GROWING, BUILDING AND REPAIRING ELITE RUGBY PLAYERS: NUTRITIONAL AND ENERGETIC CONSIDERATIONS

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AUTHORS DECLERATION

I declare that the work in this thesis was carried out in accordance with the regulations of Liverpool John Moores University. Apart from the help and advice acknowledged, the work within was solely completed and carried out by the author.

Any views expressed in this thesis are those of the author and in no way represent those of Liverpool John Moores University and the School of Sport and Exercise Science.

This thesis has not been presented to any other University for examination either in the United Kingdom or overseas. No portion of the work referred to in this research project has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

Signed

Date
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ABSTRACT

It is well documented that Rugby League requires players to experience large impact collisions, repeated multiple times during training and match play, thus requiring high amounts of lean mass. To cope with such demands the pre-season period is typically used to grow and build players' to elicit improvements in body composition profiles, ready for the start of the season. Measurements of total energy expenditure and inflammation now allows a greater understanding of the in-season demands of elite level Rugby League, therefore allowing coaches to appreciate the role nutrition can play with maintaining body composition profiles developed and helping repair players during post-match recovery.

The aim of the first study (Chapter 4) was to assess body composition using Dual Energy X-Ray Absorptiometry scans to identify the typical profiles of elite Rugby League players. One hundred and twelve players, from five different clubs, were split into positional groups and scanned for total mass, lean mass, fat mass and percent body fat. Despite small to very large inter-positional differences in all anthropometric variables (effect sizes = −0.08 to 2.56), particularly between the Prop and the other playing positions, there was large intra-position variation in body fat, lean mass (ES = -2.26 to 1.44) and total mass. When used with other key performance indicators, these data provide the first multiteam anthropometric profile of elite Super League players that can be used to guide individualised training and nutrition practices for current and aspiring athletes.

Having assessed position specific body composition profiles, the aim of the second study (Chapter 5) was to assess what changes in body composition is possible from eleven academy players over three successive pre-season periods and ninety-nine senior players from four different clubs prior to, and at the end of, a pre-season training period. There was no meaningful change in lean mass of the academy players during any of the pre-season periods (year 1 = 72.3 to 73.2 kg; ES 0.05, year 2 = 74.4 to 75.5 kg; ES 0.07, year 3 = 75.9 to 76.8 kg; ES 0.06) with small changes only occurring over the three- year study period (72.3 to 75.9 kg; ES = 0.22). The senior players showed trivial changes in all characteristics during the pre-season period (total mass = 95.1 to 95.0 kg; ES -0.01, lean mass = 74.6 to 75.1 kg; ES 0.07, fat mass = 13.6 to 12.9 kg; ES -0.17, body fat percentage = 14.8 to 14.1 %; ES -

0.19). These data suggest that academy players need time to develop towards player profiles congruent with senior players. Moreover, once players reach senior level, body-composition changes is *trivial* during the pre-season and therefore, teams may need to individualise training for players striving to gain lean mass by reducing other training loads.

Following the pre-season period, players begin the in-season period which involves approximately 8 months of competitive league and cup fixtures. During this period, it is crucial players attempt to maintain body composition profiles achieved during the pre-season period and consume required diets to cope with the demands of match play. Therefore, the aim of third study (Chapter 6) was to assess resting metabolic rate and total energy expenditure of six elite RL players over two consecutive weeks in-season including one-match per week. Fasted resting metabolic rate was assessed, followed by a baseline urine sample before oral administration of a bolus dose of hydrogen (deuterium ^{2}H) and oxygen (^{18}O) stable isotopes in the form of water ($^{2}H_2{}^{18}O$). Every 24 hours thereafter, players provided urine for analysis of total energy expenditure via the doubly labelled water method. Individual training-load was quantified using session rating of perceived exertion and data were analysed using magnitude-based inferences. There were *unclear* differences in resting metabolic rate between forwards and backs (7.7 ± 0.5 *cf.* 8.0 ± 0.3 MJ. day^{-1}). Indirect calorimetry produced resting metabolic rate values *most likely* lower than predictive equations (7.9 ± 0.4 *cf.* 9.2 ± 0.4 MJ.day^{-1}). A *most likely* increase in total energy expenditure from week-1 to week-2 was observed (17.9 ± 2.1 *cf.* 24.2 ± 3.4 MJ. day^{-1}) explained by a *most likely* increase in weekly session rating of perceived exertion (432 ± 19 *cf.* 555 ± 22 AU), respectively. The difference in total energy expenditure between forwards and backs was *unclear* (21.6 ± 4.2 *cf.* 20.5 ± 4.9 MJ. day^{-1}). We report greater TEE than previously reported in rugby that could be explained by the ability of doubly labelled water to account for all match and training-related activities that contributes to total energy expenditure.

To best cope with the demands of RL match play, many players try to adopt ideal nutritional strategies to maximise recovery post-match. With this in mind, the aim of the fourth study (Chapter 7) was to measure circulatory markers of inflammation, as a result of RL match play and investigate the efficacy of ingesting Montmorency tart cherry juice in an attempt to

facilitate recovery post-match. Eleven professional RL players competed in 2 competitive RL matches. During both matches, a randomised cross-over design was implemented with Montmorency cherry juice (MC) or a taste and colour matched placebo (PLB) supplemented for 7 consecutive days. Measures of match-play demands including total minutes (min), relative match intensity ($m.min^{-1}$), total contacts (n) and relative contacts ($con.min^{-1}$), interleukin concentration (IL-6, -8, -10), muscle soreness and sleep markers (using self-reported subjective wellness), and muscle function (using jump performance) were recorded during both matches. Average IL-6, -8 and -10 concentrations all increased post-match compared with 48 h pre-match values (IL-6 = 2.95 ± 2.62 Vs 0.66 ± 0.74, IL-8 = 3.56 ± 1.55 Vs 1.96 ± 1.09, IL-10 = 2.37 ± 2.06 Vs 0.52 ± 0.49). Mean total contacts across both matches were 28 ± 11 with 0.4 ± 0.2 $con.min^{-1}$. There were no significant effects of MC on muscle soreness, muscle function and self-reported sleep, fatigue, soreness, mood and stress, or IL-6, -8 or -10 compared with PLB at any of the time points. We report novel data showing no effects of MC on all measured markers of recovery post-match in elite rugby players and therefore question the efficacy of such supplementation in contact sports when players have followed a non-polyphenol depleted diet.

Taken together, coaches should appreciate that in both academy and senior players, meaningful changes in body composition takes time to develop and should be viewed over one to three years instead of a single pre-season period. Total energy expenditure in senior players is greater than previously reported which has direct implications on diet prescriptions and finally, unless athletes are following a low polyphenolic diet, the use of Montmorency tart cherry juice should be questioned.

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LIST OF ABBREVIATIONS

^{2}H, Deuterium

^{18}O, Oxygen

ADP, Air Displacement Plethysmography

AEE, Activity Energy Expenditure

ANOVA, Analysis of Varience

BD, Beckton Dickinson

BF%, Body Fat Percentage

BG, Beta Glucosidase

BIA, Bioelectrical Impedance Analysis

BM, Body Mass

BMC, Bone Mineral Content

BMR, Basal Metabolic Rate

CBA, Cytometric Bead Array

CG, Compression Garments

CHO, Carbohydrate

CI, Confidence Interval

CK, Creatine Kinase

CL, Confidence Limits

CM, Centimetre

CMJ, Counter Movement Jump

CO_2, Carbon Dioxide

CV, Coefficient of Variation

CWI, Cold Water Immersion

DIE, Desired Initial Enrichment

DJ, Drop Jump

DLW, Doubly Labelled Water

DOMS, Delayed Onset of Muscle Soreness

DXA, Dual Energy X-Ray Absorptiometry

EE, Energy Expenditure

EI, Energy Intake

EIMD, Exercise Induced Muscle Damage

EMG, Electromyo-stimulation

ES, Effect Size

ESL, European Super League

FACS, Fluorescence Activated Cell Sorted

FFQ, Food Frequency Questionnaire

g, Gram

G, Gravitational Force

GPS, Global Positioning Systems

H_2O, Water

Hz, Hertz

IE, Injectate Enrichment

IIMD, Impact Induce Muscle Damage

IL, Interleukin

Int, International

ISAK, International Society for the Advancement of Kinanthropometry

Jun, Junior

KG, Kilogram

Kcal, kilocalorie

LPH, Lactase Phlorizin Hydrolase

LSD, Least Significant Difference

Mg, Microgram

MJ, Mega Joules

$M.min^{-1}$, Metres per minute

$M.sec^{-1}$, Metres per second

NEAT, Non-exercise Activity Thermogenesis

NEPA, Non-exercise Physical Activity

NRL, National Rugby League

NSAID's, Non-steroidal Anti-inflammatory Drugs

NYC, National Youth Competition

O_2, Oxygen

PAL, Physical Activity Level

PE, Phycoerythrin

QC, Queensland Cup

RBE, Repeated Bout Effect

REE, Resting Energy Expenditure

RFPM, Remote Food Photographic Method

RHIE, Repeated High Intensity Efforts

RL, Rugby League

RMR, Resting Metabolic Rate

ROS, Reactive Oxygen Species

RPE, Rating of Perceived Exertion

RQ, Respiratory Quotient

RU, Rugby Union

S&C, Strength & Conditioning

SD, Standard Deviation

Sen, Senior

SENr, Sport and Exercise Nutrition Register

sRPE, Session Rating of Perceived Exertion

TEE, Total Energy Expenditure

TEI, Total Energy Intake

TEM, Technical Error of Measurement

TMA, Time Motion Analysis

VO_2, Volume of Oxygen

VCO_2, Volume of Carbon Dioxide

WBC, Whole Body Cryotherapy

CHAPTER 1.

GENERAL INTRODUCTION

1.1. Background

Rugby League (RL) requires players to perform repeated bouts of high-intensity activity including high-speed running and sprinting, separated by lower-intensity bouts of standing, walking, and jogging (Austin & Kelly, 2014; Gabbett, Polley, Dwyer, Kearney, & Corvo, 2014; Twist et al., 2014). However, what makes RL unique are the high-speed collisions from tackling, running into contact with the ball and contact with the playing surface (Takarada, 2003; Twist & Sykes, 2011). Players are typically categorised as forwards (Prop, Back Row), adjustables (Halfback, Stand-off, Hooker) and outside backs (Fullback, Winger, Centre) with the playing position dictating the specific activities performed during a match. For example, outside backs cover greater absolute distances than adjustables and forwards (Gabbett, Jenkins, & Abernethy, 2012; Twist et al., 2014), while forwards are typically involved in more physical collisions than outside backs and adjustables (Gabbett et al., 2012; Twist, Waldron, Highton, Burt, & Daniels, 2012). With this in mind, it is well known that the different playing positions require different physical qualities to allow players to perform the specific tasks required of their position (Gabbett & Seibold, 2013; Meir, Newton, Curtis, Fardell, & Butler, 2001). For example, to withstand the multiple high-speed collisions the forwards are often heavier with greater lean mass than the adjustables and outside backs.

Muscular strength and power are critical components that contribute to success in RL (Baker & Newton, 2008; de Lacey, Brughelli, McGuigan, & Hansen, 2014). As such, many academy and senior players strive to achieve optimal body composition profiles, characterised by high levels of lean mass compared with other team sports such as football. Differences in lean mass between positional groups have been observed in both elite (Lundy, O'Connor, Pelly, & Caterson, 2006; Meir et al., 2001b) and sub-elite players (Gabbett, Kelly, & Pezet, 2008), although the majority of these data have been estimated using skinfold measure and predictive equations. More recently, studies have utilised Dual Energy X-ray Absorptiometry (DXA) scan technology to assess the body composition of RL players. These studies have assessed: the changes in body composition profiles of players from the same club, at two time points over a six-year period (Jones et al., 2018), differences in body composition

between academy and senior players (Till, Jones, O'Hara, et al., 2016), or in-season changes in elite Australian NRL players (Georgeson, Weeks, McLellan, & Beck, 2012) and ESL players (Harley, Hind, & O'Hara, 2011). Therefore, longitudinal developments of academy players, and assessments of anthropometric characteristic developments of senior players, from a variety of ESL teams are required to identify changes in body composition over time and additionally, to establish position specific profiles. Although the pre-season is regarded as the optimum time period for players to increase their training volume (Dobbin, Gardner, Daniels, & Twist, 2018), and to elicit meaningful changes in body composition (Weaving et al., 2017), the extent to which these changes are possible during this period has yet to be assessed.

To allow players to gain lean mass and improve body composition profiles, it is crucial to accurately understand the energy requirements (assessment of energy expenditure alongside energy intake) of these players, however this has never been done using appropriate techniques. To date, total energy expenditure (TEE) and total energy intake (TEI) have only been reported in isolation of each other (Lundy et al., 2006) or within academy players (Smith et al., 2018). As such, data from senior professional players during a typical in-season training week is currently not available, thus making it difficult to prescribe accurate nutritional guidelines for players to support training and match-play demands.

Given the high levels of lean muscle mass combined with the intense physical demands of RL and blunt force trauma, players are regularly exposed to muscle damage and soreness (Johnston, Gabbett, Seibold, & Jenkins, 2014; Oxendale, Twist, Daniels, & Highton, 2016; Takarada, 2003; Twist & Sykes, 2011) which can persist throughout a whole season (Fletcher et al., 2016). Repeated exposure to both exercise-induced muscle damage (EIMD) and impact-induced muscle damage (IIMD) results in an inability of force production capacity in muscle (Clarkson & Hubal, 2002; Elmer, McDaniel, Mattson, & Martin, 2012; Hyldahl & Hubal, 2014) and losses in strength and power (Cheung Hume, & Maxwell, 2003; Clarkson & Hubal, 2002; Warren, Lowe, & Armstrong, 1999). Strategies to help alleviate muscle damage and inflammation are therefore essential. Specifically, nutritional strategies have received significant attention with Montmorency tart cherry juice perhaps proving to be the

strongest candidate to help RL players post-match (Bell, Walshe, Davison, Stevenson, & Howatson, 2015; Howatson et al., 2010; Howatson & Milak, 2009), as it has been reported to reduce inflammation and attenuate delayed onset of muscle soreness. However, research to date on cherry juice has been limited to laboratory-based trials (Bell, Stevenson, Davison, & Howatson, 2016) or exercise-induced muscle damage following marathon running (Howatson et al., 2010). It therefore remains unknown whether tart cherries are effective when there is a combination of EIMD and IIMD as seen in RL match play. Moreover, most of the research to date on cherry juice has removed all polyphenols from the diet prior to the intervention and consequently real-world studies with appropriate dietary controls (Close, Kasper, & Morton, 2019) are now needed.

1.2. Aims, Objectives and Structure of Thesis

The overall aim of the present thesis is to examine the body composition profiles from both professional academy and senior RL players and assess how these profiles develop as players mature and following the crucial pre-season period. A secondary aim is to investigate the efficacy of a nutritional supplement strategy during the recovery period following match-play.

This will be achieved by completion of the following objectives:

1. To assess the position specific body composition profiles in senior professional players (Chapter 4).

2. To assess the body composition changes of professional academy players over three successive seasons with particular focus given to the pre-season period (Chapter 5).

3. To assess the body composition changes in professional senior players following a pre-season period (Chapter 5).

4. To assess the total EE and total EI during a fourteen day in-season period in senior professional players using the gold standard technique of doubly labelled water (Chapter 6).

5. To assess the inflammation caused from repeated match play and then examine the effects of a nutritional intervention during the recovery period in professional academy players (Chapter 7).

CHAPTER 2.

LITERATURE REVIEW

2.1. Rugby League

Rugby League was formed in 1895 following a split from the Rugby Football Unions, primarily due to the prevention of “broken time payments” to those players who had taken time off work to play. Soon thereafter, the Northern Rugby Football Union was established and almost immediately began to modify the rules including the abolishment of the lineout in 1897 and the replacement of a ruck with the “play the ball” in 1906, amid the aim of producing a faster more entertaining game. RL match play does involve set-pieces such as scrums, penalties and “tap and go” although these remain largely uncontested and are included in the game to award one team the advantage of gaining or retaining possession of the ball following a referee’S decision. Although RL turned professional exactly a century before Rugby Union (RU), both sports have experienced increased pressures from the media for TV rights, corporate businesses for monetary sponsorship deals and loyal spectators to produce winning yet exciting performances. Both sports have seen a growing interest into the demands of the game from a research perspective although published data remains somewhat limited compared with other sports such as football. RL research within both the Northern Hemisphere (European Super League), and Southern Hemisphere (National Rugby League) has steadily increased with data now being available regarding the demands of training and match play as well as on the players themselves. As a result, more data is available to aid sport science practitioners in supporting players’ health, performance and recovery from the competitive demands of training and match play. Furthermore, this has seen an increase in the number of sport science jobs in RL.

A RL teams consists of 13 players, split into forwards (Prop, Back Row and Loose Forward), adjustable (Halfback, Stand-off, Hooker) and outside backs (Fullback, Winger and Centre). It is well known that these playing positions require unique physical qualities (Meir et al., 2001b; Twist et al., 2014) and body composition profiles (Till, Jones, O’Hara, et al., 2016) based on specific roles to cope with the demands of the game. These specific demands will be discussed later in the literature review (section 2.1.1).

A RL season is split into three components, these being the off-season, the pre-season and the in-season. The first phase is termed the off-season and is the time given for the players to be away from the club to rest and recover. The duration of the off-season is dependent on multiple factors including how successful a club has been in the preceding season (did they make the final of the league and cup competitions?), if players have been selected for international duty, and whether they have undergone surgery. Resultantly, some off-season recovery periods can be as little as 4 weeks before players are requested back into their clubs for the commencement of the next pre-season period. However, typically an off-season would last for 8 weeks.

The second phase is termed the pre-season, and this is the time when players are included in training duties but are not playing competitive league matches. A RL pre-season can last between 4-14 weeks dependent on injury status, competition success or international duty. The primary goal of the pre-season period is to increase training loads to provide improvements in fitness and conditioning, as well as implementation of new tactics for the upcoming season. Many rugby clubs use the pre-season period to optimise players' body composition, usually by decreasing fat mass and increasing lean mass. It is not uncommon for many players and coaches to believe that optimal changes to body composition can take place within just one pre-season period. However, this period of the year becomes sensitive to both injury and illness with negative dose responses associated with higher training loads (Morton, 1997).

The final phase is the in-season which is the period of time where the competitive league and cup matches are played. A typical RL in-season lasts approximately 32-36 weeks. A competitive week consists of 4-5 training days, interspersed with 1 or 2 recovery days, followed by an 80-minute match of two 40-minute halves with a 10-minute half-time break.

Many RL clubs also run an academy structure for younger players, which are typically used to provide player development from within the same club. In particular, they provide young players the opportunity to learn fundamental strength and conditioning principles, field-based skills and an accumulation of match minutes to develop match day performance. Normally

players enter academy teams at the age of under-16, progressing to under-17 and finally under-18. Players can either play for their true age group or one year up if their date of birth falls in the extra age range category. Many teams consistently attempt to develop players from within the academy age teams, through to senior level, and in doing so, many young academy players are now breaking through and representing at senior level. This can place increased pressure on academy players to gain body composition profiles to that concomitant with senior players, although to date it is not known what changes are possible during the development years of a young academy player's career.

Despite the apparent importance of longitudinal body composition developments to success in RL, current observations of anthropometric profiles have only been performed during a single time point in both academy (Till et al., 2011; Till, Cobley, O'Hara, Chapman, & Cooke, 2013) and senior players (Georgeson et al., 2012; Harley et al., 2011) or limited to one study from more than one team (Dobbin et al., 2018). Given the current body of literature, it is difficult for sport science practitioners to know the rates of development from academy player body composition profiles towards those of senior players, and therefore further research is warranted to identify these potential changes. Additionally, considering tracked body composition changes of senior players is limited to those changes seen over a whole season (Georgeson et al., 2012), current data regarding changes during the pre-season period is understudied and warrants further investigation.

This literature review will now critically appraise the existing literature on: 1) the demands of RL, 2) the physical characteristics of RL players and methods of assessment 3) the importance of nutrition for the modern day RL player and 4) the consequence of EIMD and IIMD, identifying the immediate areas for future research.

2.1.1. Positional Demands of Rugby League

Players who play in the adjustable or outside back positions cover greater distances, accelerate more into open spaces and support attacking plays whereas those players in the forward positions carry the ball into collisions accumulating advantageous field positions

(Twist et al., 2014). In support of this, data from the same authors (Twist et al., 2014) shows outside backs covering greater absolute (~7100 m) and high-speed running (~500 m) distances than adjustables (~6800 m; ~410 m) and forwards (~5700 m; ~320 m). Others (Gabbett, Jenkins, Abernethy, 2012; Gabbett, 2013; McLellan, Lovell, & Gass, 2011; Waldron, Twist, Highton, Worsfold, & Daniels, 2011) have shown considerable variation in total match distances covered (~3600 – 9700 m), although differences may be due in part to differences in technology and failure to account for actual time spent on the field. Studies have used both video-based (Sykes et al., 2009; Sirotic, Coutts, Knowles, & Catterick, 2009) and GPS-based (Gabbett, Jenkins, Abernethy, 2012; McLellan, Lovell, & Gass, 2011; Sykes, Twist, Nicholas, & Lamb, 2011; Twist et al., 2014; Waldron, Twist, Highton, Worsfold, & Daniels, 2011) methods to assess distances covered during match play. Data should be interpreted with a degree of caution considering diverse individual playing times, grouping of players and level of playing standard may all contribute to a large variability.

An overview of the reported match play demands of RL players is reported in Table 2.1. Mean running velocities between positional groups are different with adjustables (~101 $m \cdot min^{-1}$) covering more relative distances when compared to hit-up forwards (~94 $m \cdot min^{-1}$), wide-running forwards (~96 $m \cdot min^{-1}$) and outside backs (~93 $m \cdot min^{-1}$). The repeated high intensity efforts (RHIE) is defined as 3 or more maximal accelerations, high velocity sprints or contact efforts with less than 21 seconds recovery between efforts (Gabbett & Mulvey, 2008). These and collisions have shown significant variations between playing positions (Austin, Gabbett, & Jenkins, 2011; Gabbett, Wiig, & Spencer, 2013; McLellan et al., 2011). In particular, players work:rest ratios show forwards (1:6) perform more work than backs (1:7) during match play (McLellan, Lovell, & Gass, 2011). For example, hit-up forwards perform a greater number of repeated sprints and tackles, with a shorter recovery time compared to both adjustables and outside backs. Moreover, hit-up forwards are involved in a higher percentage of tackling compared with outside backs (55% *cf.* 40%, respectively) (Austin et al., 2011). These demands have been suggested to cause a greater physiological cost for forward players compared with backs who may only experience repeated sprints but without the added demand of repeated tackling and collisions (Johnston, Gabbett, & Jenkins, 2015; Johnston & Gabbett, 2011). These passages of play become pertinent considering the

outcomes of a match can be easily influenced by the ability for players to withstand RHIE demands, especially when these passages of match play occur near the try line during offensive or defensive play (Austin et al., 2011; King, Jenkins, & Gabbett, 2009).

