. & MIZAMTSIDI, M. 2006. The hypothalamic-pituitary-adrenal and the hypothalamic- pituitary-gonadal axes interplay. *Pediatr Endocrinol Rev,* 3 Suppl 1**,** 172-81.
MAZUR, A. 1985. A biosocial model of status in face-to-face primate groups. *Social Forces,* 64**,** 377-402.
MCLELLAN, C. P., LOVELL, D. I. & GASS, G. C. 2010. Creatine kinase and endocrine responses of elite players pre, during, and post rugby league match play. *J Strength Cond Res,* 24**,** 2908-19.
MEHTA, P. H., JONES, A. C. & JOSEPHS, R. A. 2008. The social endocrinology of dominance: basal testosterone predicts cortisol changes and behavior following victory and defeat. *J Pers Soc Psychol,* 94**,** 1078-93.
MEHTA, P. H. & JOSEPHS, R. A. 2011. Social Endocrinology: Hormones and Social Motivation. *In:* DUNNING, D. (ed.) *Social Motivation.* New York: Psychology Press.
MEIR, R. 1993. Evaluating players' fitness in professional rugby league: reducing subjectivity. *Strength and Conditioning Coach,* 1**,** 11-17.
MEIR, R., NEWTON, R., CURTIS, E., FARDELL, M. & BUTLER, B. 2001. Physical fitness qualities of professional rugby league football players: determination of positional differences. *J Strength Cond Res,* 15**,** 450-8.
MILLER, W. L. 2013. Steroid hormone synthesis in mitochondria. *Mol Cell Endocrinol,* 379**,** 62-73.

MILLER, W. L. & BOSE, H. S. 2011. Early steps in steroidogenesis: intracellular cholesterol trafficking. *J Lipid Res,* 52**,** 2111-35.
O'CONNOR, D. 1996. Physiological characteristics of professional rugby league players. *Strength and Conditioning Journal,* 4**,** 21-26.
PAPACOSTA, E., GLEESON, M. & NASSIS, G. P. 2013. Salivary hormones, IgA, and performance during intense training and tapering in judo athletes. *J Strength Cond Res,* 27**,** 2569-80.
PAPACOSTA, E. & NASSIS, G. P. 2011. Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science. *J Sci Med Sport,* 14**,** 424-34.
PASSELERGUE, P., ROBERT, A. & LAC, G. 1995. Salivary cortisol and testosterone variations during an official and a simulated weight-lifting competition. *Int J Sports Med,* 16**,** 298-303.
SALVADOR, A. 2005. Coping with competitive situations in humans. *Neurosci Biobehav Rev,* 29**,** 195-205.
SALVADOR, A., SUAY, F., GONZALEZ-BONO, E. & SERRANO, M. A. 2003. Anticipatory cortisol, testosterone and psychological responses to judo competition in young men. *Psychoneuroendocrinology,* 28**,** 364-75.
SAPIENZA, P., ZINGALES, L. & MAESTRIPIERI, D. 2009. Gender differences in financial risk aversion and career choices are affected by testosterone. *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 15268-15273.
SCHULTHEISS, O. C., DARGEL, A. & ROHDE, W. 2003. Implicit motives and gonadal steroid hormones: effects of menstrual cycle phase, oral contraceptive use, and relationship status. *Horm Behav,* 43**,** 293-301.
SCHULTHEISS, O. C., WIRTH, M. M., TORGES, C. M., PANG, J. S., VILLACORTA, M. A. & WELSH, K. M. 2005. Effects of implicit power motivation on men's and women's implicit learning and testosterone changes after social victory or defeat. *J Pers Soc Psychol,* 88**,** 174-88.
SENSI, S., PACE PALITTI, V. & GUAGNANO, M. T. 1993. Chronobiology in endocrinology. *Ann Ist Super Sanita,* 29**,** 613-31.
SNEGOVSKAYA, V. & VIRU, A. 1993. Steroid and pituitary hormone responses to rowing: relative significance of exercise intensity and duration and performance level. *Eur J Appl Physiol Occup Physiol,* 67**,** 59-65.
STANFIELD, C. L. 2012. *Principles of Human Physiology Fifth Edition,* Glenview; IL, Pearson Education Inc.
STANTON, S. J., LIENING, S. H. & SCHULTHEISS, O. C. 2011a. Testosterone is positively associated with risk taking in the Iowa Gambling Task. *Hormones and Behavior,* 59**,** 252-256.
STANTON, S. J., MULLETTE-GILLMAN, O. A., MCLAURIN, R. E., KUHN, C. M., LABAR, K. S., PLATT, M. L. & HUETTEL, S. A. 2011b. Low- and high-testosterone individuals exhibit decreased aversion to economic risk. *Psychological Science,* 22**,** 447-453.
TOUITOU, Y. & HAUS, E. 2000. Alterations with aging of the endocrine and neuroendocrine circadian system in humans. *Chronobiol Int,* 17**,** 369-90.
TSIGOS, C. & CHROUSOS, G. P. 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res,* 53**,** 865-71.
TWIST, C. & HIGHTON, J. 2013. Monitoring fatigue and recovery in rugby league players. *Int J Sports Physiol Perform,* 8**,** 467-74.

VIAU, V. 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. *J Neuroendocrinol,* 14**,** 506-13.
VIRU, A. M., HACKNEY, A. C., VALJA, E., KARELSON, K., JANSON, T. & VIRU, M. 2001. Influence of prolonged continuous exercise on hormone responses to subsequent exercise in humans. *Eur J Appl Physiol,* 85**,** 578-85.
WEST, D. J., FINN, C., CUNNINGHAM, D. J., SHEARER, D. A., JONES, M. R., HARRINGTON, B., CREWTHER, B. T., COOK, C. J. & KILDUFF, L. P. 2013a. The neuromuscular function, hormonal, and mood responses to a professional rugby union match. *J Strength Cond Res*.
WEST, D. W., BURD, N. A., TANG, J. E., MOORE, D. R., STAPLES, A. W., HOLWERDA, A. M., BAKER, S. K. & PHILLIPS, S. M. 2010. Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *J Appl Physiol (1985),* 108**,** 60-7.
WEST, D. W., COTIE, L. M., MITCHELL, C. J., CHURCHWARD-VENNE, T. A., MACDONALD, M. J. & PHILLIPS, S. M. 2013b. Resistance exercise order does not determine postexercise delivery of testosterone, growth hormone, and IGF-1 to skeletal muscle. *Appl Physiol Nutr Metab,* 38**,** 220-6.
WEST, D. W., KUJBIDA, G. W., MOORE, D. R., ATHERTON, P., BURD, N. A., PADZIK, J. P., DE LISIO, M., TANG, J. E., PARISE, G., RENNIE, M. J., BAKER, S. K. & PHILLIPS, S. M. 2009. Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. *J Physiol,* 587**,** 5239-47.
WEST, D. W. & PHILLIPS, S. M. 2012. Associations of exercise-induced hormone profiles and gains in strength and hypertrophy in a large cohort after weight training. *Eur J Appl Physiol,* 112**,** 2693-702.
WINGFIELD, J. C. 1990. Hormonal control of territorial behavior in birds--field and laboratory investigations. *Prog Clin Biol Res,* 342**,** 697-703.