Table 2.1 *Match-play demands in RL players by positional group. Data are shown from the European Super League and National Rugby League. Data are mean ± SD.*

Study	**Group**	**Playing time** (min)	**Distance** (m)	**Distance** ($m \cdot min^{-1}$)	**LSA** (m)	**HSR** (m)	**RHIE** bouts (n)	**Collisions** (n)	**Collisions** ($n \cdot min^{-1}$)
Austin and Kelly (2013)	NRL forwards	-	5964 ± 696	85 ± 4	4655 ± 568	432 ± 127	-	-	-
	NRL backs	-	7628 ± 744	86 ± 5	5844 ± 549	749 ± 205	-	-	-
Gabbett et al., (2012)	NRL hit-up forwards	38.0 ± 10.8	3569 ± 1177	94 ± 10	3334 ± 1082	235 ± 122	8.0 ± 5.2	42 (35-48)	1.1 (1.0-1.2)
	NRL wide-running forwards	58.5 ± 16.7	5561 ± 1579	96 ± 13	5143 ± 1474	418 ± 154	9.9 ± 6.4	45 (38-52)	0.8 (0.7-0.8)
	NRL adjustables	64.1 ± 23.0	6411 ± 2468	101 ± 19	5974 ± 2299	436 ± 198	8.6 ± 7.7	34 (28-40)	0.6 (0.5-0.7)
	NRL outside backs	73.5 ± 14.9	6819 ± 1421	93 ± 13	6235 ± 1325	583 ± 139	8.5 ± 5.4	28 (23-32)	0.4 (0.3-0.4)
Gabbett (2013)	NRL forwards	50.7 ± 13.9	5129 ± 1652	105 ± 21	4878 ± 1541	251 ± 157	11.9 ± 6.2	23.3 ± 7.6	0.5 ± 0.1
	NRL adjustables	74.9 ± 14.6	7834 ± 2207	99 ± 8	7513 ± 2138	320 ± 176	14.3 ± 5.4	16.4 ± 6.5	0.2 ± 0.1
	NRL backs	77.8 ± 10.1	7575 ± 850	94 ± 10	7123 ± 830	452 ± 112	14.5 ± 5.4	16.4 ± 6.1	0.2 ± 0.1
McLellan et al., (2011)	NRL forwards	-	4982 ± 1185	-	4664 ± 1165	232 ± 60	-	-	-
	NRL backs	-	5573 ± 1128	-	4879 ± 1339	440 ± 101	-	-	-
McLellan and Lovell (2013)	NRL forwards	-	8442 ± 812	98 ± 12	-	-	-	-	-
	NRL backs	-	8158 ± 673	101 ± 8	-	-	-	-	-
Twist et al., (2014)	NRL forwards	56.7 ± 16.4	4948 ± 1370	88 ± 8	-	-	-	-	-
	NRL adjustables	82.8 ± 8.9	7973 ± 1160	96 ± 8	-	-	-	-	-
	NRL backs	85.8 ± 3.9	7381 ± 518	87 ± 6	-	-	-	-	-
Varley et al., (2013)	NRL	64.9 ± 18.8	6276 ± 1950	96 ± 16	5950 ± 1845	327 ± 168	11.4 ± 5.9	18.9 ± 8.1	0.3 ± 0.2

Study	Group	Playing time (min)	Distance (m)	Distance ($m \cdot min^{-1}$)	LSA (m)	HSR (m)	RHIE bouts (n)	Collisions (n)	Collisions ($n \cdot min^{-1}$)
Dempsey et al., (2018)	Sen. Int. Forwards	57.6 ± 17.6	4854 ± 1506	84.6 ± 6.0	-	252 ± 164	-	18.0 ± 6.0	0.3 ± 0.2
	Sen. Int. Backs	85.7 ± 13.3	7255 ± 1206	84.9 ± 8.6	-	358 ± 204	-	12.7 ± 0.2	0.2 ± 0.1
	Jun. Int. Forwards	58.1 ± 24.1	4911 ± 2182	85.4 ± 7.2	-	246 ± 181	-	12.9 ± 6.8	0.2 ± 0.1
	Jun. Int. Backs	82.3 ± 16.4	6773 ± 1282	83.3 ± 5.8	-	279 ± 112	-	7.7 ± 5.1	0.1 ± 0.1
Oxendale et al., (2016)	ESL forwards	55.1 ± 21.3	4675 ± 1678	81.9 ± 7.3	3584 ±1254	306 ± 194	14.4 ± 10.4	54.1 ± 37.0	1.0 ± 0.6
	ESL backs	67.1 ± 25.2	5640 ± 2191	83.2 ± 10.1	4322 ± 1705	481 ± 262	10.0 ± 4.8	31.1 ± 13.1	0.5 ± 0.5
Twist et al., (2012)	ESL forwards	50.7 ± 15.7	-	-	-	-	-	38.2 ± 18.7	0.7 ± 0.3
	ESL backs	80.0 ± 00.0	-	-	-	-	-	25.2 ± 8.0	0.3 ± 0.1
Twist et al., (2014)	ESL forwards	57.9 ± 15.8	5733 ± 1158	102 ± 14	-	-	-	-	-
	ESL adjustables	69.7 ± 23.4	6766 ± 1495	104 ± 27	-	-	-	-	-
	ESL backs	83.9 ± 12.9	7133 ± 1204	86 ± 11	-	-	-	-	-
Waldron et al., (2011)	ESL forwards	44.2 ± 19.2	4181 ± 1829	95 ± 7	1723 ± 743	513 ± 298	-	-	-
	ESL adjustables	65.2 ± 12.4	6093 ± 1232	94 ± 8	2365 ± 667	907 ± 255	-	-	-
	ESL backs	77.5 ± 12.3	6917 ± 1130	89 ± 4	3262 ± 505	926 ± 291	-	-	-
Gabbett (2013)	NYC forwards	52.3 ± 25.4	4866 ± 2383	93 ± 9	4641 ± 2315	225 ± 90	7.5 ± 3.5	18.3 ± 10.5	0.4 ± 0.1
	NYC adjustables	71.3 ± 14.0	6920 ± 1481	97 ± 10	6562 ± 1297	320 ± 176	11.3 ± 6.6	19.3 ± 6.7	0.3 ± 0.1
	NYC backs	75.5 ± 15.8	7172 ± 1377	96 ± 11	6767 ± 1262	452 ± 113	8.1 ± 1.4	14.8 ± 9.1	0.2 ± 0.1
McLellan and Lovell (2013)	NYC forwards	-	4774 ± 564	82 ± 5	-	-	-	-	-
	NYC backs	-	5768 ± 765	74 ± 11	-	-	-	-	-
Gabbett (2013)	QC top 4	69.3 ± 19.6	5822 ± 1654	86 ± 8	5475 ± 1516	348 ± 186	10.9 ± 5.1	33.9 ± 12.9	0.6 ± 0.2
	QC middle 4	70.2 ± 19.0	5823 ± 1616	85 ± 7	5461 ± 1494	362 ± 193	10.6 ± 5.3	33.0 ± 12.4	0.5 ± 0.2
	QC bottom 4	68.3 ± 18.4	5880 ± 1583	87 ± 7	5547 ± 1481	334 ± 166	11.4 ± 5.7	38.6 ± 15.2	0.6 ± 0.2
McLellan and Lovell (2013)	QC forwards	-	6701 ± 678	89 ± 8	-	-	-	-	-
	QC backs	-	7505 ± 627	94 ± 8	-	-	-	-	-

Study	Group		Playing time (min)	Distance (m)	Distance ($m \cdot min^{-1}$)	LSA (m)	HSR (m)	RHIE bouts (n)	Collisions (n)	Collisions ($n \cdot min^{-1}$)
Duffield et al., (2012)	Senior players	non-elite	74 ± 10	5585 ± 1078	75 ± 14	4923 ± 935	661 ± 225	-	22 ± 9	0.3 ± 0.9
Johnston et al., (2013)	Senior players	non-elite	68.8 ± 11.2	5919 ± 872	82 ± 7	5562 ± 828	358 ± 125	1.6 ± 1.5	18.9 ± 4.9	0.3 ± 0.1
Gabbett (2013)	Junior players	non-elite	32.7 ± 8.4	2673 ± 650	83 ± 12	2529 ± 619	144 ± 82	4.5 ± 2.5	15.1 ± 7.0	0.5 ± 0.2

NRL = data on players from the National Rugby League, ESL = data on players from the European Super League, Sen = Senior players, Jun = Junior players, Int = International players, NYC = National Youth Competition, QC = Queensland Cup. LSA = Low speed activity, HSR = High speed running, RHIE = Repeated high intensity activity.

2.1.2. Activity Profiles of Rugby League Match-play

Over the last decade, technological advancements have seen a vast increase of micro-technologies (i.e. global positioning systems [GPS]), enabling data collection from multiple players simultaneously whilst providing immediate feedback. As a result, this has seen a decline in the use of time-motion analysis (TMA) which was previously a time-consuming method. Instead, it is now commonplace in elite rugby environments to utilise GPS, assisting practitioners to further understand the objective movement demands of training and competitive match play (Table 2.1). Many of the latest devices now incorporate tri-axial accelerometers (motion sensors) embedded within the units (e.g. Catapult – Minimax S4 and S5). These motion sensors are capable of measuring movement in three planes of movement (forward, lateral and vertical) simultaneously (Krasnoff et al., 2008) with movements, accelerations, and decelerations ($m \cdot s^{-2}$) measured in reference to gravitational forces ("G" forces), with 1G equating to 9.81 $m \cdot s^{-2}$ (McLellan & Lovell, 2012). Practitioners are now able to establish impacts (indicated by a rapid deceleration) in team sports (Austin & Kelly, 2014; Dwyer & Gabbett, 2012; McLellan et al., 2011; McLellan & Lovell, 2012; McLellan, Lovell, & Gass, 2011), providing information regarding body loads sustained during both training and match play (Kelly, Coughlan, Green, & Caulfield, 2012) (Norris, Highton, Hughes, & Twist, 2016). Both training and match play loads, including collisions, have been quantified via this method, especially indoors where the connectivity of GPS with satellites can be restricted. Due to limited existing research regarding the validity or reliability of current devices (Cummins, Orr, O'Connor, & West, 2013; Waldron et al., 2011), data should be interpreted with caution. In particular, the suitability of micro-technology devices to quantify loads during collisions has been questioned (Highton, Mullen, Norris, Oxendale, & Twist, 2017). Further, although measurements using micro-technology can detect differences in high-power and high-speed during linear and multi-directional running, such technology should not be used to determine the energy cost of intermittent running on their own (Oxendale, Highton, & Twist, 2017). A previously published review (Cummins et al., 2013) concludes that 10 Hz units provide more accurate and reliable data compared with lower sampling frequency devices during acceleration and deceleration. In further support, the 10

Hz units used in this thesis are two to three times more accurate at detecting changes in velocity, and up to six times more reliable than devices sampling at 5 Hz (Varley, Gabbett, & Aughey, 2014). The CV of these units across a range of speeds have been reported as 3.1 to 8.3 % at a constant velocity, 3.6 to 5.9 % for accelerations and 3.6 to 11.3 % for decelerations (Varley et al., 2014).

To allow practitioners to analyse running volumes at different intensities, specification of speed zones during different aspects of training or match play should be pre-determined. Typically, speed zones are assigned to different locomotive movement classifications. However, in recent years many practitioners have created their own 'in-house' categories, using numerous different speed zones resulting in inconsistencies in the published literature. In response to this, some researchers have attempted to standardise such speed categories for different sports (Dwyer & Gabbett, 2012), although rugby was not included and these are still based on absolute speed thresholds. Moreover, using pre-defined sprint thresholds may not accurately reflect the actual amount of sprinting a player performs. For example, a forward player with a peak running speed of 7 $m \cdot s^{-2}$ may never reach the pre-defined sprint threshold of 7 $m \cdot s^{-1}$. However, they may perform multiple amounts of sprinting at velocities less than 7 $m \cdot s^{-1}$. Observed differences of any relative distance covered at pre-defined sprinting velocity may notably be explained by different sprinting abilities between players (Gabbett, 2013) or the opportunities available during match play to reach sprinting velocity. For example, backs tend to have greater space and time (due to on-field position) to generate increased locomotive patterns, impacting overall speeds attained during training (de Lacey et al., 2014) and match play (Waldron et al., 2011). In comparison, RL forwards typically receive the ball with minimal ground to cover, sometimes from a stationary position, before colliding with an oncoming opposition player, resulting in match play that is rarely exposed to situations of maximal locomotive speeds. Taken together, GPS devices should be configured on an individual player basis in an attempt to minimise potential errors in the measurement of distance run at high intensity based on absolute speed thresholds and allow improved monitoring of loads based on measured individual physical capacities (Scott, Thornton, Scott, Dascombe, & Duthie, 2018).

2.1.3. Metabolic Demands of Rugby League Match-play

In professional RL, it has been suggested that the reduced ability to perform rugby specific demands in the second half of match play is related to the physical stress of the first half (Johnston, Gabbett, & Jenkins, 2014). In particular, a decline in both total distance and high-speed running during the second half is seen in those players who performed more physical activity in the first half (Sirotic, Knowles, Catterick, & Coutt, 2011). A potential mechanism for these reductions may be a reduction in muscle glycogen concentration. Reduced muscle glycogen has been associated with fatigue during prolonged intermittent exercise (Bangsbo, 1994; Hawley, Schabort, Noakes, & Dennis, 1997). Furthermore, reductions in both physical and technical performance from peripheral and transient fatigue occur when muscle glycogen concentrations are low (< 150 mmol.kg dry weight^{-1}) (Kempton, Sirotic, & Coutts, 2014; Sykes et al., 2011). Low muscle glycogen concentrations not only elicit fatigue through substrate depletion, but can also lead to an impaired sarcoplasmic reticulum calcium release rate (Ørtenblad, Nielsen, Saltin, & Holmberg, 2011), potentially attenuating maximal high intensity efforts such as single and repeated sprints, accelerations, contacts and sudden changes in direction. Through correctly implemented nutritional strategies, muscle glycogen concentration can be increased prior to exercise, and this may decrease decrements in performance. Recent observations from our group (Bradley et al., 2016a) have shown that even when players were fed with either a low or high CHO intake (6 g·kg^{-1} or 3 g·kg^{-1} body-weight for 24 hr), RL match play resulted in 40% muscle glycogen depletion in both conditions with similarities shown in absolute glycogen concentration between groups at the end of the match (high = 243 ± 43 compared with low = 298 ± 130 mmol kg^{-1} d.w.). Match play performance variables did not differ between carbohydrate conditions. Notably, those players who consumed a higher carbohydrate intakes pre-match presented with more homogenous pre-match muscle glycogen concentrations compared to those who consumed lower carbohydrate intakes. All players in the 6 g·kg^{-1} group presented with post-match play concentrations > 200 mmol·kg^{-1}d.w. Conversely, two players from the group consuming 3 g·kg^{-1} presented with concentrations as low as < 150 mmol·kg^{-1}d.w. at the end of the match. These observations suggest that some players were at an increased risk of muscle glycogen

depletion and premature fatigue thus highlighting the importance of appropriate fuel provision for the demands of match play.

Following the cessation of match play, an important consideration for any player is the recovery period. The ingestion of carbohydrate (or lack thereof) can reduce or exacerbate fatigue during the acute period following RL competition. To understand this further, our group conducted a RL match simulation protocol (Bradley et al., 2017), with an immediate or delayed (2 hr) carbohydrate re-feed strategy. We report a *very likely* increase (~53 %) in muscle glycogen repletion when carbohydrate was ingested immediately post-exercise compared with *unclear* differences (~ 27 %) following a delayed carbohydrate re-feed. These data highlight the importance of utilising the non-insulin dependent phase of muscle glycogen repletion (Ivy, 2007) following RL match play to restore muscle glycogen concentrations appropriately. Therefore, following a RL simulation, we show it is possible to acutely replete muscle glycogen, despite different re-feed strategies. More recently, the energetic cost of recovery from collisions and subsequent muscle damage has been examined. Using DLW to measure TEE (Westerterp, 2017), one training session involving collisions has been shown to considerably increase TEE across a 5-day training week (Costello et al., 2018). Such findings suggest that while fuelling for the kinematic demands of match play is important, it is also important to fuel appropriately to support the recovery of damaged muscle. Considering both forward and back players are involved in many high-impact collisions during match play it would be valuable to understand the magnitude of damage caused and how players can adopt nutritional strategies which may assist the recovery process. These will be discussed in sections 2.7 and 2.9.

2.2. The Physical Characteristics of Rugby League Players

2.2.1. Body Mass of Rugby League Players

Increases in body mass are evident in RL players between the age categories of under-13s (63.9kg), under-14s (71.1kg) and under-15s (77.6kg) when selected for specific Player

Performance Pathways in junior RL players (Till et al., 2013). Further increases in body mass are seen in both elite (95.3kg: Harley et al., 2011; 93.2kg: Lundy et al., 2006) and sub-elite adult players (88kg: Gabbett, Kelly, & Pezet, 2008; 85.3kg: Gabbett, 2000). Body mass is different between playing standards which has followed a progressive liner shift in the size and physical profile of rugby players exceeding that of the general population (Harley et al., 2011; Jones et al., 2015; Till, Jones, Darrall-Jones, Emmonds, & Cooke, 2015). With increased movement demands, media attention and spectators wanting to be entertained, a large emphasis is placed upon body mass in all positions, particularly lean body mass compared to other team sports such as soccer (Drust, Reilly, & Cable, 2000). Total body mass is important to match the physical qualities of different playing positions (Gabbett & Seibold, 2013; Meir et al., 2001), while the actual composition of body mass is critical for optimal performance, and for use as a key discriminator between selection and non-selection in senior elite NRL players (Gabbett, 2009; Gabbett, Jenkins, & Abernethy, 2011). This has resulted in many RL players aiming for low body fat mass and increased lean mass to improve power:weight ratios, ultimately increasing the force produced and momentum during physical collisions.

Variation in positional anthropometry illustrates the heterogeneity of contact sports such as RL, with each position requiring a unique set of physical qualities (Gabbett & Seibold, 2013; Meir, Newton, Curtis, Fardell, & Butler, 2001a). Anthropometric and physiological variations are evident between forwards and backs. Forwards are required to carry the ball into contact, gaining distance, with the objective of fatiguing the opposing defences and as such, coaches prefer these players to have high amounts of total body mass and lean mass. Conversely, wingers require acceleration and speed qualities to evade defenders and are therefore often lighter than other positions within the team (Cheng et al., 2014). It is therefore essential that players are monitored and changes in body composition are tracked as players mature and progress into senior squads. This data would allow coaches to understand the changes in body composition that may be possible during the early years of an academy player's career and plan more appropriate and timely increases in both body mass and lean mass, appreciating that this may be different between players. Although previous research has attempted to document body mass changes in RL players, these data are limited as a result

of: 1) data being collected purely on players from academy teams (Till et al., 2013), 2) sub-elite and elite players from the championship and ESL (Jones et al., 2015) or 3) players coming from one club which may reflect the training styles of that particular team and limits the number of players within each specific positional group (Harley et al., 2011). To this end, future work should now look to investigate body mass differences in senior grade players firstly, between positional groups and secondly, from multiple teams from the same league.

2.2.2. Height of Rugby League Players

Height increases linearly with age (under 13's 171 ± 7 cm *cf.* under 15's 178.6 ± 5.7 cm) (Till et al., 2013), and when combined with other variables that are deemed important for RL demands, can successfully discriminate playing success between teams and regional and national level players with taller players being selected for national level (Dobbin, Highton, Moss, & Twist, 2019; Till et al., 2011) . Unsurprisingly, senior players report taller in stature than academy players (Till, Jones, O'Hara, et al., 2016), with height differences also seen between positional groups of senior squads (Greene, Varley, Duncan, & Gabbett, 2017). Smaller and shorter players may be able to avoid tackles and evade contact far better than taller slower players, and as such a smaller stature would suit these positions, such as in the adjustable positions. In contrast, taller players would have a better advantage of jumping in the air to retrieve the ball from opposing kicks than shorter players. Data on position specific height of players from more than one team is limited and as such warrants further investigation.

2.2.3. Body Composition of Rugby League Players

Body composition refers to specific structures of the human body and individual tissues of which these structures are comprised. In order to examine changes or differences in these structures, a five tiered approach of body composition analysis is proposed, sectioned into the atomic, molecular, cellular, tissue/system and whole body sections (Wang, Pierson, & Heymsfield, 1992). The majority of research with RL players has employed level two validation measurements which employ anatomical or chemical measure of assessment

(Ackland et al., 2012; Eston & Reilly, 2009). These consist of using a single-compartment model (i.e. whole-body stature), with either a two-compartment model (i.e. fat mass and lean mass), or three-compartment model (i.e. fat mass, lean mass and bone mineral content). It has been well documented that, to enhance accuracy of measurement with level two methods, standardisation of protocols should be tightly followed (Nana et al., 2016).

RL match play involves reduced set-piece plays, such as line-outs and scrums, and increased movement patterns such as the "play the ball" at tackle breakdowns than its parent sport RU. This has resulted in RL adopting a faster style of play and therefore players across all positions require high levels of mobility, strength and power to be successful (Waldron et al., 2014; Kempton, Sirotic, et al., 2015). Forwards are involved in more collisions and repeated high-intensity efforts and required to perform more physical collisions than backs, who are required to cover more absolute metres (Meir et al., 2001b; Oxendale, Twist, Daniels, & Highton, 2016; Twist et al., 2014). Therefore, there is a large requirement of strength needed to successfully complete repeated tackles on opposing players. For players to achieve increased strength and power during competition (Gabbett, King, et al., 2008), there is a need for players to achieve high lean mass profiles. During the pre-season, there tends to be no fixtures (unless clubs have arranged warm-up friendly matches) and as such players perform high-load volume training, followed by adequate recovery to elicit greatest body composition changes and fitness improvements (Weaving et al., 2017). Therefore, the pre-season period seems the most logical period of the season to try and increase lean mass profiles, however research during this crucial part of the season is limited (Dobbin et al., 2018).

Some researchers have suggested that those players who have high amounts of fat mass may be considered advantageous when withstanding the impact forces associated with tackles and collisions (Gabbett, King, & Jenkins, 2008). However, excessive fat mass has a detrimental effect upon performance due to a reduced heat dissipating ability, increased metabolic demands (Meir et al., 2001b) and has been associated with reduced tackling ability (Gabbett, Jenkins, & Abernethy, 2011a). Furthermore, increases in fat mass may be an unwanted consequence of attempting to gain lean mass too quickly with the aim of reaching a specific body mass target, however this is speculative and requires more research. To understand

changes in fat mass, it is therefore essential that studies now look to track fat mass from academy and senior players across all positional groups and from multiple teams who have different training programmes.

An understanding of the possible changes that can be made with body composition is clearly important for the RL player, however studies are currently somewhat limited. Although some have suggested RL players present with homogenous anthropometric profiles, rather than clear position specific profiles that are seen in RU forwards and backs, these data were collected from a single team in the ESL, Championship League and NRL, and so may not represent the different changes possible from other clubs which follow different strength and conditioning programmes (Duthie, 2006; Harley et al., 2011; Jones et al., 2015). Furthermore, forwards have been shown to have greater lean mass than backs in both the ESL and NRL and greater fat mass than backs in the ESL and Championship League (Jones et al., 2015; Lundy et al., 2006), although these data have been performed at different time-points during a season. To this end, research assessing what changes are possible during the crucial pre-season period is currently poorly understood. If this data was available, it may allow coaches to appreciate that pre-season programmes could be tailored to individual players ensuring sufficient time is allowed for meaningful changes to occur, although this is yet to be performed.

Pre-season periods typically last 10-14 weeks depending on international duty, injury or success in cup competitions. Recently the importance of pre-season training has been highlighted with associations between the number of pre-season training sessions completed and a reduction with in-season injuries (Windt, Gabbett, Ferris, & Khan, 2017). Despite the apparent importance of body composition to success in RL, research into the longitudinal developments of players during the crucial pre-season period is limited to one study showing changes during a 6-year window from the 2008 to 2014 ESL season in academy players (Jones et al., 2018). Players showed increases in body mass (3.4 %), fat mass (5 %) and lean mass (3.3 %), respectively, with no data in-between these time points. In summary, research into the maturation and development of academy players, year-to-year, as they progress from academy squad players into selection for senior squads is yet to be defined. Further,

considering clubs routinely use the pre-season period in an attempt to improve body composition profiles in senior players, there is currently no data showing the possible changes during this crucial period of the whole season from the start to end of a single pre-season period. A summary of body composition data is outlined below in Table 2.2.

Table 2.2 *Body composition of professional Rugby League players, mean + SD. ESL = data on players from the European Super League, NRL = data on players from the National Rugby League, NYC = data on players from the National Youth Competition, C = Championship players, S = Senior players, A = Academy players.*

Study	Position (Number of players)	DXA		
		Lean mass (kg)	Fat mass (kg)	Body Fat (%)
Barlow et al., 2015 ESL, S A	Unspecified (n = 21 S, 24 A)	70.0 ± 8.0	17.0 ± 5.0	19.1 ± 4.4
Georgeson et al., 2012 NRL, S	Unspecified (n = 19)	81.5 ± 7.8	10.7 ± 3.1	-
Harley et al., 2009 ESL, S	Unspecified (n = 23	-	-	15.8 ± 4.0
Harley et al., 2011 ESL, S	Unspecified (n = 20)	76.9 ± 9.5	14.0 ± 3.9	15.6 ± 3.7
Jones et al., 2015 ESL, S	Backs (n = 2)	73.2 ± 7.9	12.7 ± 3.4	14.8 ± 3.6
	Forward (n = 1)	78.5 ± 6.4	16.8 ± 4.2	17.5 ± 3.7
Jones et al., 2015 ESL, C	Backs (n = 4)	68.6 ± 5.7	18.2 ± 4.5	20.8 ± 3.8
	Forwards (n = 3)	73.9 ± 7.6	20.1 ± 4.4	21.4 ± 4.3
Jones et al., 2017 ESL, S	Unspecified (n = 12)	78.5 ± 9.1	14.0 ± 4.3	14.9 ± 3.5

2.3. Methods to Assess Body Composition

Anthropometric (i.e. height and body mass), body composition (i.e. lean mass versus fat mass ratio) and physical characteristics (i.e. strength, power and speed etc) can directly relate to successful outcomes during RL competition (Johnston, Gabbett, & Jenkins, 2014; Till et al., 2015). Many clubs realise the importance of assessing and monitoring body composition profiles during both the pre-season and in-season competition periods in an attempt to firstly validate prescribed strength and conditioning programmes and secondly, track any changes (if any) that may occur throughout the season. Methods that have been used to assess body composition in rugby players include bioelectrical impedance analysis (BIA), densitometry, skinfold measurement and DXA scan technology.

2.3.1. Bioelectrical Impedance Analysis

The use of BIA within RL players is limited with only one study comparing BIA, lean mass index, skin fold assessment and DXA from one Australian RL team (Delaney et al., 2016). Similarly to anthropometric skinfold assessment, BIA calculates body composition via equations, which are population specific and cannot be applied to generalised demographics (Ward et al., 2000). There have been a number of studies showing the accuracy and reliability of BIA is reduced further when exercise (Caton, Molé, Adams, & Heustis, 1988), body temperature (Gudivaka, Schoeller, & Kushner, 1996), hydration (Lukaski, Bolonchuk, Hall, & Siders, 1986), and nutritional status (Slinde & Rossander-Hulthén, 2001) are not standardised prior to assessment. When performing BIA analysis, practitioners may be able to standardise nutrition and hydration status by asking players to arrive to the morning assessment following an overnight fast protocol. However, this has known limitations to scheduling, timing and player adherence. Although exercise status on the day may be controlled by performing assessments first thing in the morning, controlling exercise status 24 hrs prior to the assessment would be very difficult during both the pre-season period and in-season periods due to congested training and match schedules. Finally, considering that

changes in vasoconstriction, vasodilation and overall blood flow can potentially influence impedance measurements (Gudivaka et al., 1996), it would be near impossible to control individual player temperature prior to assessment without the use of ingested core temperature pills or muscle temperature probes. Therefore, other methods to assess body composition should be explored.

2.3.2. Densitometry

Densitometry follows a two compartmental indirect measurement method, calculating the density of a given mass by either water volume or air displacement and is considered as one of the gold standard methods to measure body composition at the cellular and tissue level. Hydrodensitometry (hydrostatic or underwater weighing) calculates body density via the Archimedes principle where a mass will displace its own given volume within water. However, this method is particular time consuming and requires specialist equipment and as such, has not been performed within elite RL players. Another displacement method using a similar principle of body volume calculation, is air displacement plethysmography (ADP). To date, only one study has assessed the validation of ADP via a commercial unit called BODPOD (COSMED, Rome, Italy) against DXA. In this population of athletes, measurement of body fat percentage by ADP does not provide similar results to DXA assessment (coefficient of determination 82%, standard error of estimate 1.71) (Harley, Hind, Hara, & Gross, 2009). One area of consideration with the use of BODPOD is that all volumetric calculations must occur under adiabatic conditions (without the transfer of heat), which usually involves volunteers wearing a swim camp and lycra clothing. In the aforementioned study, authors do not state what clothing the RL players wore and this may be a contributing factor to their contrasting results when compared to DXA values for measuring body fat percentage. Despite this, where more accurate estimations of body fat percentage are required, DXA may be the preferable method of assessment.

2.3.3. Skinfold Assessment

To assess body composition in RL players, studies have previously utilised surface anthropometry assessment via skinfold measurement and predictive equations (Delaney et al., 2016; Gabbett, Jenkins, & Abernethy, 2011b; Till et al., 2011; Till, Jones, & Geeson-Brown, 2016). Such measurements of skinfold thickness have even been used as a tool to identify those players for selection and have been shown to be different between playing standards of RL (Gabbett et al., 2011a; Till et al., 2011). Measurement involves applying callipers to a double fold of gripped skin (Martin, Ross, Drinkwater, & Clarys, 1985) using a two compartmental indirect (i.e. measured in sum of skinfolds) and doubly indirect (if a percentage of fat mass is calculated) method. Although skinfold assessment does have advantages in relation to time and ease of use, the accuracy and reliability of estimating body fat percentage and lean mass is limited (Harley et al., 2011). Moreover, skinfold assessment assumes a constant skinfold compressibility and constant skin thickness in the double fold between differing sites. Such limitations can be exaggerated by age, gender, measurement site, skin temperature, hydration status, and the grip of the anthropometrist and pressure applied to the calliper (Martin, Drinkwater, Clarys, Daniel, & Ross, 1992).

In an attempt to standardise surface anthropometry skinfold protocols among practitioners, the International Society for the Advancement of Kinanthropometry (ISAK) was established. The aims of ISAK are to regulate assessments of body composition via skin callipers. However, body composition, including fat mass and lean mass are calculated from predictive equations which have been based on the general population, and not athletic populations (Reilly et al., 2009). To this end, the use of skinfold protocols for the assessment of body composition in elite RL players is questionable and has resulted in researchers using alternative methods.

2.3.4. Dual Energy X-Ray Absorptiometry

DXA scan technology uses a three compartmental indirect method of assessment, passing high and low energy X-ray photons through the body via a fan beam. Both the density and

volume of differing body tissues affects the energy of these beams, i.e. soft tissues (fat mass & lean body mass) allow a greater passage of photons and harder tissues (bone mineral contect) allow less. An image estimation of the differing compartment tissues is then generated via pixilation, of which 40-45% contains bone mineral content, and as such the remaining pixels are then utilised to calculate both fat mass and lean mass with valid and reliable measures of regional body compositions (Nana et al., 2012).

Previous studies in elite RL players has used DXA scan technology to assess body composition of RL players from one club (Georgeson et al., 2012), during one season (Harley et al., 2011) or from two time points six seasons apart (Jones et al., 2018). Although these data show RL players possess high lean mass and total mass body compositions, the majority have not differentiated between positional groups or have been performed on Australian NRL players (Barlow et al., 2015; Georgeson et al., 2012; Harley et al., 2011; Till, Jones, O'Hara, et al., 2016). A profile of body composition characteristics in a large group of academy and senior players from a variety of ESL teams and positional groups would therefore be useful to enable position specific anthropometric characteristics to be established. Such data might be useful for sport science support staff with talent identification, and could assist training and nutrition strategies to achieve optimal positional body composition profiles for players and provide known position-specific profiles for young academy players to develop towards as they mature through academy systems into senior squads.

The use of DXA does have known limitations and recently a number of standardisation issues have been raised in the literature. Such issues include pre-scan hydration (Toomey, McCormack, & Jakeman, 2017), nutritional intake (Bone et al., 2017) and exercise status (Nana, Slater, Hopkins, & Burke, 2012). As such, this has led to the development of a best practice protocol (Nana, Slater, Stewart, & Burke, 2015; Nana et al., 2016). Many comparative studies assessing the relationships between all cellular and tissue body composition methods refer to hydrodensiometry or DXA as the criterion method, against which other measures are compared. If possible, the use of DXA may therefore provide the best method of body composition analysis if known particular limitations can be controlled in RL players.

2.4. Nutrition and Rugby League

2.4.1. Overview of the Nutritional Recommendations for Rugby League

Sufficient EI is important for normal growth and development, maintaining health and well-being, reducing the risk of illness and injury, and optimizing sporting performance (Thomas, Erdman, & Burke, 2016). Therefore, understanding the metabolic demands of both training and match play in RL becomes vital in allowing performance nutritionists to correctly prescribe EI for rugby players. Not only is this important to provide essential micronutrients for general health, but the correct EI will support players during the pre-season period when an emphasis is placed on body composition manipulation. Subsequently, once the season begins, EI during training days and the days leading into competition to fuel higher intensities (Burke, Kiens, & Ivy, 2004) becomes imperative. Finally, following EIMD and IIMD from match play, effective EI including recommended protein intake to facilitate accelerated adaptation and repair (Tipton & Wolfe, 2001) during recovery days is essential to ensure players stay fit and healthy throughout the whole season. Team sport metabolic and match demands data have allowed physiological training programmes and nutritional strategies to be implemented to enhance performance and/or delay fatigue. Such data exists in soccer (Maughan & Shirreffs, 2007; Ono, Kennedy, Reeves, & Cronin, 2012; Russell & Pennock, 2011) and other team sports (Mujika & Burke, 2011). These data have also formed the basis of nutritional position stands (Thomas et al., 2016), of which many rugby players and practitioners have attempted to translate for use within RL. However, there are distinct differences in the amount of distance covered by soccer players (Anderson et al., 2017) when compared with RL players (Gabbett, Jenkins, Abernethy, 2012), and in particular the most noticeable difference between match demands includes the repeated high-impact physical collisions that exist in RL (Austin et al., 2011; Oxendale, Twist, Daniels, & Highton, 2016; Twist et al., 2012) and not soccer. To this end, to support such demands, RL players possess greater lean mass and total mass body composition profiles when compared to soccer players (Milsom et al., 2015). Therefore, the suitability of using studies from other team sports to inform nutritional guidelines for RL players is questionable.

To allow practitioners to implement correct nutritional guidelines for players, it is important that reliable methods of EI collection are adopted. This would aid in improving body composition and supporting recovery from training and competition. With this in mind, EI of non-elite (Smith, Jones, Sutton, King, & Duckworth, 2016) and elite players have been performed but remains limited in professional senior players during training and competition (Costello, Deighton, Dyson, Mckenna, & Jones, 2017; Tooley, Bitcon, Briggs, West, & Russell, 2015), and therefore warrants further research. Methods employed to assess EI will be discussed in the next section.

2.5. Total Energy Intake in Rugby League Players

Total energy intake has previously been described as one of most challenging physiological assessment methods within human science (Hackett, 2009). One proposed reason, is the difficulty with securing accurate and reliable data (Capling et al., 2017), which can be influenced by the investigators choice of collection method dependant on the population being studied (Magkos & Yannakoulia, 2003). Secondly, once the data is obtained, the evaluation of the information requires special expertise and is typically labour intensive and time consuming. Diet recall, food frequency questionnaires and diet history are categorised as retrospective methods, and depend on the athlete's memory and honesty to assess recent or less recent food diaries. Duplicate portion, diet records and the remote food photographic method are categorised as prospective methods and monitor current and ongoing food consumption. However, due to the degree of subject cooperation required, and burden incurred, many athletes under-report total EI, which raises concern over the accuracy of the data (Capling et al., 2017), including studies on rugby players (Bradley, Cavanagh, Douglas, Donovan, Twist, et al., 2015; Bradley, Cavanagh, Douglas, Donovan, Morton, et al., 2015; Costello et al., 2017; Lundy et al., 2006). The difficulties in accurately assessing EI has resulted in substantial discrepancies between self-reported EI and estimated EE across a variety of sports, genders and age-groups (Burrows, Harries, Williams, Lum, & Callister, 2016; Fogelholm et al., 1995; Hassapidou & Manstrantoni, 2001) and suggested under-reporting (~30%) or substantial differences (11 – 44 %) being observed between studies (Hill & Davies, 2001; Magkos & Yannakoulia, 2003). This can be explained by intentionally or

unintentionally omitting foods consumed (Naoyuki Ebine et al., 2002; Magkos & Yannakoulia, 2003; Trabulsi & Schoeller, 2001), and possible changes in typical food intake or dietary patterns due to the nature of the dietary intervention itself (Jones & Leitch, 1993). With this in mind, many practitioners working with elite athletes accept the best option it to select the method that is most appropriate to suit the situation, whilst clearly acknowledging the limitations of the chosen method alongside careful interpretation of the data. An overview of dietary assessment methods is provided in Table 2.3.

Table 2.3. *Dietary assessment methods: overview, period of interest, advantages, disadvantages and applications in clinical practice and research, adapted from Burke (2015).*

Method	Overview of method	Period of Interest	Advantages	Disadvantages	Applications
			Retrospective		
24 h recall	Subject describes foods consumed over the last 24 h period or a typical day	24 h	Speedy to implement Low burden for the subject Interview can be structured around daily activities Does not alter intake Suited to epidemiological research	Relies on subject's honesty, memory and food knowledge Requires trained interviewer Day for recall may be "atypical" Suitable for group surveys, but not representative of individuals normal intake	Mainly used to rank food or nutrient intakes of groups of people. May not be suitable for individual assessment
Food Frequency Questionnaire (FFQ)	Subjects asked how often they eat foods from a standardised list and to estimate portion sizes often using photos of food models as a prompt	From 24 h period to open-ended	Can be self-administered to lower burden on the investigator Can be used to cross-check data obtained from other methods Validated for ranking individuals Can be modified to target certain nutrients Can be automated to allow quick processing by investigator	Relies on responder's honesty, memory literacy and food knowledge Validity dependent on the food list and the quantification method	Mainly used to detect, measure or rank specified nutrients or food intake in groups or individuals. Used for cross-checking data obtained from other methods
Diet History	Open-ended interview concerning food use, food preparation, portion sizes, food like/dislikes and food checklist	Open-ended or over a specific period	Accounts for daily variation in food intake by investigating a "typical" day Can target contrasts between periods of interest as a sub-theme Collects information on timing of intake and factors that influence food patterns	Relies on responder's honesty, memory, food knowledge Labour intensive and time consuming Requires trained interviewer Mostly appropriate for qualitative assessment rather than quantitative	Mainly used to assess usual intake of individuals in clinical practice. Good for longitudinal studies.

Prospective						
Written diary record)	food (diet	Weighed	May be undertake for 1-7 days, with increasing ability to track usual intake as duration increases, but reduced compliance	Provides a more accurate quantification of foods than household measures Considered the "gold standard for dietary assessment"	Relies on participants' honest and food knowledge Time consuming for subjects to keep and investigator to process Distorts food choice and quantity: subject alters their diet to improve their intake or to reduce the workload of recording	Mainly used to determine eating habits for 1-7 days. Used for validating other methods
		Household measures (descriptions of cups, teaspoons, dimensions of food portions etc.)		Improved compliance with subjects compared with weighed record Less alteration of normal eating pattern compared to weighted or semi-weighed records	See comments for weighed record Requires checking by trained person Needs standardised set of household measures Subjective/inaccurate assessment of portion sizes	Mainly used for invested athletes who have access to correct serving utensils

2.5.1. Food Record (Diet History)

Currently there is no gold standard measure for assessing EI, but by far the most common method in applied sports nutrition research and practice is the food record. Food records are considered accurate enough for dietary assessment of individuals and groups (Magkos & Yannakoulia, 2003), but different time-frames of recording are required in each case. All food, drinks and the amount of each consumed are recorded by the participant for a specified number of days (i.e. 3-7 days) (Thompson & Byers, 1994), ideally with recordings scribed at the time of consumption in order to avoid the reliance on memory. Quantification is achieved by the weighed food method or in terms of other household measures (e.g. cups or tablespoons) or estimated using pictures. In order to gain an overall insight into players' nutritional practice, a 7-day period is typically used by practitioners to allow assessment of EI from training, competition and recovery days. Extensions in the assessment period unquestionably increases the reliability of collected data but at the expense of greater demands on subject cooperation to document information truthfully and accurately as well as a reliance on practitioners to code the data correctly, using appropriate databases (Braakhuis, Meredith, Cox, Hopkins, & Burke, 2003).

Further challenges include, the burden of recording large food intakes and frequent eating occasions to support training and competition demands, irregular meal patterns, contribution of sports foods and supplements, and the difficulty of reporting meals consumed at restaurants during social occasions (Magkos & Yannakoulia, 2003). Accurate details of food descriptions, brands, amount consumed, preparation methods, recipes for food mixtures, portion sizes and left overs all should be provided to support accurate analysis. The number of days required to measure dietary intake reliably varies between young and inexperienced athletes compared with elite professional athletes who are competent in completing food records. Assessment of group dietary intake requires considerably fewer days of data collection than individual assessment, as does the estimation of macronutrient intakes (carbohydrate, protein, fat) in comparison with micronutrient (vitamin and mineral) intakes (Basiotis, Thomas, Kelsay, & Mertz, 1989; Basiotis, Welsh, Cronin, Kelsay, & Mertz, 1987;

Beaton, Milner, McGuire, Feather, & Little, 1983; Marr & Heady, 1986; Nelson, Black, Morris, & Cole, 1989).

A 3-7 day monitoring period is believed to provide reasonably accurate and precise estimations of habitual energy and macronutrient consumption (Braakhuis et al., 2003), and undeniably requires less burden than the weighed-food inventory method. However, it is possible that by the time respondents near the end of a 7-day food record, fatigue is experienced through completing multiple records, which in turn may increase the likelihood of incomplete and retrospective scribed, rather than concurrently scribed records (Gersovitz, Madden, & Smiciklas-Wright, 1978). Additionally, if the participant is aware that the intake is being recorded, this can alter the typical dietary behaviours (Vuckovic, Ritenbaugh, Taren, & Tobar, 2000) and affect both the types of food chosen and the quantities consumed (Andersen, Johansson, & Solvoll, 2002; Kristjansdottir, Andersen, Haraldsdottir, de Almeida, & Thorsdottir, 2006; Rebro, Patterson, Kristal, & Cheney, 1998). This may result in an awareness of the food record leading to non-habitual intakes, and affect the reason for the investigation in the first place. It is possible for practitioners to increase the reliability of the food record method. Firstly, by providing training to the athlete prior to the monitoring period of how to complete food records properly. Secondly, by dissecting the food record with the athlete to clarify food entries and also any entries that may have been forgotten. Finally, by reviewing the food record in combination with a catalogue of pictures that may have been taken to allow cross-examination. Although this method has been implemented previously in RL players, observations were performed in NRL players (Lundy et al., 2006) and academy ESL players (Smith et al., 2016). Data from Smith and colleagues (2016), show players consuming adequate EI compared to suggested adolescent guidelines (Desbrow et al., 2014), with greater EI in older players compared to younger players. With the known body composition, training and match play differences between academy and senior players it would be valuable for practitioners to understand the EI patterns of senior players to match these demands. Currently there is no data on the EI of senior professional RL players playing in the ESL, and therefore work should now look to explore the EI patterns of RL players.

2.5.2. 24-hour Diet Recall

Performing a 24-hour dietary recall requires low athlete burden, minimal distortion of food intake, and are relatively easy to administer, either face to face or over the telephone, meaning multiple recalls can be collected from a larger number of athletes. 24-hour recalls have been shown to be effective in assessing the EI of a group of athletes given that increasing the number of subjects measured decreases the variability (Magkos & Yannakoulia, 2003) and have been shown in some situations to be more accurate than food diaries (Sawaya et al., 1996). One reason for this, is the ability of a practitioner to dissect greater detail from the athlete via a 1-1 conversation, compared to the same athlete attempting to complete the food diary alone, without the opportunity to confirm inputs with the practitioner. With the advancements of technology, it is not uncommon for many athletes to use specific tools (i.e. food quantification applications) or photographs in order to better estimate portion size and quantity (i.e. placing a small portion or large portion of rice on the same plate to estimate portion size). The 24-hour dietary recall method does not require any scribing from the athlete and recall is always performed after the diet has been consumed, limiting any interference with dietary behaviours associated with food records. However, a disadvantage of the 24-hour dietary recall, is that athletes may not report food intake accurately. This may be a result of players perceiving certain foods to be a poor choice and so they fail to disclose this intake, or mask the poor choice with a perceived better choice. Finally, as it suggests, the 24-hour recall only provides a snapshot view of the athlete's dietary intake, and therefore does not allow practitioners to establish dietary intakes during different days of the week (i.e. training, recovery and match days) which would better reflect an athlete's habitual EI patterns.

2.5.3. Food Frequency Questionnaire

Food frequency questionnaires (FFQs) are a low burden method of dietary assessment due to self-administration and are particularly helpful when assessing the status of nutrients with a limited number of rich dietary sources such as vitamin D (Larson-Meyer, Woolf, & Burke, 2018). Briefly, the process requires athletes to identify how often they eat a specific list of

individual foods/beverages (of food categories) with frequency recorded in times per day, week, month or year. Qualitative FFQs ask respondents to describe the size of usual servings relative to typical servings; semi-qualitative states standard servings and non-qualitative does not address portion sizes (Lee & Nieman, 2013; Thompson & Subar, 2008). Furthermore, the structure of FFQs neglects essential information such as the timing of the food/beverage intake and the combination of foods/beverages consumed in the same meal/snack (Larson-Meyer et al., 2018). Although FFQs have been utilised within athletic populations (Braakhuis, Hopkins, Lowe, & Rush, 2011; Fogelholm et al., 1992), and collegiate athletes (Sunami et al., 2016), due to the logistic and technical requirements of appropriate questionnaire design and validation (Cade, Thompson, Burley, & Warm, 2002), the development of athletic group specific FFQs is currently lacking. Moreover, it would be difficult to utilize a non-rugby specific FFQ within a rugby population. RL players possess particular dietary requirements to ensure unique body composition profiles (i.e. high lean mass and total body mass) are maintained during competition periods (Till, Jones, O'Hara, et al., 2016), and particular nutritional intakes are consumed to support recovery from both EIMD and IIMD from training and match play. Finally, many professional rugby players now follow strategic carbohydrate intake (Bradley, Cavanagh, Douglas, Donovan, Twist, et al., 2015) planned around timing of training and match play to in an attempt to maximise performance, however FFQs fail to capture such diurnal variation in EI.

2.5.4. Weighed Food Inventory

The weighed food inventory involves precise measurements and recordings of foods before consumption and also once again following the cessation of each meal. This allows the assessment of any food left over to be calculated allowing the practitioner to analyse daily nutritional intake with reasonable accuracy when compared to estimated diet records (Magkos & Yannakoulia, 2003). Usually respondents complete this method over a period of 7-days providing practitioners with an appropriate quantitative log of food intake (Hackett, 2009). Weighing of individual food items at the time of consumption may not be convenient for athletes before, during or after irregular training and eating schedules, alongside the regularity of eating requirements and lack of access to equipment to physically weigh the

food especially when athletes may be “on the move” (Thompson & Subar, 2008). To this end, it would require a highly motivated individual to weigh-out and weigh-in every food item from each meal over a 7-day period, which places a large burden on what is typically a already busy lifestyle. Lastly, the completion of a weighed food inventory places restrictions on normal social periods of the day which athletes use to relax with family and friends (i.e. dining out), altering normal eating behaviours and subsequently reducing the validity of the data.

2.5.5. Remote Food Photographic Method

A method that allows assessment during free-living conditions, whilst removing any emphasis on individuals estimating portion size, is the remote food photographic method (RFPM). This method allows athletes to capture pictures of individual nutritional intake and non-intake using smartphone applications and send to the researcher at the time of consumption, which then allows a time stamp for each meal. Normally the investigator will use reference or standard portions of known quantities of foods to estimate the portion size of the foods consumed by the athlete. This method has been found to be highly reliable when used to measure EI in adults (Williamson et al., 2003) and similar to two laboratory based validity tests with underestimation of EI by -4.7% and -5.5%, respectively, and -6.6% during free-living conditions (Martin et al., 2009). More recently, this method has been used in soccer (Anderson et al., 2017) and rugby players (Costello, Deighton, Dyson, Mckenna, & Jones, 2017). In particular, Costello et al. (2017) found this to be an accurate measure to assess the nutritional intake of elite RL players with a small mean bias for under reporting across a 96-h assessment when compared to a researcher-observed weight method. However, during this study, athletes were provided with all food and beverages to consume, and questions whether similar results would be seen if athletes were to purchase and consume their own food, which is far more reflective of what happens in a real-world rugby setting.

2.5.6. Dietary Analysis

Regardless of the nutritional intake collection method chosen, the next step is to analyse each meal and specific food type for nutritional value. This is performed by undertaking estimation of the energy and nutrient content from food composition tables or databases, which provide information on the average composition of particular food. Even with the most experienced practitioner, variable or erroneous coding is commonplace with analysing dietary intakes due to the need to match to the closest item in databases when specific foods are not listed, as well as errors in the food composition values on such databases (Larson-Meyer et al., 2018). Further errors include, systematic bias of food types that are missing from databases, in particular common sports foods or supplements that many athletes now consume regularly, misreading of the food records or error in data entry, and the difficulty in trying to analyse meals with multiple ingredients. Such errors have been highlighted with variability in estimates of energy and nutritional intakes from athletes over a 7-day period resulting in substantial differences in the variability of nutrients analysed (Braakhuis et al., 2003). As technology advancements have progressed, so too has the use of modern software such as Nutritics (Nutritics Ltd, Ireland) to quantify both pre-match nutritional plans and to assess normal EI in elite rugby players (Bradley et al., 2016b; Bradley, et al., 2015). Such software also allows manual addition of new food, meals and sport supplements including individual nutrient composition in an attempt to improve reliability. However, in instances where manual entries are not possible, practitioners may substitute the recorded food with another judged to have similar nutritional characteristic, although this practice increases inter-practitioner variability. Each practitioner should be trained in using computerised software in order to make accurate recommendations based on dietary analysis, otherwise professional interpretation when trying to quantify food intakes to match foods from online databases, may result in further errors (Braakhuis et al., 2003). Finally, practitioners should understand that all methods of EI collection have positives and negatives, and therefore to obtain the most accurate results, decisions of which method to use should be made in consideration of the research setting.

2.6. Total Energy Expenditure

TEE is reflected in heat production by the human body for daily function and can be broken down into the below key components:

1. Exercise – the energy expended during physical activities termed activity energy expenditure (AEE), ~15-30% of TEE, which will vary between individuals depending on intensity and duration of exercise (Binns, Gray, & Di Brezzo, 2015),
2. Energy intake – the energy expended through increased metabolism in order to digest, absorb and convert food termed diet-induced thermogenesis, ~10% of TEE (Heydenreich, Kayser, Schutz, & Melzer, 2017),
3. Processes essential for life – the energy expended for every day normal functions including resting metabolic rate (RMR), basal metabolic rate (BMR), non-exercise activity thermogenesis (NEAT) and non-exercise physical activity (NEPA) contributing to ~60-80% of TEE in humans (Ferro-Luzzi, 2005; Genton, Melzer, & Pichard, 2010; Keys, 1950),
    - BMR – the energy required to maintain the body's basic cellular metabolic activity and organ functions,
    - RMR – the energy required by the body in a resting condition (Ainsworth et al., 2000)
    - NEAT – energy expended through sub-conscious movement such as fidgeting (Levine, Schleusner, & Jensen, 2000)
    - NEPA – energy expended through non-formal, yet intentional movement, such as carrying a bag (Levine et al., 2000)

By definition, BMR should be measured under "resting conditions" including five hours of fasting and no physical activity. Because these conditions are rarely met in most athletic populations, RMR is more commonly used to represent EE in the applied practical setting. RMR tends to be the largest component (60-75%) of daily EE in humans (Speakman & Selman, 2003) and is often estimated using prediction equations (e.g. Cunningham, 1980), of which some are dependent on the accurate understanding of lean muscle mass. Although organs account for approximately 75% of resting EE, they only constitute 10% of total body

weight. In contrast resting skeletal muscle consumes only 20% of RMR but represents approximately 40% of total body weight. Adipose tissue consumes less than 5% of RMR but usually accounts for approximately 20% of body weight (Blundell et al., 2012) Although EE of metabolically active organs is responsible for a large component of resting EE, fat-free mass, which is composed primarily of skeletal muscle, accounts for most of the variability in EE between individuals. Although some equations have been validated in athletic populations (Cunningham, 1991; Ten Haaf & Weijs, 2014; Thompson & Manore, 1996) the lean mass of the athletes in these studies was 46-63kg which is substantially lower than the lean mass (62-82kg) previously reported in RL players (Harley et al., 2011; Smith et al., 2018). Therefore, the appropriateness of these prediction equations for RL players is questionable.

Advancements in technology has seen RMR measured in elite RL players using indirect calorimetry via metabolic carts (Smith et al., 2018). There are a number of factors that may result in unreliable participant RMR values during measurement with indirect calorimetry, including measurement protocol used, meal composition the evening prior and morning of assessment, caffeine intake, nicotine intake, exercise pre-assessment and measurement duration, all of which need to be considered if accuracy of data obtained is to be improved (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). For example, indirect calorimetry assesses the amount of heat generated indirectly according to the amount and pattern of substrate use and by-products produced. Therefore, nutrition intake alone can alter values obtained. In brief EE can be calculated by measuring the amount of oxygen used, and carbon dioxide released by the body:

$$\text{Substrate} + O_2 \xrightarrow{\text{oxidation}} CO_2 + H_2O + \text{Heat}$$

The specific amount of oxygen used can be measured and is called oxygen consumption (VO_2), whereas the amount of carbon dioxide gas produced by the cells is called carbon dioxide production (VCO_2). The total average daily EE in kilocalorie (kcal) is usually calculated using the modified Weir equation with substitution of the measured VO_2 and VCO_2 values usually including a urinary nitrogen component (Weir, 1949). However, within

the applied setting, the collection of urine samples may not always be available and considering the urinary component of EE only contributes to a small error of 1-2% in the calculation of final EE, an abbreviated equation is commonly used (Haugen, Chan, & Li, 2007):

Energy expenditure (kcal/day) = [($\dot{V}O_2$ X 3.941) + ($\dot{V}CO_2$ x 1.11)] x 1440

Following this, the ratio between $\dot{V}CO_2$ and $\dot{V}O_2$ can be defined as the respiratory quotient (RQ) which reflects substrate use. Use of different substrates is associated with different $\dot{V}O_2$ and $\dot{V}CO_2$ and therefore different RQ values are represented as seen in Table 2.4.

Table 2.4. *Comparison of oxygen uptake, carbon dioxide released, respiratory quotient and heat generation during oxidation of 3 main biological substrates (adopted from Haugen et al., 2007).*

Substrate	Oxygen consumed	Carbon dioxide produced	Respiratory quotient	Heat produced per g oxidized (kcal)
Glucose	0.746	0.746	1.00	3.75
Lipid	2.2029	1.430	0.69	9.30
Protein	0.966	0.782	0.81	4.30

2.6.1. Methods to Assess Total Energy Expenditure

Assessment of TEE would help inform appropriate training loads and nutritional requirements for RL players in an attempt to maximise performance (Fowles, 2006). Although the most precise method of analysis is direct calorimetry, its application is limited to the laboratory setting and, as such, is rarely used in studies with professional athletes. An overview of the advantages and disadvantages to each method is provided in Table 2.5. Measurement of EE commonly employed in both athletic and non-athletic populations can be assessed by commercially available portable wearable micro-technology units (e.g. heart rate monitors and GPS). Although financial costs and ease of use are positives to these

methods of measurement, individual calibration (Strath, Brage, & Ekelund, 2005) and increases in heart rate via thermoregulation (Brage, Brage, Franks, Ekelund, & Wareham, 2005) can largely affect the accuracy of such devices. Accelerometers that have inbuilt GPS can monitor duration of physical activity and distances covered at varying speeds. Tri-axial accelerometers, housed within these devices can also be used to rate the level of impacts in a collision by dividing the rate of the acceleration and deceleration on each axis by gravity (Roe, Halkier, Beggs, Till, & Jones, 2016), and the further inclusion of gyroscopes and magnetometers allows the assessment of orientation and rotation during acceleration and deceleration events (Gastin, Mclean, Breed, & Spittle, 2014). However, the use of GPS and accelerometers have been shown to underestimate EE when calculated using metabolic power (estimation of the metabolic 'internal' cost of activities from players' 'external' movements) in RL players (Highton, Mullen, Norris, Oxendale, & Twist, 2017), and is in agreement with other team sports (Buchheit, Manouvrier, Cassirame, & Morin, 2015; Walker, McAinch, Sweeting, & Aughey, 2016). Previous work by our group (Bradley, et al., 2015) and others (Zanetti, Pumpa, Wheeler, & Pyne, 2014) have utilised SenseWear Armbands to assess EE in rugby players. However, given that such armband devices are made of plastic and contain battery compartments it is not safe, nor feasible, to wear during water-based activity or contact activity and as such the data obtained through this device may not reflect the true energetic cost of rugby.

Table 2.5. *Methods to assess energy expenditure: Advantages and limitations of each, adapted from Pinherio Volp et al. (2011).*

Method	Advantages	Limitations
Direct calorimetry	Highly sophisticated method considered gold standard for measuring total energy expenditure (TEE)	Highly complex method, high cost and requires the confinement of the subject for 24-hr or more
Indirect calorimetry	This method is considered gold standard for measuring resting energy expenditure (REE) and resting metabolic rate (RMR). It is a non-invasive method, reasonably accurate and has high reproducibility. It also allows to quantify and to identify energy substrates oxidation. Allows short-term measurement of EE	High cost, relatively complex. Requires trained personnel for its correct use and would be difficult to administer in the field
Doubly labelled water	This is the gold standard method with accuracy between 97-99%. It measures TEE precisely in free living subjects and because it uses deuterium (H^2) and oxygen-18 (O^{18}), is a safe method.	It is costly and requires both sophisticated equipment and trained personnel. It does not provide information on energy expended during physical activity neither does it provide information about substrate oxidation. Cannot analyse day-to-day variations in EE

Accelerators and heart rate monitors	Inexpensive and lightweight method to assess total distances covered, distance covered at varied speeds and durations of exercise.	Accuracy depends largely on calibration of individual devices. Unable to detect changes in direction or collisions or eccentric contractions
Wearable micro technology (i.e. SenseWear Armbands)	A relatively cheap and reusable alternative to DLW. Day-to-day variations in EE can be observed, alongside specific snapshots in the day such as around tough training sessions	Potential damage to the device and/or subject whilst performing contact-based activity means it must be removed during these periods. Similarly, device must be removed when bathing or exposed to water
Physical activity records	Low cost method that estimates EE from an extremely detailed registry of all physical activity performed daily. Wide variety of types of activities listed which is frequently updated allowing the correction of or inclusion of typical activities from specific regions or countries	The comparison of results between different studies is limited due to various existing codes for activities. The estimated EE does not consider inter-individual differences which may affect the energetic cost of a movement

2.6.2. Doubly Labelled Water

Given the difficulty in measuring TEE in contact sport, it is now more common to see indirect calorimetry, in particular doubly labelled water (DLW), being implemented in professional athletic environments (Sagayama et al., 2017; Smith et al., 2018). For over three decades DLW has been used for the assessment of free-living EE in individuals (Schoeller & van Santen, 1982) whilst being acknowledged as the criterion or "gold standard" approach in free-living individuals (Buchowski, 2014). Originally, radioactive isotopes of hydrogen and oxygen were ingested, but for safety reasons non-radioactive forms of deuterium oxide and water containing the isotope oxygen-18 have now replaced these markers. The development of the DLW method originated in 1949 (Lifson, Gordon, Visscher, & Nier, 1949). Using the stable isotope of oxygen, researchers administered ^{18}O-labelled water to animals and showed that the ^{18}O-label appeared in expired CO_2. This clearly demonstrated that expired CO_2 was derived from body water. For this to occur, rapid isotopic equilibration occurred between the oxygen atoms of body water and CO_2 through the action of the enzyme carbonic anhydrase (Figure 2.1). Further experiments showed that total daily CO_2 production could be measured from the differential elimination of water labelled with stable isotopes of hydrogen and oxygen. After the administration of DLW, the labelled hydrogen ($^2H_2$) would be eliminated as water (^2H_2O), corresponding to water output, whereas the oxygen isotope would be eliminated as water ($H_2{}^{18}O$) and as expired carbon dioxide ($C^{18}O_2$). By measuring the difference between the elimination rates of labelled oxygen and hydrogen, the carbon dioxide production rate can be calculated. A plot of the change in concentrations of the two isotopes in body fluids, from which the rate of loss of these isotopes from the body fluid can be calculated, as shown in Figure 2.2. The carbon dioxide production rate is converted to EE by knowing the RQ of the food ingested during the observation period. One advantage of using this method in human populations is that the subjects can continue with habitual daily activities between the collection of initial and final samples which is clearly an advantage in a collision based sport like rugby, however due to financial restrictions (almost £1000 per sample) data on elite rugby players are somewhere limited.

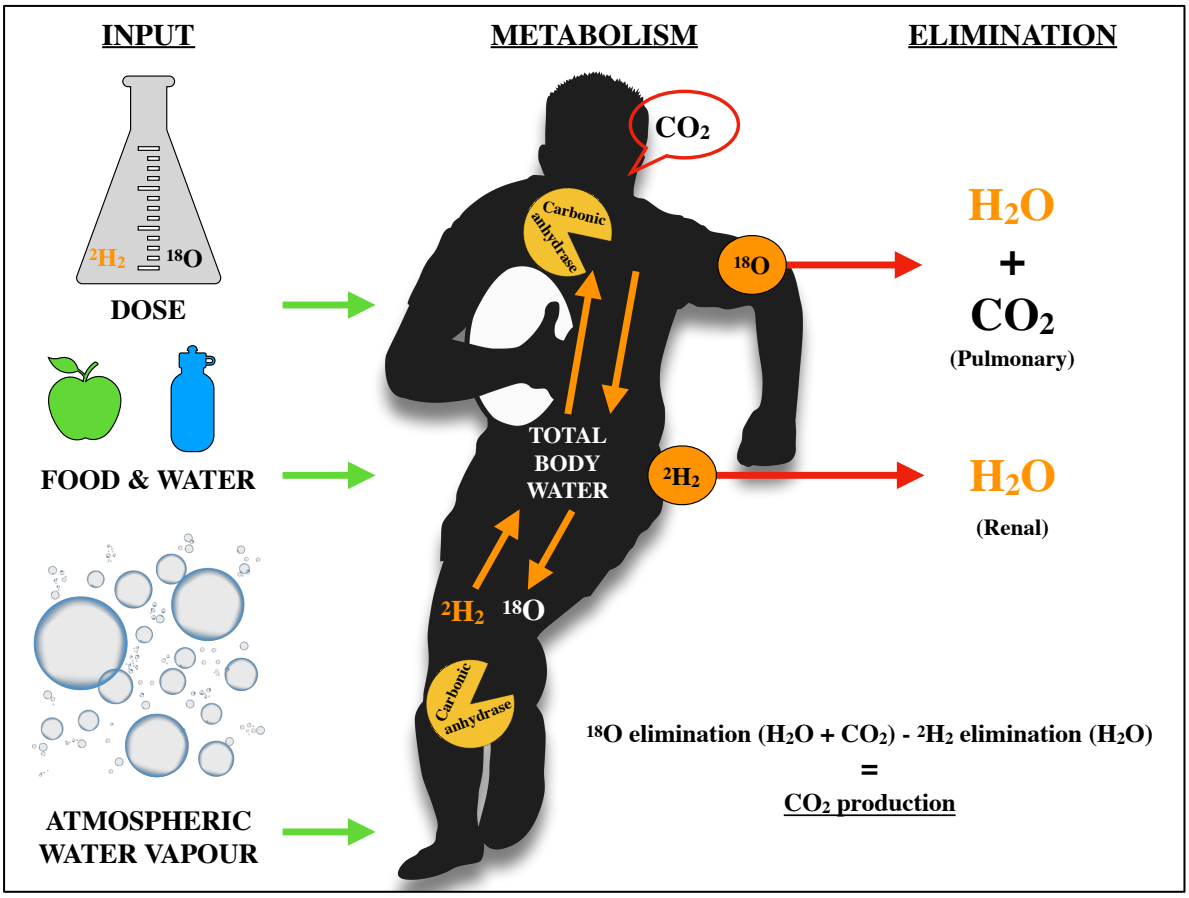


Figure 2.1. *The theory of doubly labelled water method*

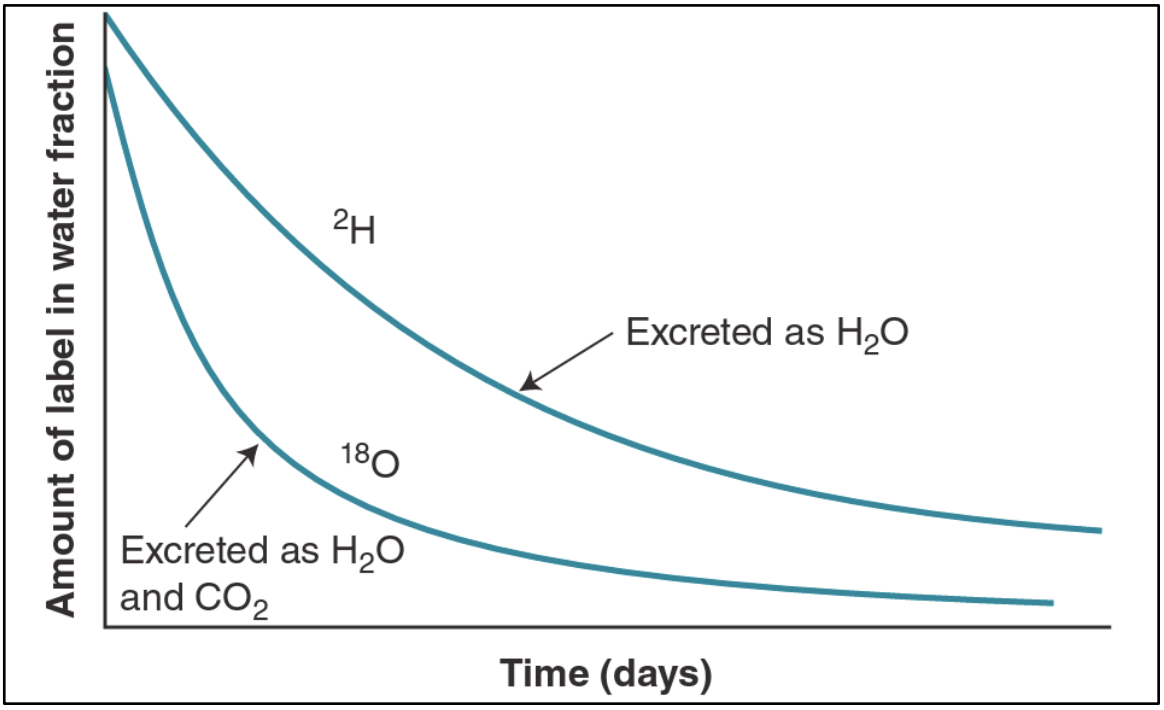


Figure 2.2. *Doubly labelled water technique. Excretion of ^{2}H (deuterium) and ^{18}O (oxygen) occurs at different rates. The more rapid the drop in ^{18}O relative to the drop in ^{2}H, the higher the EE (adapted from Ainslie, Reilly, & Westerterp, 2003).*

The desired dose of the isotopes is determined prior to consumption and is calculated according to body mass using the following equation:

$$^{18}O \text{ dose} = [0.65 \text{ (body mass, g)} \times \text{DIE}]/\text{IE}$$

Where DIE is the desired initial enrichment (DIE = 618.923 x body mass ($kg^{-0.305}$)) and IE is the enrichment of the injectate (10%) 100,000 part per million.

Although DLW is considered the gold standard and a non-invasive method accounting for all daily activities in free-living individuals (Schoeller et al., 1986; Westerterp, 2017) previous studies have only assessed TEE using DLW in academy players (Smith et al., 2018). DLW was used to examine in-season EE in rugby players (Smith et al., 2018). This study quantified TEE of 27 elite male English academy (U16 and U20) and senior (U24) RL and RU players during a 14-day in-season period. Predicted TEE, determined by published

equations, was compared to measured TEE by age group. Differences were found between TEE estimated by the Schofield, Cunningham and Harris-Benedict equations (Cunningham, 1991; Schofield, 1985) compared with DLW. Due to the large variability between individuals, negligible differences in TEE were observed between RL and RU and furthermore, approximately ~350-400 kcal·day^{-1} differences were reported between consecutive age groups from the same rugby code. Considering this study was performed using academy players, future work should now be performed on senior level RL players to assess TEE with players who possess greater body composition profiles than academy players. Additionally, the assessment of EI as well as TEE would provide much greater insights into whether senior players currently match TEE demands with adequate EI values.

Although DLW is considered the criterion method for the assessment of TEE, the measurement of TEE is assessed over a number of days or weeks, from which a mean daily TEE can be calculated. This results in a few limitations, in particular an inability to determine and therefore examine individual periods of elevated expenditure or individual training sessions (DeLany & Lovejoy, 1996). Moreover, considering the stable isotope enrichment is calculated individually to each participants total mass, these studies can carry huge financial cost, especially if subjects weigh in excess of 100kg which is common with professional elite level rugby players. Additionally, once the isotopes have been administered and samples collected, specialist expertise and equipment are required for the analysis of isotope concentrations in body fluids via mass spectrometry resulting in limited studies within elite rugby settings. Finally, it is suggested that a 5% error is introduced within field studies when the RQ is not known, because CO_2 production and not oxygen utilisation is being measured (Westerterp, 2017). Taken together, current literature agrees that measurement of free-living TEE via DLW, would provide the most accurate data in athletes and could be used for a reference technique for validating estimates of energy requirements obtained via other methods (Westerterp, 1999; Westerterp & Plasqui, 2004). Considering studies have only been performed with academy players (Smith et al., 2018), it is therefore crucial that future work should now be performed with senior level RL players to assess TEE in players who possess greater body composition profiles than academy players. Additionally, the

assessment of EI as well as TEE would provide much greater insights into whether senior players currently match TEE demands with adequate EI values.

2.7. Damage and Inflammation in Rugby League Players

For RL players, it is widely accepted that skeletal muscle damage and soreness is consistently experienced following training and matches (Fletcher et al., 2016; Twist, Waldron, Highton, Burt, & Daniels, 2012). The training and match demands routinely lead to EIMD from repeated high intensity exercise, often involving eccentric actions and IIMD from collisions with opposing players and resulting impacts with the playing surface. To date, the vast majority of the research into the damaging effects of RL match play has focused upon EIMD (Oxendale et al., 2016; Twist & Sykes, 2011), however there is a growing appreciation of the importance of IIMD (Naughton, Miller, & Slater, 2017). Both forms of muscle damage and their associated inflammations to skeletal muscle will be discussed below.

2.7.1. Aetiology of Exercise-Induced Muscle Damage

Given that professional RL involves many eccentric contractions and repeated high intensity muscle contractions, muscle damage or injury is inevitable (Clarkson & Hubal, 2002). If contractions are performed during longer muscle lengths, with greater forces (Nosaka & Sakamoto, 2001), and at faster angular velocities (Chapman, Newton, Sacco, & Nosaka, 2006), the magnitude of damage resulting from eccentric actions is amplified. The severity tends to range from mild EIMD through to varying degrees of muscular strain, muscular tears, significant contusions and lacerations (Järvinen, Järvinen, Kääriäinen, Kalimo, & Järvinen, 2005). Overload of mechanical stress via EIMD weakens the ultrastructure and membrane integrity of muscle tissue and typically presents as tender, aching or swelling muscles (Cheung Hume & Maxwell, 2003). Such myofibrillar disruption leads to the increased release of intracellular proteins (e.g. creatine kinase, myoglobin and interleukins) into the bloodstream (Hyldahl & Hubal, 2014), which have been used as indirect markers of the damage (Oxendale et al., 2016).

Following primary damage, a secondary mechanism commences to drive the removal, regeneration and remodelling of damaged tissue, and encourage skeletal muscle tissue adaptation to protect against subsequent muscle damaging exercise. This is characterised by an acute inflammatory response of specific binding proteins (i.e. neutrophils and macrophages) to the area of damage and the subsequent release of cytokines and other pro-inflammatory factors (Smith, Kruger, Smith, & Myburgh, 2008). The subsequent cascade of inflammatory proteins is a vital, well-orchestrated process that clears necrotic tissue, and initiates tissue repair and adaptation (Chazaud, 2016). Neutrophils are suggested to be the first immune cells to infiltrate muscle following the initial insult (Hyldahl & Hubal, 2014), activated in part by augmented intracellular Ca^{2+} signalling pro-inflammatory cytokine release (Butterfield, Best, & Merrick, 2006); in particular, the production of the pro-inflammatory cytokine interleukin-6 (IL-6), which is presented as the first cytokine in the circulation. IL-6 is synthesised and secreted by skeletal muscle during prolonged exercise and consistently increases more than any other IL (Fischer, 2006). Furthermore, IL-6 is the only myokine released into the circulation in appreciable concentrations (Chan McGee, Watt, Hargreaves & Fabbraio, 2004). IL-6 concentrations between 3-6 $pg{\cdot}ml^{-1}$ have been reported immediately following a single rugby match (Cunniffe et al., 2010) and following the cessation of repeated matches (Cunniffe et al., 2011) although this was performed in RU players. These values are similar to concentrations of IL-6 from semi-professional football players (Bell, Stevenson, Davison, & Howatson, 2016) and resistance-trained subjects (Nieman, et al., 2004), with concentrations returning to baseline in the acute hours following exercise (Hennigar & Pasiakos, 2017).

Secondary increases in IL-6 may be observed if tissue injury has occurred. This result in the movement of immune cells to infiltrate and secrete IL-6, encouraging the repair of skeletal muscle damage (Pedersen & Hoffman-Goetz, 2000; Pedersen, Steensberg, & Schjerling, 2001). This secondary release also signals the acute-phase response and tissue repair, represented by a lower magnitude, but more sustained release of IL-6 into the circulation. Following increases in circulating concentrations of IL-6, the release of IL-8 acting as a chemokine, and anti-inflammatory cytokine IL-10 begins (Ostrowski, Asp, Schjerling &

Pedersen, 1999) which is suggested to initiate the beginning of skeletal muscle recovery and regeneration. While there is research highlighting IL-8 and IL-10 changes following exercise in humans in sports such as semi-professional football (Bell et al., 2016) and marathon running (Ostrowski, Asp, Schjerling, & Pedersen, 1999), data in RL following training and match play is currently limited and warrants further research.

2.7.2. Aetiology of Impact-Induced Muscle Damage

In addition to EIMD from locomotive passages of play, another source of large physical stress and muscle damage involves training and match play interactions such as running into contact with the ball, tackles being received or delivered on an opposing player and subsequent contacts with the playing surface (Takarada, 2003). Differences between IIMD and EIMD revolves around IIMD interactions causing the potential to damage underlying muscle tissue at or adjacent to the receiving impact site.

In RL, high-impact collisions, associated with G forces as high as 10.1 G (McLellan & Lovell, 2012) cause extensive tissue trauma (Johnston, Gabbett, Seibold, & Jenkins, 2014) and associated inflammation (Cunniffe et al., 2010). Compressions to the muscle membrane typically occur from blunt force trauma when players collide with one another during tackles, but insults also occur from impact collisions with the playing surface itself. These can result in mild contusions through to large haematoma formation and clinically serious complications such as proliferation of bone within muscle tissue (Järvinen et al., 2005), disruption to capillary networks, intramuscular bleeding, oedemas and inflammation (Elmer et al., 2012). Such abuse results in both primary and secondary acute cellular injuries, encompassing direct myofibrillar damage, tissue necrosis and invasion of proinflammatory factors (Smith et al., 2008) illustrated in Figure 2.3.

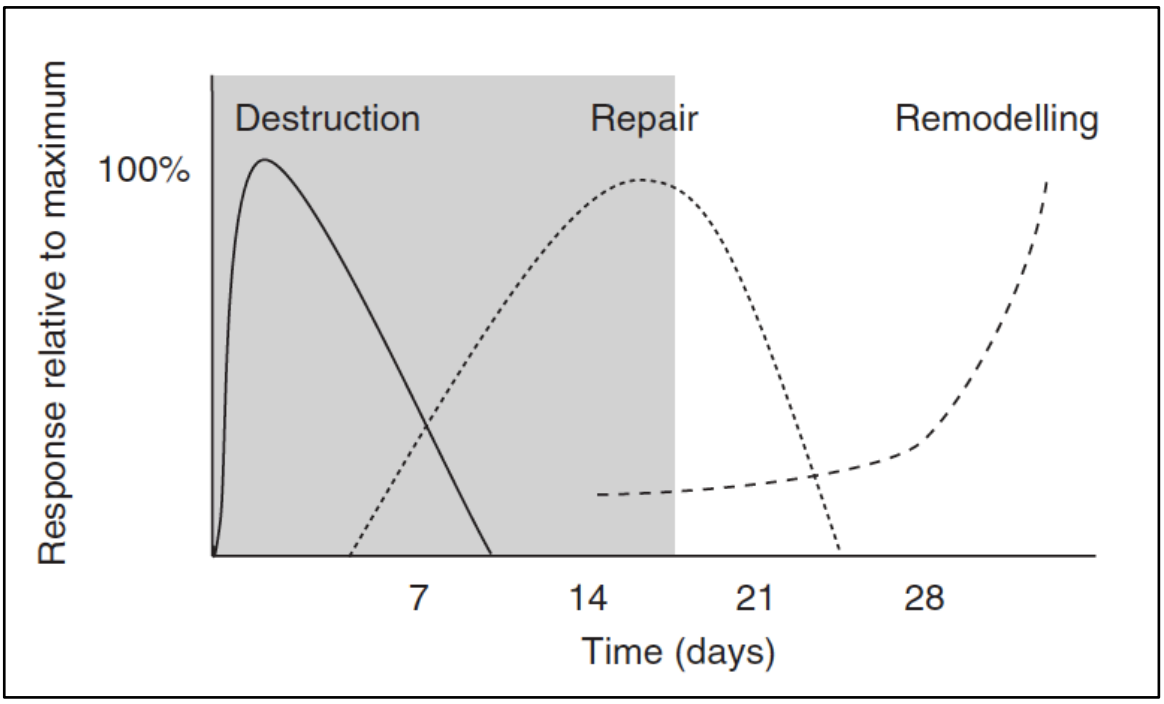


Figure 2.3. *Illustrative time-course of regeneration of injured muscle includes three chronologically overlapping, but distinct, physiological events: destruction, repair and remodelling (adopted from Smith et al., 2008).*

Mild contusions, similar to IIMD, result in augmented blood flow located in close proximity to the injured site in an attempt to facilitate muscle repair. During a well-orchestrated process, increased capillary permeability allows an increase in leukocyte number, levels of plasma proteins (Tidball, 2005) and the release of prostaglandins via inflammatory cells which are thought to sensitise local nociceptors to induce the common sensation of soreness (Hyldahl & Hubal, 2014). Localised myokines adjacent to the impact site, signal the migration of neutrophils, as immune cells, whose actions are implicated in free radical proliferation and secondary damage formation (Smith et al., 2008; Tidball, 2005). Clearly, a strong link between muscle damage and collisions is apparent. This is supported by *large* and significant associations between tackles and damage presented with increases in both myoglobin (r = 0.852) and CK (r = 0.922) (Oxendale et al., 2016; Takarada, 2003; Twist et al., 2012), the latter of which has been consistently identified in several subsequent investigations (Lindsay et al., 2016; McLellan et al., 2011; Oxendale et al., 2016; Shearer et al., 2015; Smart, Gill,

Beaven, Cook, & Blazevich, 2008; Twist et al., 2012). If injury is sustained to skeletal muscle then infiltration of immune cells would occur to encourage the removal, repair and regeneration of skeletal muscle damage.

2.7.3. Effects of EIMD & IIMD on Neuromuscular Function & Recovery

The recovery process between EIMD and IIMD is similar, although recovery from EIMD is often associated with accelerated recovery and decreased primary and secondary injury (Järvinen et al., 2005; Smith et al., 2008). Although EIMD and IIMD have similarities in regeneration and remodelling following injury, the infiltration of inflammatory markers and subsequent secondary damage response associated with IIMD is thought to differ to EIMD (Merrick, 2002). Decrements in neuromuscular function, following EIMD, are often reported using measures of various jump tests, sprint performance and dynamometry. For example in overreached players, maximal running speed is reduced by ~5% and ~10% following 5 and 6 weeks of intensified training (Twist & Highton, 2013). Moreover, CMJ performance is decreased following match simulation protocols using both a soft tackle bag (5.4%) and weighted tackle sled (4.9%) (Norris et al., 2016) Immune cell-derived IL-6 repairs skeletal muscle damage and enhances regeneration and growth after exercise. Although muscle derived IL-6 positively affects metabolism by stimulating glucose availability during exercise, if repeated bouts of exercise are performed without adequate rest and nutrition, this may result in detrimental performance outcomes, particularly if muscle glycogen concentrations are low before the onset of exercise i.e. as a results of inadequate recovery (Hennigar & Pasiakos, 2017). From a RL perspective, players perform training and match play demands throughout a 38-42-week season, potentially developing persistent elevations in circulating concentrations of IL's. Such persistent circulating IL's may result in catabolic effects and muscle atrophy, causing attenuations in muscle strength and function and increases in muscle soreness (Clarkson & Hubal, 2002). However, at present no research group has investigated this hypothesis following repeated match play and therefore this remains purely speculative. Taken together, nutritional practices leading into match play to

ensure adequate fuelling for and following match play to ensure appropriate and adequate recovery are of high importance for the professional RL player.

Typically, the extent of muscle damage is measured by assessing several indirect markers. The most appropriate indirect marker following eccentric exercise is reduced muscle force (Damas, Nosaka, Libardi, Chen, & Ugrinowitsch, 2016). Losses in the production of force after exercise and in the days following damaging exercise is suggested to be a consistent and robust marker of tissue damage (Hyldahl & Hubal, 2014). Data in RL shows strong relationships between match-related demands and decreases in jump flight time following match play (McLellan & Lovell, 2012; Oxendale et al., 2016; Twist et al., 2012) which is consistent with RU (West et al., 2014). Additionally, agility and sprint performance, two key locomotive patterns in RL match play, are reduced following EIMD, providing more evidence that performance of activities requiring rapid generation of force is impaired following muscle-damaging exercise (Highton, Twist, & Eston, 2009). Currently the literature examining IIMD in RL has been undertaken following single matches or match simulation protocols. This typically involves evaluation of IIMD on the neuromuscular function and recovery from one competitive match or simulated collisions. Significant decrements in functional measures have been observed including power and mean sprint time following IIMD compared to non-impact control conditions (Johnston, Gabbett, Seibold, et al., 2014) and is further shown in other contact simulations (Pointon & Duffield, 2012; Singh, Guelfi, Landers, Dawson, & Bishop, 2011).

Concomitantly, delayed onset of muscle soreness, which is suggested to be the most commonly assessed marker of damage (Warren et al., 1999) shows a consistent similar pattern with players reporting augmented levels of soreness in the acute hours and days following match play (Johnston, Gabbett, Seibold, et al., 2014; Oxendale, Twist, Daniels, & Highton, 2016; Twist et al., 2012). In particular, the work by Fletcher and colleagues (2016) provides robust data showing severe levels of muscle soreness in the first day after match play, improving in proceeding days and persisting for the entire season. This shows the consequence of frequent exposures to EIMD and IIMD and the negative effect this may potentially have on losses in muscle function and performance. For example, repeated

exposure to muscle damage can increase the chance of minor injury and feelings of tender, aching or swelling muscle's or in some circumstances muscular strain, muscular tears, contusions and laceration injuries (Cheung, & Maxwell 2003; Järvinen et al., 2005). Observations in RL have shown an average of 34 days are missed from training and match play following an injury (Fitzpatrick, Naylor, Myler, & Robertson, 2018). To this end, it is clear that increases in damage and soreness are going to be experienced following RL match play, and it is important for coaches to understand how this may affect recovery. However, research assessing the impacts of both EIMD and IIMD on muscle soreness and function during the recovery from match play are limited.

2.7.4. Adaptation to EIMD and IIMD

Where possible, it is important that players perform the correct training stimulus to elicit improvements in cardiorespiratory fitness, agility, ball skills, strength, power and body composition changes (Johnston, Gabbett, & Jenkins, 2014). This can be achieved through the implementation of periodised training programmes (Turner, 2011). However, it is just as important that players perform the correct amount of contact training during a pre-season period to ensure players are best prepared for the demands of repeated match play once the season begins. Anecdotally evidence from professional clubs suggests coaches adopt strategic timing and implementation of IIMD for players. For example, low velocity soft contacts with tackle bags combined with floor-based technique wrestling lessons at the beginning of pre-season periods, are a common feature with many Super League teams. As players progress through the pre-season, the intensity and trauma of collisions becomes amplified through greater accelerations into player-on-player contacts. IIMD developments of this nature continue towards the end of the pre-season phase accumulating with daily high-intensity full contact training sessions ready for the first game of the in-season period. The underlying rational behind such pre-season training evolves around the phenomenon of the repeated bout effect (RBE) (McHugh, Connolly, Eston, & Gleim, 1999) and the newly coined term, '*contact adaptation'* (Kraemer et al., 2009).

The RBE has been identified as a protective adaptation whereby prior exposure to eccentric exercise may alleviate a muscle's sensitivity to damage from consecutive bouts of similar intensity (Stupka, Tarnopolsky, Yardley, & Phillips, 2001). Such adaptations have been suggested to persist up to six months following the initial bout of damaging exercise and supported by significant reductions in force loss, muscle damage markers and soreness following a secondary bout (Naughton et al., 2017). It is believed that the remodelling of the extracellular matrix and adaptation in the inflammatory response contributes to the mechanisms of the RBE, although a unifying theory remains elusive due to many contributing factors including neural, mechanical and cellular changes (Hyldahl, Chen, & Nosaka, 2017; McHugh, 2003). Comparable adaptations from EIMD have been postulated following IIMD by way of compression or trauma to skeletal muscle and termed contact adaptation. In other contact sport populations, researchers reported no change in CK concentration immediately post-exercise in collegiate-level American Football players and hypothesised that, due to it being the final game of a 12-week season, an adaptation of IIMD occurred over the previous matches, resulting in decreased muscle damage (Hoffman et al., 2002). Challenging the idea of contact adaptation, is a previous study from our research group, whereby season-long subjective soreness responses to RL match play persisted all season. Taken together, this highlights that the use of CK as a marker of muscle damage is limited, especially given the known limitations of varying individual resting values and the ability of individuals to clear CK from the blood at different rates (Baird, Graham, Baker, & Bickerstaff, 2012). This data clearly demonstrates irrespective of the in-season period, i.e. from the start to the end of the season, players reported similarities in perceived soreness following match play (Fletcher et al., 2016).

Due to the high-intensity accelerations and repeated physical collisions of professional RL training and match play, conflation of both EIMD and IIMD exists. Therefore, the underlying mechanisms behind adaptive responses involving repair, regeneration and recovery is currently equivocal and complex. To truly investigate any adaptive response, performance-based metrics are necessary, and may provide a holistic model when combined with indices such as subjective soreness and markers of skeletal muscle damage. Furthermore, it has been suggested that the theory of EIMD may not be applicable to other forms of muscle damage

including that which occurs following blunt force trauma and thus supports the contention that IIMD is distinct from EIMD, with independent, but yet to be fully defined, changes in markers of inflammation and changes in muscle soreness and function. To this end, research assessing the magnitude of inflammation present following RL match play, concurrently with match play demands, muscle function and soreness should now be performed.

2.8. EIMD and IIMD Recovery Strategies

Identifying the best recovery strategies from training and match play demands is somewhat complex. It is clear the overall demands of a RL match lead to fatigue (McLellan et al., 2011; McLellan et al., 2011; Twist et al., 2012) often reported as sensations of tiredness and accompanying decrements in muscle performance and function. Moreover, known associated damage and inflammation is experienced following repeated accelerations, decelerations, eccentric muscle actions and impacts with opposing players and the playing surface. Additional contributions to tissue damage are suggested to arise from metabolic high-intensity exercise (Tee Bosch & Lambert, 2007). When combining this with the irregular number of days between games that is commonly seen during in-season periods, and the subsequent availability of rest days between matches, then making the correct decision on the most effective recovery strategy is multifaceted. As such practitioners currently adopt multiple recovery strategies, in an attempt to decrease the magnitude of initial damage and thereby reduce the subsequent inflammatory cascade. Some of the most common recovery strategies currently used in rugby in an attempt to reduce symptoms of swelling, soreness and damage include: compression garments (Duffield, Cannon, & King, 2010; Tavares, Smith, & Driller, 2017), cryotherapy (Selfe et al., 2014), cold water immersion (Tavares et al., 2018) and electromyo-stimulation (Beaven et al., 2013). At present, although evidence for these methods remains equivocal, an emerging area of research gaining wide interest for its purported effects on recovery strategies is the use of specific nutritional compounds found in normal food types.

2.9. Nutritional Strategies for Recovery

Nutritional interventions that accelerate recovery of muscle function and ameliorate soreness are used frequently in athletic populations and may be beneficial for athletes when rapid recovery between competitive events is necessary. In particular, protein, omega-3 polyunsaturated fatty acids and vitamins C, D, and E have all received great interest in recent years to enhance the adaptive response to exercise due to their proposed individual anti-inflammatory properties (Owens, Twist, Cobley, Howatson, & Close, 2018). Attention has also been given to those foods that may exert beneficial effects on reducing secondary phase skeletal muscle damage and will be discussed below.

2.9.1. Antioxidants

It has been suggested that antioxidants can reduce reactive oxidative species (ROS) and therefore decrease the oxidative stress imposed upon tissue (Howatson & van Someren, 2008; Sousa, Teixeira, & Soares, 2014). As such, these compounds might be a candidate for attenuating the secondary damage to tissue membranes caused from RL demands that may impact upon subsequent training and matches. Although a number of studies have examined the effectiveness of vitamin C and E supplementation in preventing or attenuating muscle damage, the findings of these studies are equivocal (Bryer & Goldfarb, 2006; Connolly, Lauzon, Agnew, Dunn, & Reed, 2006; Howatson & van Someren, 2008). With this, a recent shift towards examining 'functional foods' rich in antioxidant compounds known as polyphenols has taken place due to the potential synergistic solutions they may provide in managing the negative effects associated with RL demands.

2.9.2. Polyphenols

Polyphenols are abundant micronutrients which are found in many common foods consumed in the diet of humans (e.g. coffee, beans, wine, fruits and vegetables). They can be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another (Manach, Scalbert, Morand, Rémésy,

& Jiménez, 2004). Over the last decade, interest in food sources rich in polyphenols, mainly in plants, has increased due to findings that they exhibit antioxidant and anti-inflammatory properties in human and animal models (Bowtell, Sumners, Dyer, Fox, & Mileva, 2011). One of the chief reasons is the recognition of the antioxidant properties of polyphenols, and the great abundance in various food sources including fruits and vegetables typically consumed within a balanced diet (Manach et al., 2004).

2.9.1. Polyphenol Metabolism

Successful effects of dietary polyphenols rely extensively on achievable concentrations in the circulation after both ingestion and metabolism. Depending on origin, the structural diversity of dietary polyphenols can alter the overall bioavailability and therefore intestinal absorption of individual polyphenols (Marín, Miguélez, Villar, & Lombó, 2015). An understanding of the absorption of dietary polyphenols is an essential step for biological activity and effects on health, performance and recovery. In brief, following ingestion of flavonoids, sugar molecules are cleaved from the phenolic backbone in the small intestine and are absorbed here. Lactase phlorizin hydrolase or B-glucosidase acting as enzymes hydrolyse glycosylated flavonoids and then aglycones enter epithelial cells by passive diffusion (Marín et al., 2015). However, flavonoids linked to a rhamnose molecule must reach the colon and be hydrolysed here by the colon microbiota in order to proceed to its absorption (Figure 2.4).

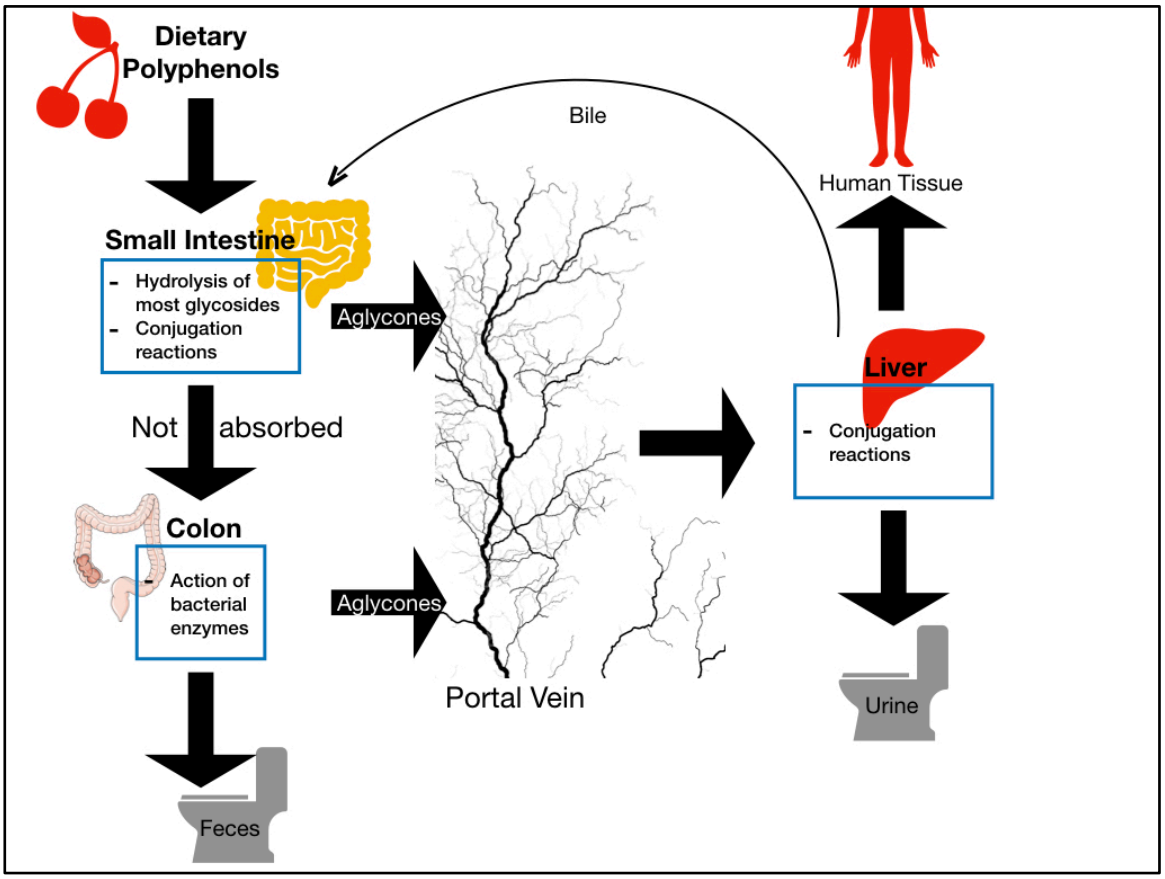


Figure 2.4. *Absorption and metabolism routes for dietary polyphenols and their derivatives in humans adapted from Marín et al., 2015.*

2.9.2. Polyphenol Effects on Recovery

Although polyphenols have been described as a nutrient source that can reduce damage to muscles, it is generally acknowledged that a dietary intervention is unlikely to interact with the primary phase of mechanical stress during an exercise bout (Bell, McHugh, Stevenson, & Howatson, 2014; Howatson & van Someren, 2008). A more likely interaction with the secondary inflammation and damage is much more widely accepted, whereby positive effects of polyphenol supplementation during the recovery phase can be seen. In particular, a rich polyphenol source and an emerging food of interest is Montmorency tart cherries (*Prunus cerasus*) which have been shown to improve strength recovery, inflammatory markers, oxidative stress markers, and soreness when compared to a placebo (Connolly, McHugh, & Padilla-Zakour, 2006; Howatson et al., 2010). Subsequently, the positive effects of

Montmorency tart cherry juice blends (Kuehl, Perrier, Elliot, & Chesnutt, 2010), and concentrates (Bell et al., 2014; Bell, Walshe, Davison, Stevenson, & Howatson, 2015; Bowtell, Sumners, Dyer, Fox, & Mileva, 2011) have been demonstrated following various exercise paradigms. Furthermore, following oxidative stress and inflammation as a result of marathon running (Connolly, McHugh, et al., 2006; Howatson et al., 2010), Montmorency tart cherry supplementation has been shown to attenuate post-race concentrations of IL-6 and, as such, the authors concluded that the phytochemicals were modulating EIMD. Another food rich in polyphenols are blueberries which have been found to supress oxidative stress-induced muscle damage *in vitro* (Hurst et al., 2010). Following 300 eccentric contractions of the quadriceps muscles with 10 female participants, indices of soreness, muscle damage and inflammation were not different between groups, although recovery of isometric torque and plasma oxidative capacity were significantly greater with a blueberry smoothie compared with a placebo (McLeay et al., 2012). Given that the current research is limited to studies on non-RL players, research now needs to be performed with RL players to realise the benefits of polyphenolic compounds in this population of athletes.

Although interventions using polyphenol supplementation show positive effects following EIMD, research on the effectiveness following IIMD is limited. In an animal model, following a contusion injury and supplementation with grape seed derived polyphenols, a decreased inflammatory cell response was seen, suggesting an accelerated recovery (Kruger & Smith, 2012). However, research investigating polyphenol supplementation following IIMD in humans is currently limited. The efficacy of polyphenols to exert beneficial effects in professional rugby players following both training and match play EIMD and IIMD is currently unclear and warrants research.

In several human studies, the active metabolite of polyphenols, anthocyanins, are shown to be rapidly absorbed but with low efficiency (Manach & Donovan, 2004). Single doses of 150 mg to 2 g of total anthocyanins were given to subjects, in the form of berries, berry extracts or concentrates. Measurements of anthocyanins in the plasma were low (0.01 – 0.02 μmol/L) and the mean time taken to reach maximum concentration was 90 minutes following ingestion. Observations from these studies therefore challenge the proposed effectiveness of

commonly consumed supplements by many athletes, for example the commercially available cherry concentrate supplement (Active Edge™, Cherry Active) which contains 320 mg anthocyanins per 30 ml serving. Of particular interest, although other research groups have shown positive effects on reducing soreness and improving performance outcomes following ingestion of Cherry Active® (Bowtell et al., 2011; McCormick, Peeling, Binnie, Dawson, & Sim, 2016) such observations were reported without measuring plasma concentrations of anthocyanins. Furthermore, the methods employed by these studies included an exclusion period of 5 days prior to exercise stress whereby subjects refrained from consuming any foods containing natural sources of polyphenols. This methodological design raises a several questions. Firstly, is excluding foods rich in natural polyphenols (i.e. fruit and mixed berries) really representative of what athletes do habitually in the real world? Secondly, are the positive effects seen therefore just a result of repleting an artificially-induced deficient polyphenol status or do supra-physiological doses of polyphenols offer additional protection?

Considering the great abundance and bioavailability in various food sources including fruits and vegetables normally consumed within a balanced diet by many athletes (Manach et al., 2004), future work should look to investigate the effectiveness of Cherry Active® supplementation versus a placebo group, whilst following normal habitual dietary intakes. In particular, such work would be much more representative of real-world RL environments (Close et al., 2019) and provide data on the effectiveness of commercial supplements versus real food following both training and competition demands which cause both EIMD and IIMD to players.

2.10. **Perspectives**

In summary, RL players require body composition profiles that reflect the modern-day player, however what these profiles look like at senior level across all positions and multiple teams is currently unknown. The pre-season period is a pertinent period of the whole season for coaches to implement training programmes that elicit body composition changes to allow players to begin the in-season period in the best possible condition. However, an

understanding of the time required for developing academy players to mature towards body compositions concomitant with senior players is yet to be defined. Furthermore, once players are selected and play at senior level, the possible changes in body composition that can occur within one pre-season period are currently poorly understood. In an attempt to maintain body compositions achieved during the pre-season, and inform appropriate nutritional intakes required, it is important that coaches understand the RMR and TEE of training and match play during the in-season period in senior RL players. During RL match play, it is well established that demands result in EIMD and IIMD, including blunt force trauma from repeated high impact collisions. Currently, the magnitude of inflammation caused from these demands is limited to measurements of CK which has known limitations, and as such, studies should look to alternative markers of inflammation, for example interleukins which are able to provide a more meaningful data. Finally, in an attempt to facilitate recovery, many team-sport athletes, including RL players, supplement Montmorency tart cherries into their diets. However, the efficacy of such supplements on reducing markers of inflammation, muscle soreness and function following RL demands is yet to be investigated and as such questions the use of supplements in elite athletes who may already be consuming polyphenolic rich diets from natural food sources.

CHAPTER 3.

GENERAL METHODS

This chapter describes general methodologies and theory of methodologies adopted in the investigations undertake for this thesis.

3.1. Ethical Approval and Location of Testing

The local ethical committee of Liverpool John Moores University approved all the studies in this thesis. All subjects were fully informed, both in writing and, verbally towards the nature of the testing procedures and were free to withdraw at any time during the studies. Training and match load data for study three (chapter 6) took place at Widnes Viking Rugby League home stadium (Figure 3.1). The training load data, 48 h pre-match and 48 h post-match blood collection for study four took place at Warrington Wolves Rugby League training facility (Figure 3.2) whilst the match load data and half-time and full-time blood sampling took place at Victoria Park stadium (Figure 3.3), Huddersfield Rugby Union stadium (Figure 3.4) and Castleford Tigers Rugby League stadium (Figure 3.8), respectively. Gatekeeper consent was attained, and risk assessments were thoroughly conducted for all venues.

Figure 3.1. *Widnes Vikings Rugby Club training and match day facilities used for study three. A 4G playing surface was utilised and provided a consistent training and playing surface.*

Figure 3.2. *Warrington Wolves Rugby Club indoor training facilities used for study five. A 4G playing surface was utilised and provided a consistent training and playing surface.*

Figure 3.3. *Victoria Park Stadium, Warrington. This outdoor pitch was played on for the first match in study five. Half-time and full-time blood sampling took place in the home changing room.*

Figure 3.4. *Huddersfield Rugby Union club. This pitch was played on during the second match in study five. Half-time and full-time blood sampling took place in the away changing room.*

Figure 3.5. *Castleford Tigers Rugby Club. This stadium was the facility where half-time and full-time blood samples were collected for the final match in study five. Samples were collected in the away changing room.*

3.2. Participants

All participants were male and free from any known illness or injury at the time of testing. The total number or players participating in these studies was 239. Overall 217 senior and 22 academy players were recruited from 6 different professional ESL clubs. The characteristics of all professional players for the 4 studies can be seen in Table 3.1.

Table 3.1 *Summary of subject characteristics from all 4 studies. All subjects in every study were professional players registered with the Rugby Football League. * denotes academy players. Data are mean ± SD.*

	N	Age (yrs)	Height (cm)	Weight (kg)
Study 1 (Chapter 4)	112	25 ± 5	179 ± 0.5	89.5 ± 7.7
Study 2	11*	18 ± 1	185 ± 0.6	93.2 ± 9.3
(Chapter 5)	99	25 ± 6	181 ± 0.8	95.1 ± 9.4
Study 3 (Chapter 6)	6	29 ± 3	182 + 0.2	94.9 ± 6.7
Study 4 (Chapter 7)	11*	18 ± 1	182 ± 0.4	92.0 ± 8.6

3.3. Assessment of Anthropometry

3.3.1. Dual Height and Weight Stadiometer

In all studies, wearing shorts only, players were weighed in the morning before the commencement of any training or club requirements. Weight and height were recorded using a dual body mass and height stadiometer (SECA, Birmingham, UK) to the nearest 0.1 kg and 0.5 cm, respectively. The mean coefficient of variation (CV) for body mass and height was 0.00 % and 0.23 %, respectively.

3.3.2. Dual Energy X-Ray Absorptiometry

During studies 1, 2 and 3 players underwent a whole-body fan beam DXA measurement scan (Hologic QDR Series, Discovery A, Bedford, MA, USA) analysed using QDR for Windows software version 12:4:3. The effective radiation dose was approximately 0.01 mSv per person with only shorts being worn and removal of all jewellery and metal objects ensured before each scan. Prior to each set of data acquisitions, calibration was carried out using an anthropometric spine and step phantom with a subsequent radiographic uniformity scan following the Hologic guidelines. All scans were performed and analysed by the same trained operator (Ionising Radiation Medical Exposure Regulations, 2006), according to both the college of Radiographers and in-house protocols to achieve high precision scans.

Players arrived at Liverpool John Moores University between 07:00 – 09:00 on the morning of each scan, approximately within 1-2 hours of waking in a fasted state and rested having not performed any exercise for the previous 16 hrs (Nana et al., 2015), having been instructed not to consume any food after 21:00 the previous evening. Players laid in a supine position on the DXA scanner bed and were positioned within the scanning area with arms by the side of the body, with the palmer surface of the hand facing and orientated towards the *vastus lateralis* muscle, fingers pointed and toes plantar flexed to ensure standard positioning. Foam blocks were placed between the palmer surfaces of the hand to ensure even spacing between the lateral aspect of the thigh. The duration of the scan was ~ 180 s and players were instructed to remain in position until otherwise instructed.

Scans were automatically analysed by the software, however the trained operator subsequently adjusted, if needed and confirmed regions of interest. In study 1, 2 and 3 the percentage of adipose tissue is reported as sub-total, i.e., whole body minus the head to provide stronger associations and reduced measurement error than with DXA defined total (whole body) adiposity, as previously used (Doran, Mc Geever, Collins, Quinn, McElhone, & Scott, 2014). Values were obtained for total mass (kg), lean mass (kg), fat mass (kg) and per cent body fat (%) data.

The mean CV and technical error of measurement whilst using DXA scan technology for whole body fat mass, lean mass and percent body fat has previously been reported as follows: 1.9 % and 0.37 kg, 1.0 % and 0.44 kg and 1.9 % and 0.41 %, respectively. Further, regional reliability estimate are also reported: upper limb fat mass (2.8 %, 0.06 kg), lower limb fat mass (2 %, 0.15 kg), trunk fat mass (1.9 %, 0.42 kg), upper limb lean mass (4.5 %, 0.05 kg), lower limb lean mass (2.8 %, 0.11 kg), trunk lean mass (3.2 %, 0.26 kg) (Milsom et al., 2015). Additionally, the mean CV of the scanner using software version 12:4:3 QDR for Windows during the testing period for the players in study 1, 2 and 3 was 0.37 %.

3.4. Quantification of Training and Match Load

3.4.1. Global Positioning Systems

During study 4, GPS micro-technology was used to evaluate relative and absolute number of tackles including physical collisions, and distance covered, as previously described (Bradley, Cavanagh, Douglas, Donovan, Twist, et al., 2015; Bradley, Cavanagh, Douglas, Donovan, Morton, et al., 2015). The selection of players to wear the GPS units was guided by the head coach of the squad on his recommendation as to those who would play the most minutes in the match and therefore provide the respective study with substantial data. During study 4, the Optimeye S5 (Catapult Innovation, Melbourne Australia) was fitted to all players prior to kick off. Acquisition of satellite signals and synchronisation of the GPS clock, with the satellite's atomic clock was performed 30 minutes prior to data collection (Maddison & Ni Mhurchu, 2009). Units were placed in a custom designed neoprene vest (Catapult Innovation, Melbourne, Australia) positioned between the scapulae with movements recorded sampling at 10 Hz. Tri-axial accelerometers and gyroscope sampling at 100Hz, provided data on the number of physical collisions. (see Figure 3.6).

Figure 3.6. *Catapult Optimeye S5 unit being fitted in a neoprene vest attached to players back during study four and five.*

3.4.2. Session Ratings of Perceived Exertion

Quantification of gym and pitch training loads in study 2 was assessed using the session rating of perceived exertion (sRPE; Foster et al., 2001), which has previously been used in both professional RU (Bradley, Cavanagh, Douglas, Donovan, Twist, et al., 2015) and RL (Lovell, Sirotic, Impellizzeri, & Coutts, 2013; Weaving, Marshall, Earle, Nevill, & Abt, 2014). Using a modified 10-point Borg Scale (Borg, Hassmén, & Lagerström, 1987) individual RPE's were provided by each player following cessation of each training session from which sRPE (AU) was calculated by multiplying RPE by duration of session for field and gym based training accumulatively. An example of a typical training week consisting of typical strength and conditioning, training, fitness and skills is shown in Table 3.2.

Table 3.2. *A typical in-season training week. Training days are shown in relation to game day rather than days of the week. Number in parentheses indicates the duration in minutes of the particular activity. Swimming was performed off site whilst all other activities were performed on site at the respective rugby club.*

	Game Day-5	**Game Day-4**	**Game Day-3**	**Game Day-2**	**Game Day-1**	**Game Day**	**Game Day +1**
AM	Swim (30) Weights (40)	Weights (40)	Rest	Mobility (15)	Captains Run (30)	Game	Recovery
Mid-AM	Skills (40)	Skills (30)	Rest	Power Weights (30)	Rest	Game	Recovery
PM	Rest	Rugby (45)	Rest	Rugby (45)	Rest	Game	Recovery

3.5. Psychometric Perceptual Responses

During study 4, based on methods adapted from others (Mclean et al., 2010), and those that have previously been used in rugby (Twist et al., 2012)**,** players provided ratings of perceived sleep quality, muscle soreness using a 1-5 Likert scale. In study 4 players provided perceptual responses 48 h pre-match and 48 h post-match. Higher values were indicative of a positive response to the question, while lower values reflected a negative outcome with similar scales showing good reliability and validity (De Vries Michielsen, & Van Heck, 2003). Players were familiar with this procedure as part of their normal club requirements and were instructed to complete individually to avoid any potential influence from other players or coaching staff.

3.6. Countermovement Jump and Drop Jump Performance

Countermovement jump height was estimated from an individual's flight time (s) (the difference between take-off and landing time) using two photoelectric parallel bars (Optojump™, Microgate, Bolzano, Italy) in study four (see Figure 3.7). All players were accustomed to jump procedures as part of the clubs regular monitoring process. Players began in an upright position and were instructed to place and keep both hands on their hips throughout testing. They were then required to flex the knees rapidly ~90° before jumping for maximal height and landing back onto the mat or floor. Take-off and landing position of the lower limbs was assumed to be the same, with any jumps that deviated from the described procedures repeated. Players performed three jumps with the maximum flight time used for later analysis in line with previous RL data (Twist et al., 2012), and recommendations (Cormack Newton, McGuigan, & Doyle, 2008). During the drop jump performance, players were allowed three jumps, dropping from a height of 30 cm each time. Similar to CMJ, players began in an upright position and instructed to place and keep both hands on their hips throughout testing. Players then were asked to step off the 30 cm box without lifting their centre of gravity, and land in-between the parallel bars before jumping as high as they could whilst minimising ground contact time, before landing on both feet to end the trial. The Optojump™ bars include resolution of 96 diodes, at a 1 kHz sampling rate.

Figure 3.7. *Countermovement jump and drop jump being performed by an academy player using two photelectric bars.*

3.7. Venous Blood Collection and Storage

Following ethical approval and informed consent, whole blood samples (10ml) were drawn from a superficial vein in the antecubital crease of the forearm using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson). In study 4, blood samples were withdrawn two days before and two days after the match (48 h pre-match and 48 h post-match) as soon as players arrived for normal club training commitments (see Figure 3.8).

Figure 3.8. *An example of study 4 blood samples being drawn from players. This occurred at 48 h pre-match and 48 h post-match time points.*

On match-day in study 4 blood samples were also collected during the normal 10-minute half-time interval period within the rules of the game and governed by the Rugby Football League. Following the full-time whistle, post-match blood samples were collected in the changing rooms within ~30 minutes of the match finishing, whilst players performed normal post-match routines and listened to match-feedback from the head coach. Successful blood collection was performed 48 h pre-match, half-time, full-time and 48 h post-match in study four during three consecutive in-season competitive scheduled fixtures (one home, two away), with a team of six trained researchers in attendance.

Blood samples were collected into serum separating tubes (Nu-care Products, UK), stored on ice (for up to a maximum of 3 hrs) until centrifugation. Centrifugation (Sigma 4- 16KS, SIGMA, Germany) was performed at 1500 RCF for 15 min at 4° C. Following centrifugation, duplicate aliquots of serum were stored at -80° C for later analyses of interleukins. All samples were recorded and tracked using the ProCuro database (see Figure 3.9).

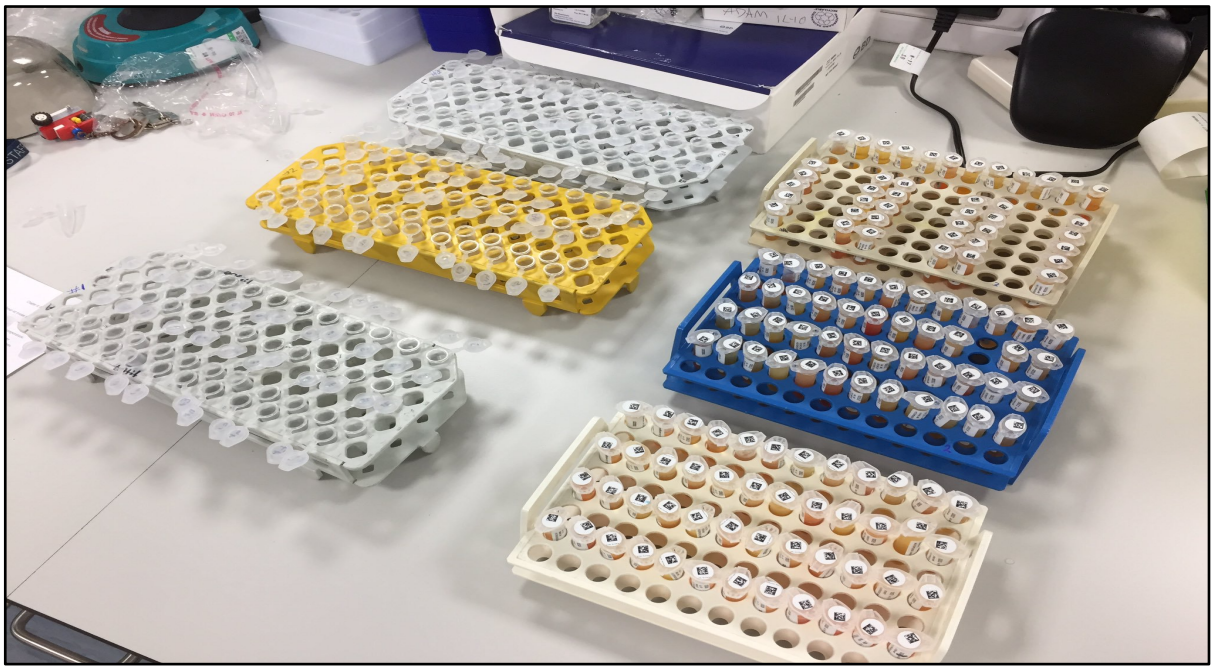


Figure 3.9. *Aliquots of samples ready for analysis. Bar code stickers on each lid represent the Pro Curo system in operation.*

3.8. Flow Cytometry

In study 4, interleukin concentrations were measured using flow cytometry. Flow cytometer is a tool which can be used for the interrogation of characteristics and phenotyping the cells. Flow cytometry aids in the identification of different cell types of a heterogenous population and can be coupled with cell sorting as an output application. When protein is combined with a dye that emits a fluorescent signal, a flow cytometer or fluorescence activated cell sorted (FACS) can measure the size and amount of fluorescence associated with a particular molecule analysing a light scatter. The investigations in this thesis used fluorescence activated cytometry using protocols developed by Becton Dickinson (see Figure 3.10), in this case, to allow multiplexing of analytes within the samples collected. The combination of antibody conjugated beads with the potential to be of differing sizes or to be conjugated to secondary antibodies linked to different fluorophores provides capacity to multiplex samples.

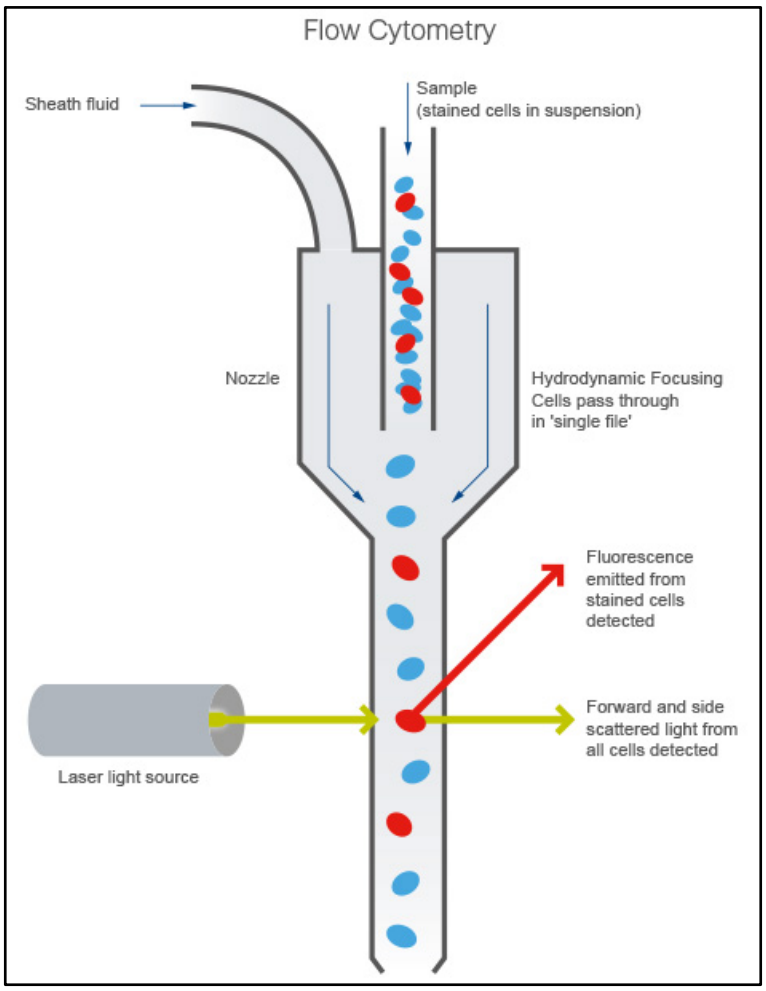


***Figure 3.10**. A schematic of fluorescence detection by a flow cytometer.*

Both the capture beads and detection reagents were prepared according to manufacture guidelines and were incubated with standards or unknown analytes, in which sandwich complexes (capture bead + analyte + detection reagent) are formed. These complexes can be measured using flow cytometry to identify particles with fluorescence characteristics of both the bead and the detector. This method significantly reduces sample requirements and experimental time in comparison with traditional ELISA techniques.

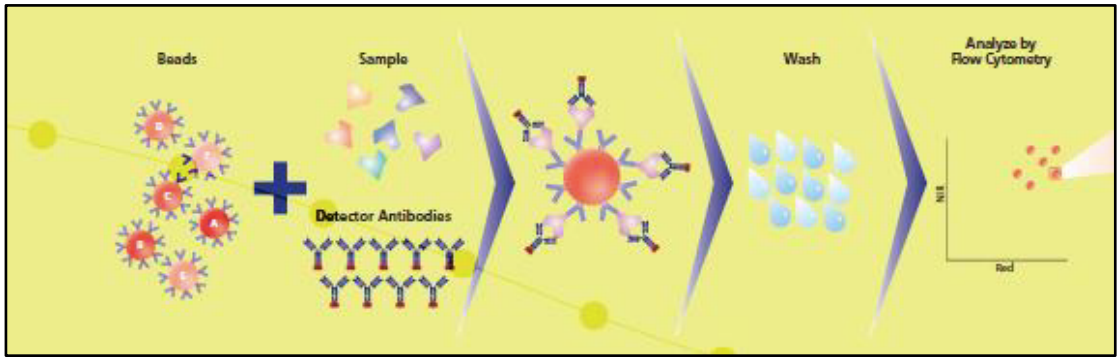


Figure 3.11 *Cytometric Bead Array (CBA).*

The capture beads for the proteins of interest (provided in BD CBA Human Soluble Protein Master Buffer Kit) were prepared according to manufacturing guidelines and final serum samples were analysed on a BD FACSCalibur™ (Becton Dickinson, Franklin Lakes, NJ, USA), supported by Cell Quest Pro Software (Becton Dickenson, Franklin Lakes, NJ, USA). An example of a sample being processed is shown in Figure 3.13. Data were uploaded from Cell Quest Pro and Filtered using FCS Filter™ and analysed using FCAP array software (Hungary Software Ltd, for BD Biosciences, San Jose CA, USA). According to manufacture's instructions, 200 events were captured per analyte per sample (see Figures 3.13 and 3.14).

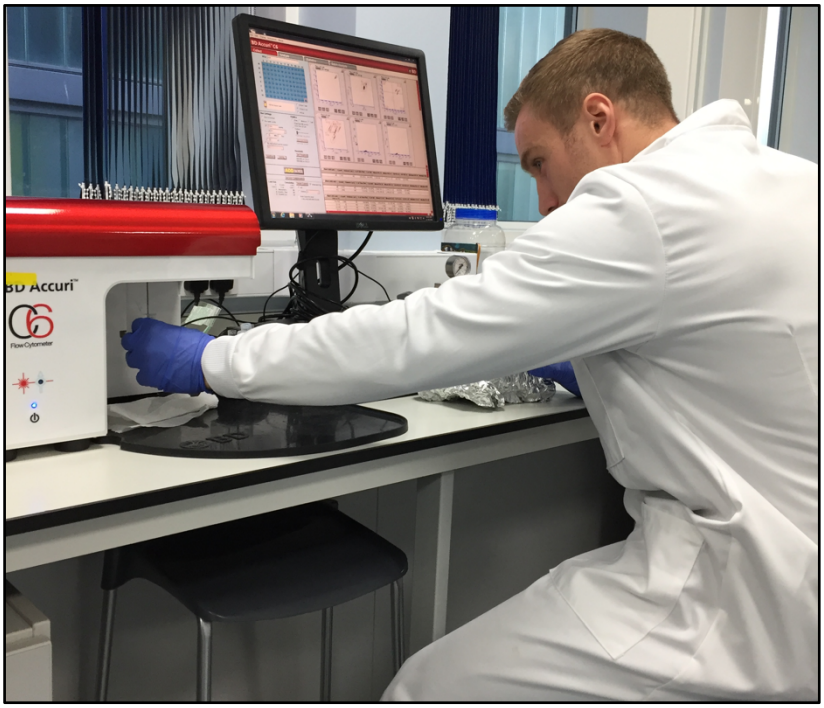


Figure 3.13. *Samples being run with 200 events using the Cell Quest Pro on the BC FACSCalibur.*

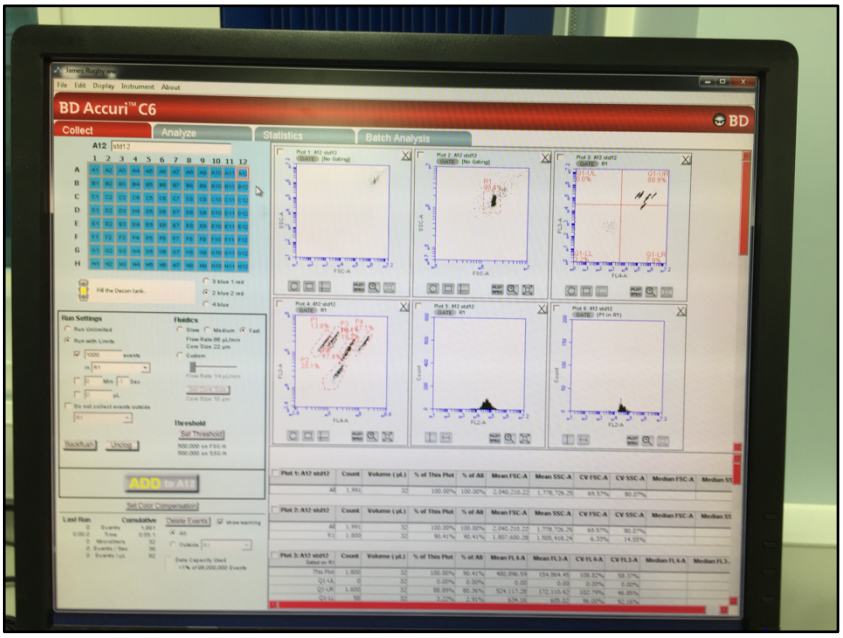


Figure 3.14. *An example of the data output from the Cell Quest Pro when running the interleukin samples at 200 events per analyte per sample.*

3.9. Statistical Analysis

In chapter four, differences between positional groups were compared using a one-way analysis of variance with least significant difference post hoc. Significance was set as $P < 0.05$ and all statistical analysis was conducted using SPSS v20 for Windows (IBM, New York, NY, USA). In all chapter's magnitude-based inferential (MBI) statistics were employed to provide a more meaningful explanation of the data in all studies. Data were analysed using the Cohen's effect size (ES) statistic ± 90% confidence limits (CL) and magnitude-based inferences, as suggested previously (Batterham & Hopkins, 2006). Thresholds for the magnitude of the observed change for each variable was determined as the between participant standard deviation (SD) in that variable x 0.2-0.6, 0.6-1.2 and 1.2-2.0 for a *small*, *moderate* and *large* effect, respectively (Hopkins, Marshall, Batterham, & Hanin, 2009). Threshold probabilities for a meaningful effect based on the 90% confidence limits (CL) were: <0.5% *most unlikely*, 0.5-5% *very unlikely*, 5-25% *unlikely*, 25-75% *possibly*, 75-95% *likely*, 95-99.5% *very likely*, >99.5% *most likely*. Effects with confidence limits across a *likely small* positive or negative change were classified as *unclear* (Hopkins et al., 2009). All calculations were completed using a predesigned spreadsheet (Hopkins, 2006).

Recently the use of MBI's has received critiques due to the increased chance of false positive rates compared to traditional hypothesis testing (Welsh & Knight, 2015). However, the decision to use MBI's and effects sizes throughout this thesis was made on the basis of providing physical magnitudes and applicable meaningful percentage changes to the data presented. If presented with confidence limits, the authors suggest more meaning can be drawn from presented data (Batterham & Hopkins, 2006). Moreover, it is hoped that the data in this thesis will be read and interpreted by support staff working with professional players and players themselves. Taken together, the use of traditional null hypothesis testing, drawing a line in the sand to report something to be significant or not, would not be useful for those trying to apply the data with professional rugby players.

CHAPTER 4.

POSITION SPECIFIC DIFFERENCES IN THE ANTHROPOMETRIC CHARACTERISTICS OF ELITE EUROPEAN SUPER LEAGUE RUGBY PLAYERS

This chapter explores the position specific body composition profiles of 112 players from five different European Super League clubs. Findings report all positional groups to be similar in anthropometric characteristics apart from the Prop forwards, suggesting an ideal position specific body composition profile is difficult to establish.

This study was published in the European Journal of Sport Science in 2015.

Morehen, J. C., Routledge, H. E., Twist, C., Morton, J. P., & Close, G. L. (2015). Position specific differences in the anthropometric characteristics of elite European Super League rugby players. *Eur J Sport Sci,* 15(6), 523-529.

4.1. Abstract

RL is a collision sport which traditionally adopts a large emphasis on lean muscle mass. Currently there is limited research on the anthropometry of ESL players. The aim of this study was to assess body-composition using DXA scans to identify the typical profile of elite rugby league players. One hundred and twelve players from five different clubs competing in the ESL were recruited for the study. DXA scans were performed and the total mass, lean mass, fat mass and percentage body fat were reported for each positional group. For the Fullback and Winger's, Centre's, Halfback's, Hooker's, Prop's and Back Row Forward's the mean (SD) body fat % was 13 (2.1), 13 (2.4), 12 (3.4), 15 (3.9), 16 (4.3) and 15 (2.1) %, respectively, and total mass was 86 (8.2), 91 (6.6), 81 (8), 84 (9.5) 102 (8.5) and 93 (5.5) kg, respectively. Despite *small* to *very large* inter-positional differences in all anthropometric variables (effect sizes = -0.08 to 2.56), particularly between the Prop and the other playing positions, there was large intra-position variation in body fat, lean mass and total mass making a standardized position specific profile difficult to establish. When used with other key performance indicators, these data provide the first multi-team anthropometric profile of elite ESL players that can be used to guide individualized training and nutrition practices for current and aspiring players.

4.2. Introduction

In section 2.1.1, we show it is well known that the various playing positions in RL require unique physical qualities based on their specific roles (Gabbett & Seibold, 2013; Meir et al., 2001). While outside backs (~7,000 m) cover greater absolute distances than adjustables (~6,000 m) and forwards (~4,000 m) (Gabbett, Jenkins, Abernethy, 2012; Waldron et al., 2011) , total distance covered relative to match time ($m \cdot min^{-1}$) is similar between positions (~90-95 $m \cdot min^{-1}$) (Gabbett, Jenkins, Abernethy, 2012; Waldron et al., 2011). On average, forwards are also involved in around one physical collision (tackle or being tackled) with the opposition per minute of playing time, whereas this occurs less frequently for outside backs (~0.3 min^{-1}) and adjustables (~0.6$\cdot min^{-1}$), respectively (Gabbett, Jenkins, Abernethy, 2012; Twist et al., 2012). Props are required to carry the ball forward into the defence, make distance and tire opposing defenders, meaning coaches prefer these players to have high total body and lean mass. Conversely, wingers require acceleration and speed qualities to evade defenders and, as such, are often lighter than other positions within the team (Cheng et al., 2014b). These differences in anthropometric characteristics are therefore observed between positional groups in both elite (Lundy et al., 2006; Meir et al., 2001b) and sub-elite (Gabbett, Kelly, et al., 2008) players. Low skinfold thickness (as a proxy marker of body fat) is one of the most important discriminators between selection and non-selection in senior elite NRL players (Gabbett, 2009; Gabbett et al., 2011b) and differentiates between higher and lower playing standards (Gabbett et al., 2011b; Till et al., 2011). Higher skinfold thicknesses and lower estimated lean mass are also related to poorer tackling ability (Gabbett et al., 2011b). In juniors, anthropometric data have been used to predict player selection in the UK, highlighting the importance of body composition to talent development (Till et al., 2013).

Despite the apparent importance of body composition to success in RL, to date there are limited studies reporting these measures in elite ESL players from a number of teams. Indeed, with coaches adopting different styles of play, player profile preferences and tactics during matches, it is important to assess players from more than one team from within the ESL (Georgeson et al., 2012; Harley et al., 2011; Jones et al., 2015). Additionally, within one club there may be a limited number of players for each playing position and so a typical body

composition profile is hard to establish. Furthermore, many of the previous studies assessing body composition in RL players have only utilised skinfold measures and predictive equations, which have obvious limitations (Doran et al., 2014; Reilly et al., 1995). While previous studies have used DXA scan technology to assess the body composition in elite RL players, these have not differentiated between positional groups (Harley et al., 2011; Kelly et al., 2012), or have been on Australian NRL players (Georgeson et al., 2012). A profile of body composition characteristics in a large group of players from a variety of ESL teams would therefore be useful to enable position specific anthropometric characteristics to be established. Such data might then be used for talent identification and to assist in individual training and nutrition practices. Accordingly, the aim of this study was to assess the anthropometric data of elite RL players taken from several ESL teams to identify the typical positional profiles.

4.3. Methods

4.3.1. Participants

One hundred and twelve elite RL players currently playing in the ESL volunteered for this study. Data were collected on the first team squad members of five teams. Players were categorized into six positional groups based on where they played at club and international standard, these being: Fullback and Winger's (24), Centre's (10), Halfback's (18), Hooker's (10), Prop's (24), and Back Row Forward's (26). If players played in multiple positions, they were asked to self-select their predominant position. All testing took place during the final weeks of pre-season or in the first two weeks of the season in accordance with the availability of the selected clubs. All players from the same club were tested on the same day. The local ethics committee of Liverpool John Moores University granted ethical approval for the study.

4.3.2. Study Design

All players attended the university laboratory between 07:00-10:00 in a fasted and hydrated condition having refrained from exercise in the previous 12 h. Players height and weight was

recorded using a dual height/body mass stadiometer (see section 3.3 for height/body mass assessment). Players then underwent a whole-body fan bean DXA measurement scan (see section 3.3 for methods of body composition measurement). Scans were analysed using QDR for Windows software version 12:4:3 by the same trained operator, according to in-house protocols to achieve high precision scans.

4.4. Results

The physical and anthropometric characteristics of 112 elite ESL players by position can be seen in Tables 4.1 and 4.2.

4.4.1. Height

There was a main effect of player position on height ($F_{5, 108} = 14.07$, $P < 0.0005$), with *post-hoc* analyses revealing *small* to *large* differences between positional groups. The Fullback and Winger's were shorter than Centre's ($P = 0.019$; effect size = -0.77), Prop's ($P < 0.0005$; effect size = -1.20) and Back Row Forward's ($P = 0.01$; effect size = -0.96), but taller than Halfback's ($P = 0.015$; effect size = 0.65) and Hooker's ($P = 0.012$; effect size = 0.96). Centre's were taller than Halfback's ($P < 0.0005$; effect size = 1.33) and Hooker's ($P < 0.0005$; effect size = 1.77), but similar to Prop's ($P = 0.458$; effect size = -0.29) and Back Row Forward's ($P = 0.846$; effect size = -0.08). Halfback's were shorter than Prop's ($P < 0.0005$; effect size = -1.77) and Back Row Forward's ($P < 0.0005$; effect size = -1.56), but similar in height to Hooker's ($P = 0.671$; effects size = 0.14).

Table 4.1 *Mean (±SD) [range] for age and height of Professional rugby league players by position. Differing letters denote significant difference from other.*

Position	Fullback and Winger (*n* = 24)	Centre (*n* = 10)	Halfback (*n* = 18)	Hooker (*n* = 10)	Prop (*n* = 24)	Back Row Forward (*n* = 26)
Age (y)	25 (±5) [16-35]	24 (±5) [18-34]	25 (±6) [16-37]	26 (±6) [18-33]	24 (±5) [18-37]	24 (±5) [19-34]
Height (cm)	181.1 (±5.5)[a] [171-191]	185.4 (±5.7)[b] [176-197]	177.1 (±6.7)[c] [165-192]	176.3 (±4.5)[c] [170-185]	186.8 (±3.9)[b] [180-194]	185.8 (±4.2)[b] [178-198]

* Halfbacks include the stand-off and scrum-half positions combined. Back Row Forward includes the second row and lock forwards combined.

4.4.2. Total Mass

There was a main effect of player position on total mass ($F_{5,108} = 20.74$, $P < 0.0005$), with *post-hoc* analyses revealing *small* to *very large* differences between positional groups. While Fullback and Winger's were not different to Hooker's ($P = 0.44$; effect size = 0.25) or Centre's ($P = 0.062$; effect size = -0.71) they had lower total mass than Prop's ($P < 0.0005$; effect size = -1.95) and Back Row Forward's ($P = 0.001$; effect size = -1.06), but higher total mass than Halfback's ($P = 0.048$; effect size = 0.59). Centre's total mass was not different to Back Row Forward's ($P = 0.448$; effect size = -0.35), but greater than Halfback's ($P = 0.001$; effect size = 1.38) and Hooker's ($P = 0.025$; effect size = 0.92), and lower than Prop's ($P < 0.0005$; effect size = -1.45). Halfback's total mass was lower than Prop's ($P < 0.0005$; effect size = -2.56) and Back Row Forward's ($P < 0.0005$; effect size = -1.78), but not different than Hooker's ($P = 0.374$; effect size = -0.30). The total mass of the Prop's was higher than Hooker's ($P < 0.0005$; effect size = 2.05) and Back Row Forward's ($P < 0.0005$; effect size = 1.24).

4.4.3. Lean Mass

There was a main effect of player position on lean mass ($F_{5,108} = 16.58$, $P < 0.0005$) with *post-hoc* analyses revealing *small* to *very large* differences between positional groups. Fullback's and Winger's possessed lower lean mass than Centre's ($P = 0.046$; effects size = -0.71) and Back Row Forward's ($P = 0.008$; effect size = -0.77), although their lean mass was greater than Halfback's ($P = 0.033$; effect size = 0.61). There was no significant difference in lean mass between Fullback's and Winger's and the Hooker's ($P = 0.087$; effect size = 0.58). Centre's had higher lean mass than Halfback's ($P < 0.0005$; effect size = 1.44) and Hooker's ($P < 0.0005$); effect size = 1.42), but had lower lean mass than Prop's ($P = 0.007$; effect size = -1.01). There was no difference in lean mass between Centre's and Back Row Forward's ($P = 0.944$; effects size = -0.02). Halfback's had similar lean mass values to Hooker's ($P = 0.904$; effect size = -0.03), but possessed lower lean mass than Prop's ($P < 0.0005$; effect size = -2.26) and Back Row Forward's ($P < 0.0005$; effect size = -1.54).

4.4.4. Fat Mass

There was a main effect of player position on fat mass ($F_{5,108} = 9.93$, $P < 0.0005$), with *post-hoc* analyses revealing *small* to *large* differences between positional groups. Fullbacks and Winger's had lower fat mass than Prop's ($P < 0.0005$; effect size = -1.41) and Back Row Forward's ($P = 0.01$; effect size = -1.25), but were not different to Centre's ($P = 0.571$; effect size = -0.33), Halfback's ($P = 0.456$; effect size = 0.28) and Hooker's ($P = 0.237$; effect size = -0.44). Centre's had lower fat mass than Prop's ($P < 0.0005$; effect size = -1.19), but were similar to Halfback's ($P = 0.252$; effect size = 0.53), Hooker's ($P = 0.597$; effect size = -0.21) and Back Row Forward's ($P = 0.143$; effect size = -0.80). Halfback's had lower fat mass than Prop's ($P < 0.0005$; effect size = -1.47) and Back Row Forward's ($P = 0.002$; effect size = -1.24) but were not different to Hooker's ($P = 0.084$; effect size = -0.59).

4.4.5. Body Fat Percentage

There was a main effect of player position on body fat percentage ($F_{5,108} = 50.7$, $P < 0.0005$), with *post-hoc* analyses revealing *small* to *moderate* differences between positional groups. Fullback's and Winger's had a lower body fat percentage than Prop's ($P < 0.0005$; effect size = -1.06) and Back Row Forward's ($P = 0.038$; effect size = -0.86), but were not different to Centre's ($P = 0.97$; effect size = 0.0), Halfback's ($P = 0.686$; effect size = 0.14) and Hooker's ($P = 0.088$; effect size = -0.61). While Centre's had lower body fat percentage than Prop's ($P = 0.002$; effect size = -1.03), they were not different to Halfback's ($P = 0.715$; effect size = 0.14), Hooker's ($P = 0.153$; effect size = -0.59) or Back Row Forward's ($P = 0.109$; effect size = -0.80). Halfback's were similar to Hooker's ($P = 0.052$; effect size = -0.63), but had lower body fat percentage than Prop's ($P < 0.0005$; effect size = -1.03) and Back Row Forward's ($P = 0.02$ effect size = -0.78).

Table 4.2. *Mean (±SD) [range] total mass, lean mass, fat mass and body fat percentage values of Professional rugby league players by position groups. Differing letters denote significant difference from other.*

Position	**Fullback and Winger (*n* = 24)**	**Centre (*n* = 10)**	**Halfback (*n* = 18)**	**Hooker (*n* = 10)**	**Prop (*n* = 24)**	**Back Row Forward (*n* = 26)**
Total Mass (kg)	85.9 (±8.2)a [71-99]	91.2 (±6.6)ab [79-103]	81.1 (±8.0)c [65-91]	83.7 (±9.5)ac [69-98]	102.2 (±8.5)d [89-128]	93.3 (±5.5)b [85-106]
Lean Mass (kg)	71.9 (±6.6)ac [61-82]	76.1 (±5.1)b [66-86]	68.0 (±6.1)c [55-78]	68.2 (±6)c [60-77]	81.8 (±6.1)d [71-99]	76.2 (±4.4)b [69-86]
Fat Mass (kg)	10.9 (±2.2)a [7-17]	11.7 (±2.6)ac [8-16]	10.1 (±3.4)ac [6-16]	12.5 (±4.6)ac [7-22]	16.8 (±5.5)d [10-35]	13.6 (±2.1)c [9-18]
Body Fat (%)	12.7 (±2.1)a [9-17]	12.7 (±2.4)ac [9-17]	12.3 (±3.4)ac [8-19]	14.6 (±3.9)ac [10-23]	16.3 (±4.3)d [11-27]	14.5 (±2.1)c [10-18]

* Halfbacks include the stand-off and scrum-half positions combined. Back Row Forward includes the second row and lock forwards combined.

4.5. Discussion

The primary aim of the present study was to characterize the anthropometric characteristics of elite ESL players using DXA scan technology in an attempt to identify position specific profiles. To this end we recruited and tested 112 RL players from five different ESL first team squads (all with different nutrition and strength coaches) to ensure a heterogeneous player group. We report *small* to *very large* anthropometric differences between playing positions and, perhaps more importantly, there was considerable variation in anthropometric characteristics within playing positions. These data therefore provide a base for talent identification and player profiling, although caution must be taken given the prevalence for deviations from the standard position profile.

As expected, differences in body fat (percentage and total) between the Outside Backs (i.e. Fullback, Wingers, Centres) and Forwards (i.e. Props, Back Row) were typically *small* to *large* in magnitude, with Prop forwards showing larger body fat values than all other playing groups. These findings reaffirm previous data using skinfolds that show forward players being the heaviest and with the greatest body fat (Cheng et al., 2014b; Gabbett, 2002; Morgan & Callister, 2011; Till et al., 2013). The additional fat mass in the Props likely represents the unique positional demands of this group of players, who are required to withstand high speed physical collision on a more regular basis than the other groups (Twist et al., 2012). Moreover, the detremental effects of higher body fat on athletic performance in the Props might be compensated by the fact that they have less game time than the other groups through tactical substitutions (Gabbett, Jenkins, Abernethy, 2012; Waldron, Highton, Daniels, & Twist, 2013).

The present data also confirms the large intra-position variation in body composition (Cheng et al., 2014b; Gabbett, 2006). For example, whilst the mean percentage body fat of the Hookers was ~14%, the maximum percentage body fat was as high as 23% and the lowest as little as 10%. Whilst one could argue that reducing body fat might be beneficial to performance in players that exhibit higher than average values (i.e. an improved power-to-

mass ratio), extra fat mass might be advantageous in collisions. However, this suggestion remains speculative and requires further investigation. These data also suggest that an ideal body fat value for elite RL players within a positional group does not exist. Accordingly, the assessment of body fat might be best employed as a monitoring tool to track individual changes due to nutritional or training interventions, rather than as a selection tool. These data also suggest that body fat assessment in RL should be used as a confirmatory tool rather than diagnostic, and that coaches should be encouraged to assess the whole physiological profile.

One advantage of assessing body composition using DXA is that lean mass can also be calculated. As anticipated, *small* to *very large* position specific differences were observed in the present study with the Prop Forwards demonstrating the greatest lean mass and the Halfbacks the lowest. However, as with body fat, there was again large intra-position variation with as much as a 35 kg difference in lean mass within some playing positions. Such a range in lean body mass makes a 'typical' or 'ideal' profile difficult to establish and might reflect the differing playing styles that can exist within positional groups. For example, some Prop Forwards are often used as 'impact players' with their on-field time controlled through tactical substitutions (Waldron et al., 2013), whereas other players within the same position often play substantially more minutes and as such might not be able to carry the same absolute mass. It could be argued that this is somewhat unique to RL where other team sports (e.g. football) demonstrate a more homogenous anthropometric profile (Iga, Scott, George, & Drust, 2014). Such findings again suggest that anthropometry cannot be used on its own for player selection and should always be viewed alongside other key performance indicators.

4.6. Practical Applications

Interestingly, we observed similarities in anthropometric profiles between several positional groups. With the exception of the Hookers and Halfbacks the mean height of the playing groups was very similar. These data might represent the fact that in the modern game all players are involved in high-speed physical collisions and are expected to carry the ball aggressively and be strong in defence. Therefore, there are fewer opportunities for the smaller

player to be competitive in the modern game. Similarities in most anthropometric characteristics of Centres and Back Row Forwards perhaps reflect the modern game and the need for players to be interchangeable in these two positions. Similarly, the recent trend for coaches to use ball-playing Halfbacks in the Hooker position to ensure dynamic play and ball distribution around the ruck is supported by the similar anthropometric traits between these two positional groups. The only truly unique positional group identified in the present study was that of the Prop Forwards who were consistently taller, possessed more lean mass, more body fat and more total mass than all other playing groups. Taken together, the current data reflect the modern game of RL where every player must tackle, make large impact collisions with the ball in hand whilst remaining fast and agile. To enable versatility within a squad, coaches would seem to require the 'complete' player with the ability to play in several positions rather than focusing on one particular skill set. It would be interesting to establish if the reasons that players migrate to a given position (especially Prop Forward) is due to a genetic predisposition for these physical characteristics or due to a lifetime of training to play this role. Such genetic studies are now possible and provide an exciting opportunity for future research (Heffernan, Kilduff, Day, Pitsiladis, & Williams, 2015).

4.7. Conclusion

In conclusion, the present study has for the first time assessed the body composition of elite ESL players from more than one club using DXA scan technology. We report that there are significant differences between player positions, especially separating the Prop Forwards from the other positional groups. Perhaps most importantly however, there are large differences within playing positions and therefore it is not possible to identify an ideal position specific anthropometric profile. Using the first large scale analysis on the anthropometric profile of elite senior ESL RL players, these data can be used by coaching staff working with academy and senior players to inform individualized player training and nutrition strategies. These data allow clear targets for junior players to develop towards as they progress into senior squad selection and a benchmark to aim at. It is now crucial that future studies look at how academy players develop from season to season and also the

potential changes in body composition that can be achieved within a single pre-season period within senior players.

CHAPTER 5.

DEVELOPMENTS OF ANTHROPOMETRIC CHARACTERISTICS IN PROFESSIONAL ACADEMY AND SENIOR RUGBY LEAGUE PLAYERS: IS THERE TOO MUCH EMPHASIS ON THE PRE-SEASON PERIOD?

This chapter investigates firstly, the body composition changes over a 3-year period in academy players as they progress towards senior squad selection. Secondly, this chapter explores the changes in anthropometric characteristics of senior players during a single pre-season period. Findings firstly suggest it takes 1-3 years for academy players to develop profiles similar to senior players and secondly findings show no meaningful change in body composition profiles during a single pre-season period in senior players

This study is currently under review in the European Journal of Sport Science

Morehen, J. C., Clarke, J., Batsford, J., Erskine, R. E., Morton, J. P., & Close, G. L. (2019). Developments of anthropometric characteristics in professional academy and senior rugby league players: Is there too much emphasis on the pre-season period? *Eur J Sport Sci,* under review.

5.1. Abstract

RL is a team sport requiring players to experience large impact collisions, thus requiring high amounts of lean mass. Many players (academy and senior) strive to increase their lean mass during the pre-season, however, quantification of changes during this period have not been thoroughly investigated. We therefore assessed changes in body-composition using DXA in eleven academy players over three successive pre-seasons and ninety-nine senior players from four different ESL clubs prior to, and at the end of, a pre-season training period. There was no meaningful change in lean mass of the academy players during any of the pre-season periods (year 1 = 72.3 to 73.2 kg; ES 0.05 ± 0.05, year 2 = 74.4 to 75.5 kg; ES 0.07 ± 0.03, year 3 = 75.9 to 76.8 kg; ES 0.06 ± 0.05) with *small* changes only occurring over the three-year study period (72.3 to 75.9 kg; ES = 0.22 ± 0.08). The senior players showed *trivial* changes in all characteristics during the pre-season period (total mass = 95.1 to 95.0 kg; ES -0.01 ± 0.03, lean mass = 74.6 to 75.1 kg; ES 0.07 ± 0.03, fat mass = 13.6 to 12.9 kg; ES -0.17 ± 0.05, body fat percentage = 14.8 to 14.1 %; ES -0.19 ± 0.06). These data suggest that academy players need time to develop towards player profiles congruent with senior players. Moreover, once players reach senior level, body-composition changes are *trivial* during the pre-season and therefore teams may need to individualise training for players striving to gain lean mass by reducing other training loads.

5.2. Introduction

In chapter 4, anthropometric characteristics from professional senior RL players were reported showing large intra and inter-position variations for total mass, lean mass, fat mass and body fat percentage. Given the physical demands of the sport, these data provide greater insights into the body compositions that senior players strive to achieve to competitively play RL. It is therefore crucial that developing RL players increase their lean mass to that of senior players, as outlined in chapter 4, in order to improve body compositions. Indeed, at senior level, success has been attributed to players who possess greater muscular strength and power (Baker & Newton, 2008; de Lacey et al., 2014).

To allow lean mass to be tracked, body composition must be assessed alongside total mass. Sum of skinfolds has been previously used to track changes in academy players (Till et al., 2013; Till, Jones, Darrall-Jones, Emmonds, & Cooke, 2015; Waldron et al., 2014), however, neglecting the opportunity to assess lean mass. In comparison to skinfold assessments, a more valid and reliable method of body composition is the use of DXA. DXA scanning has gained acceptance as a reference method for body composition analysis (Nana et al., 2012). The use of DXA provides an accurate assessment of total and regional body composition measurements (Barlow et al., 2015) and would therefore be useful for sport science support staff to identify whole body lean mass, fat mass and body fat percentage changes in both academy and senior RL players. However, studies using DXA to identify changes in body composition during the pre-season period are yet to be performed.

Considering the in-season period includes weekly matches, and during certain periods of the year, multiple matches per week (Twist, Highton, Daniels, Mill, & Close, 2017) many teams limit their in-season resistance training to between one-three sessions per week (Baker, 2001). Therefore, the pre-season is regarded as the optimum time period for players to increase their training volume (Dobbin et al., 2018) to make meaningful changes in body composition (Weaving et al., 2017). Despite the importance of the pre-season period, to date there are no data quantifying the changes in body composition over this period in both

academy and senior players. Such data are essential to guide support staff with regards to realistic targets during the pre-season period and help to reduce the pressure that may be placed on youth players to gain mass too quickly. Moreover, in senior players, if meaningful changes are not made during the pre-season period, perhaps time could be better spent developing other key areas of success in RL players, for example, rugby specific skills and/or physical training to reduce injury risk.

To this end, the aim of this study was twofold: 1) To track, for the first time using DXA, the changes in body composition during three consecutive pre-season periods in elite academy players from two different European Super League clubs and 2) assess for the first time changes in body composition in elite senior players from three different European Super League clubs at the beginning and end of the pre-season period.

5.3. Methods

5.3.1. Participants

Part One

Eleven academy players (age 18 ± 1 years at the start of the study) from two ESL academy teams volunteered for this study. At the beginning of the study, all players were competing regularly at academy level, with all players progressing to senior RL by the end of the three-year study period.

Part Two

Ninety-nine first team senior professional RL players (age 25 ± 6 years) from three different ESL teams, including forty-four current/former Internationals volunteered for this study. Players were categorised into six positional groups based on their predominant match-day position and asked to self-select their position if they played in multiple. In total, players were split as follows: Fullbacks and Wingers (18), Centres (11), Halfbacks (6), Hookers (11), Props (19) and Back Row Forwards (28).

5.3.2. Study Design

All players' attended the university laboratory between 07:00-09:30 in a fasted and hydrated condition having refrained from exercise in the previous 18 h. Players' height and weight were recorded using a dual height/body mass stadiometer (see section 3.3 for height/body mass assessment). Testing took place within the opening week and final week of the teams' respective pre-seasons. All players completed the whole pre-season training phase (10-12 weeks), which consisted of typical strength and conditioning training, fitness and skills (Dobbin et al., 2018). Players consumed nutritional intakes suggested and recommended by the clubs Sport and Exercise Nutrition register nutritionist and in line with those previously reported for professional rugby players (Bradley et al., 2015). Players then underwent a whole-body fan bean DXA measurement scan (see section 3.3 for methods of body composition measurement). Scans were analysed using QDR for Windows software version 12:4:3 by the same trained operator, according to in-house protocols to achieve high precision scans. Ethical approval, for Parts one and two, was granted from the National Research Ethics Service (17/WM/0014).

5.3.3. Part One – Academy Players

At the start and end of the pre-season, players visited the laboratory for body composition assessment, using a DXA scanner, along with basic anthropometrical analysis. This process was repeated for three consecutive seasons generating six assessment points for analysis.

5.3.4. Part Two – Senior Players

At the start and end of the pre-season, players visited the laboratory for body composition assessment, using a DXA scanner, along with basic anthropometrical analysis.

5.4. Results

5.4.1. Part One - Academy Players

The total mass, lean mass, fat mass and percentage body fat of the academy players can be seen in Figures 5.1A, 5.1B, 5.2A and 5.2B, respectively. There was no change in the height of the academy players from the start of the pre-season in year 1 to the end of pre-season in year 3 (183.6 ± 5.9 vs 185.5 ± 6.0 cm; 0.8%, ES 0.03 ± 0.01, *very unlikely*) and therefore these data are not graphically reported.

5.4.1.1. Total Mass

There was no change in the total mass from the start of the pre-season to the end of the pre-season in either year 1, 2 or 3 (year 1: -1.6 %, ES 0.11 ± 0.13, *very unlikely*; year 2: 0.7 %, ES 0.06 ± 0.06 *very unlikely* and year 3: 0.1 %, ES 0.00 ± 0.09, *most unlikely*). There was no change in total mass between the start of pre-season in year 1 compared with the start of pre-season in year 2 (1.6 %, ES 0.11 ± 0.13, *most unlikely*). Similarly, there was no change in total mass from the start of pre-season in year 2 compared with the start of pre-season in year 3 (1.2 %, ES 0.09 ± 0.11, *very unlikely*). Collectively, this resulted in a *small* 2.7 % increase in total mass from 91.7 kg in year 1 to 94.4 kg in year 3 (2.7 %, ES 0.22 ± 0.15, *likely*). Total mass is shown in Figure 5.1A.

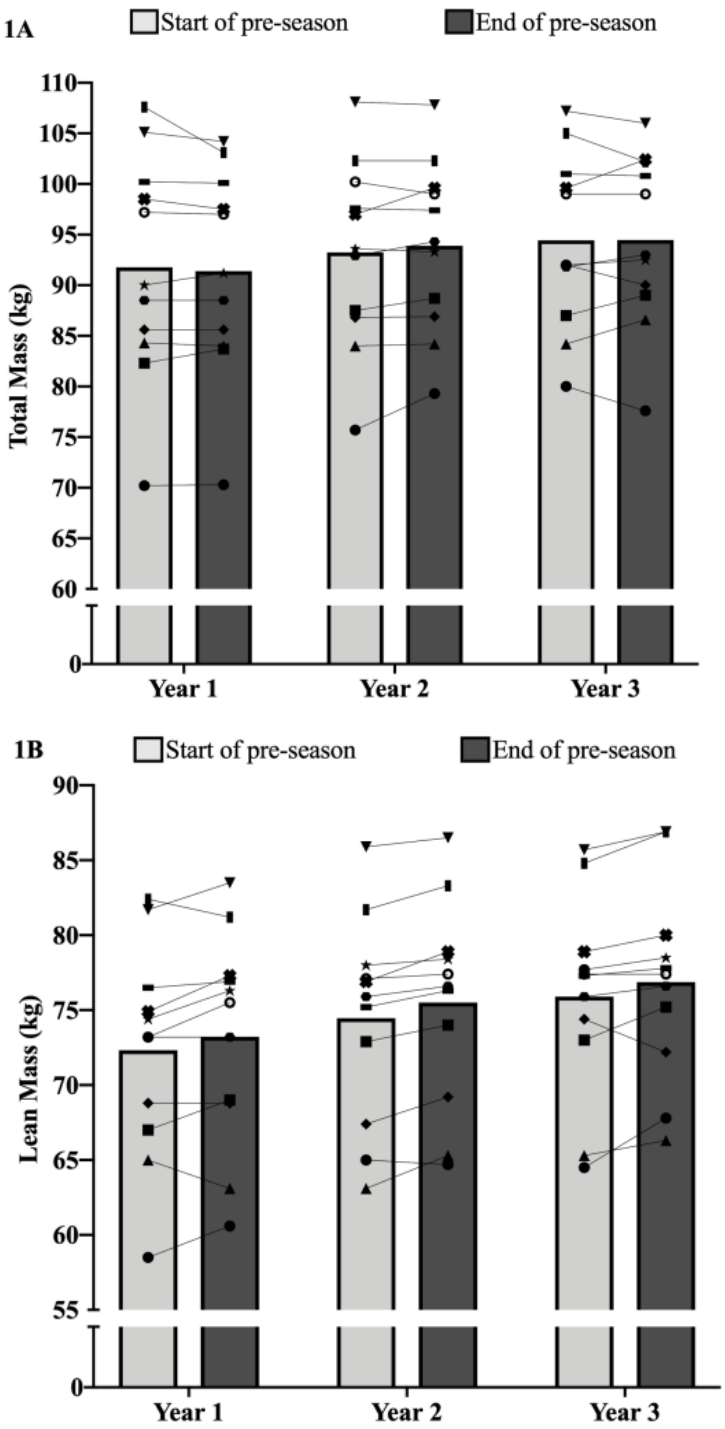


Figure 5.1. *Change in total mass (1A) and lean mass (1B) from the start of pre-season to the end of pre-season over three consecutive rugby league seasons. Individual shapes represent each player. Light grey bars represent start of pre-season average whilst dark grey bars represent end of pre-season average.*

5.4.1.2. Lean Mass

There was no change in lean mass from the start of pre-season to the end of pre-season in year 1, 2 or 3 (year 1: 1.1 %, ES 0.05 ± 0.05, *very unlikely*; year 2: 1.2 %, ES 0.07 ± 0.03, *very unlikely* and year 3: 1.1 %, ES 0.06 ± 0.05, *very unlikely*). There was no change in lean mass from the start of pre-season in year 1 to the start of pre-season in year 2 (2.6 %, ES 0.13 ± 0.11, *very unlikely*) and the start of pre-season in year 2 compared to the start of pre-season in year 3 (1.7 %, ES 0.09 ± 0.08, *very unlikely*). Collectively, this resulted in a *small* increase of lean mass from 72.3 kg in year 1 to 75.9 kg in year 3 (4.3 %, ES 0.22 ± 0.08, *likely*). Lean mass is shown in Figure 5.1B.

5.4.1.3. Absolute Fat Mass

There was no change in fat mass from the start of pre-season to the end of pre-season in year 1, 2 or 3 (year 1: 1.0 %, ES 0.01 ± 0.06, *very unlikely*; year 2: 1.6 %, ES 0.02 ± 0.05, *very unlikely* and year 3: 3.1 %, ES 0.03 ± 0.03, *very unlikely*). There was a small change in fat mass from the start of pre-season in year 1 to the start of pre-season in year 2 (17.6 %, ES 0.15 ± 0.13, *unlikely*) and no change from the start of pre-season in year 2 compared to the start of pre-season in year 3 (2.8 %, ES 0.03 ± 0.05, *very unlikely*). Collectively, there was a *trivial* increase from 12.1 kg in year 1 to 14.4 kg in year 3 (20.9 %, ES 0.18 ± 0.12, *unlikely*). Absolute fat mass is shown in Figure 5.2A.

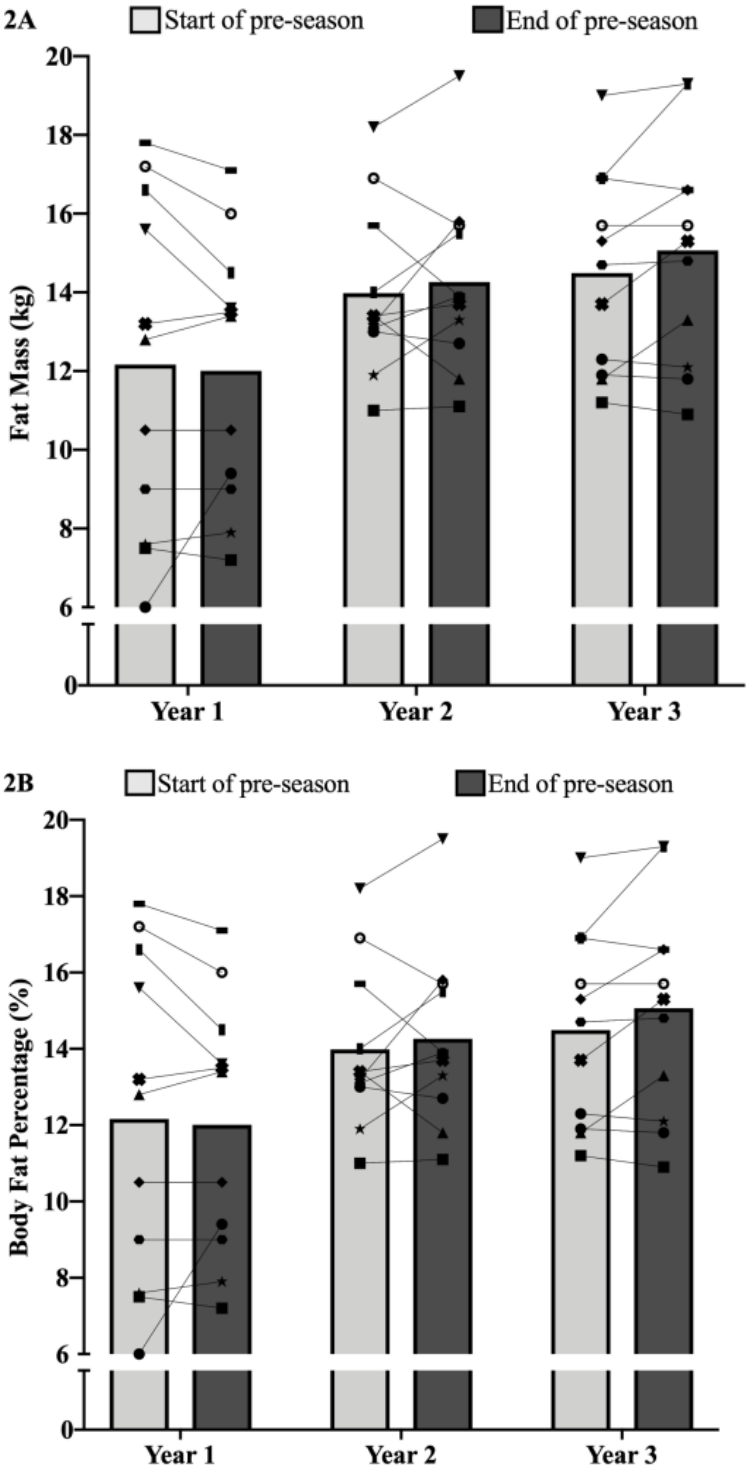


Figure 5.2. *Change in fat mass (2A) and body fat percentage (2B) from the start of pre-season to the end of pre-season over three consecutive rugby league seasons. Individual shapes represent each player. Light grey bars represent start of pre-season average whilst dark grey bars represent end of pre-season average.*

5.4.1.4. Body Fat Percentage

There was no change in body fat percentage from the start of pre-season to the end of pre-season in year 1, 2 or 3, respectively (year 1: 0.2 %, ES 0.01 ± 0.23, *unclear*; year 2: -0.2 %, ES -0.01 ± 0.31, *unclear* and year 3: 1.5 %, ES 0.02 ± 0.03, *very unlikely)*. Similarly, no change in body fat percentage was seen from the start of pre-season in year 1 to the start of pre-season in year 2 (13.0 %, ES 0.12 ± 0.10, *unlikely*) or the start of pre-season in year 2 to the start of pre-season in year 3 (0.1 %, ES 0.00 ± 0.04, *most unlikely*). Collectively, there was no change in body fat percentage from the start of pre-season in year 1 to the start of pre-season in year 3 (13.1 %, ES 0.13 ± 0.10, *unlikely*). Percentage body fat is shown in Figure 5.2B.

5.4.2. Part Two - Senior Players

5.4.2.1. Total Mass

There were trivial changes in total mass from the start of pre-season to the end of pre-season in all players (-0.2 %, ES -0.01 ± 0.03, *most unlikely*) and all positional groups (Full Back and Winger: 0.6 %, ES 0.05 ± 0.09, *very unlikely*; Centre: 0.2 %, ES 0.03 ± 0.15, *most unlikely*; Halfback: -0.1 %, ES -0.01 ± 0.10, *most unlikely*; Hooker: -0.3 %, ES -0.05 ± 0.19, *most unlikely*; Prop: -0.7 %, ES -0.11 ± 0.11, *most unlikely* and Back Row Forward: -0.3 %, ES -0.04 ± 0.08, *most unlikely*), (Table 5.1).

5.4.2.2. Lean Mass

There were trivial change in lean mass from the start of pre-season to the end of pre-season in all players (0.7 %, ES 0.07 ± 0.03, *very unlikely*) and all positional groups (Full Back and Winger: 1.5 %, ES 0.09 ± 0.05, *very unlikely*; Centre: 0.3 %, ES 0.02 ± 0.04, *most unlikely*; Halfback: 0.4 %, ES 0.01 ± 0.06, *most unlikely*; Hooker: 0.8 %, ES 0.04 ± 0.05, *very unlikely*;

Prop: 0.3 %, ES 0.03 ± 0.05, *most unlikely* and Back Row Forward: 0.5 %, ES 0.04 ± 0.06, *most unlikely*), (Table 5.1).

5.4.2.3. Fat Mass

There were trivial changes in fat mass from the start of pre-season to the end of pre-season in all players (-5.4 %, ES -0.17 ± 0.05, *very unlikely*) and all positional groups (Full Back and Winger: -3.5 %, ES -0.04 ± 0.04, *very unlikely*; Centre: -4.4 %, ES -0.04 ± 0.04, *very unlikely*; Halfback: -3.2 %, ES -0.02 ± 0.03, *very unlikely*; Hooker: -6.2 %, ES -0.06 ± 0.06, *unlikely*; Prop: -3.8 %, ES -0.05 ± 0.05, *very unlikely* and Back Row Forward: -5.1 %, ES -0.07 ± 0.04, *unlikely*), (Table 5.1).

5.4.2.4. Body Fat Percentage

There were trivial changes in body fat percentage from the start of pre-season to the end of pre-season in all players (-5.3 %, -0.19 ± 0.06, *unlikely*) and all positional groups (Full Back and Winger: -4.4 %, ES -0.05 ± 0.04, *very unlikely*; Centre: -3.9 %, ES -0.04 ± 0.04, *very unlikely*; Halfback: -3.1 %, ES -0.02 ± 0.04, *very unlikely*; Hooker: -6.1 %, ES -0.06 ± 0.06, *unlikely*; Prop: -3.4 %, ES -0.05 ± 0.05, *very unlikely* and Back Row Forward: -4.9 %, ES -0.07 ± 0.03, *very unlikely*) (Table 5.1).

Table 5.1. *Mean (±SD) [range] total mass, lean mass, fat mass and body fat percentage values of senior Rugby League players by positional groups from the start of pre-season and the end of pre-season period.*

Position	**Full Back and Winger (*n* = 18)**		**Centre (*n* = 11)**		**Halfback (*n* = 6)**		**Hooker (*n* = 11)**		**Prop (*n* = 19)**		**Back Row Forward (*n* = 28)**	
Pre-season Period	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
Total Mass (kg)	90.6 (±8.3) [79-108]	91.1 (±8.4) [80-110]	91.6 (±3.7) [87-99]	91.8 (±4.3) [87-100]	86.5 (±7.2) [80-100]	86.4 (±7.1) [80-100]	87.2 (±4.9) [81-96]	86.9 (±4.0) [81-94]	107.2 (±5.0) [97-115]	106.5 (±5.0) [97-115]	98.8 (±6.0) [87-115]	98.5 (±5.5) [87-113]
Lean Mass (kg)	71.2 (±5.3) [64-82]	72.4 (±5.7) [65-83]	73.5 (±3.1) [70-79]	73.8 (±4.0) [68-80]	67.9 (±5.2) [61-77]	68.2 (±6.7) [62-77]	68.9 (±3.8) [63-75]	70.0 (±3.7) [64-76]	82.2 (±4.0) [73-90]	82.5 (±4.0) [75-90]	77.7 (±5.1) [66-93]	78.1 (±4.7) [67-93]
Fat Mass (kg)	12.5 (±3.5) [8-19]	12.0 (±3.6) [7-20]	11.4 (±1.4) [9-14]	11.0 (±1.8) [8-14]	12.6 (±3.3) [9-18]	12.1 (±3.3) [8-17]	11.8 (±2.0) [8-15]	10.8 (±1.0) [9-12]	17.8 (±2.8) [14-25]	17.1 (±2.9) [13-23]	14.0 (±2.7) [9-20]	13.3 (±2.8) [8-20]
Body Fat (%)	14.2 (±2.9) [9-19]	13.6 (±3.0) [9-19]	13.0 (±1.6) [10-16]	12.5 (±2.0) [9-17]	15.0 (±3.1) [10-18]	14.4 (±3.3) [10-18]	14.2 (±2.0) [11-17]	13.1 (±1.2) [11-15]	17.2 (±2.3) [14-23]	16.6 (±2.4) [13-22]	14.8 (±2.5) [10-20]	14.0 (±2.7) [8-20]

5.5. Discussion

The aim of the present study was to assess the changes in body composition (total mass, lean mass, fat mass, and body fat percentage) in professional RL players with a specific focus on what changes can be made during the crucial pre-season period. To this end, using DXA, we tracked eleven academy players over three successive pre-seasons, and we assessed ninety-nine senior players prior to and following a single pre-season training programme. Surprisingly, we report for the first time in academy players (17-19 years old) *trivial* anthropometric differences throughout the pre-season periods with *trivial* to *small* differences observed following three-years' worth of pre-season training. Similarly, in senior players we also report *trivial* changes across all body composition characteristics, in all positional groups, measured prior to and following a pre-season training period. Collectively, these data contradict the assumption that significant gains in lean mass are made during a RL pre-season and highlights the importance of looking at changes in body composition over the entire season, along with the need for player specific training and nutrition strategies to maximise training adaptations.

As expected, previous research in academy players aged 13-17 has shown incremental increases in total mass as players mature (Till et al., 2013; Waldron et al., 2014). We confirm and extend this by showing increases in total mass (year 1; 91.6 kg, year 2; 93.6 kg, year 3; 94.5 kg) over three consecutive years in academy players. The range of total mass changes within a single pre-season period was between 0 and 3.6 kg, whilst over the three-year study period this increased between 0.6 and 7.4 kg. Interestingly, minimal changes in lean mass were reported during the pre-season period in academy players with *small* changes only occurring over a full season. For example, within a single pre-season period, the amount of lean mass gained between players ranged between 0 to 3.3 kg with larger increases of 1.3 to 9.3 kg built over three consecutive years of data collection (year 1; 72.7 kg, year 2; 74.5 kg; year 3; 76.4 kg). This may reflect that, during the pre-season period, players are also subjected to increased training loads (Dobbin et al., 2018) in terms of on-field running as well as increased gym work, and consequently gains in lean mass may be difficult to achieve due to inadequate nutrient intake (Howarth et al., 2010) combined with the opposing demands

of concurrent training (Coffey & Hawley, 2016). Indeed, we have previously shown that the daily energy expenditure of some players during a normal training week could be as high as 5374 Kcal with approximately an additional 500 Kcal per day being required to increase lean mass. This would require a daily intake of 5874 Kcal which is substantially more than has been documented in elite rugby players (Bradley et al., 2015). It may therefore be prudent for coaches to consider reducing the training load of players whose main priority is to gain lean mass to facilitate this growth along with age-specific nutrition support given during this crucial period.

Fat mass increased in the academy players over the three-year study period, something that none of the players were striving to achieve. This increase in body fat may be detrimental to performance and is likely to be an unwanted consequence of attempting to gain lean muscle mass too quickly with the aim of reaching a specific body mass target. Coaches should now use these data to help plan more appropriate and timely increases in lean muscle mass appreciating that for some players, meaningful changes in lean mass can take one to three years to achieve. It should be noted, however, that these data are only on eleven academy players given that we attempted to track players from two different academies through to first team, and future studies may wish to evaluate such suggestions using a larger group of players.

The senior players in the present study also demonstrated no meaningful changes in any anthropometric characteristic, from the start to the end of pre-season. These data confirm and extend previous research, which has used skinfold thickness and DXA scan technology showing either non-meaningful or *trivial* changes in body composition in RL players (Dobbin et al., 2018; Georgeson et al., 2012; Harley et al., 2011). Although the mean gain in lean mass was only 0.7 kg, the maximum gain in lean mass over the pre-season was approximately 3 kg which, whilst certainly meaningful, is lower than what some coaches strive to achieve during this period, especially given it is possible that 1-2 kg of this lean mass could be attributable to changes in hydration status, muscle glycogen concentration and creatine (Bone et al., 2017). Therefore, despite many clubs using the pre-season period to make significant changes in body composition (Weaving et al., 2017), a longer time period may be required

(Georgeson et al., 2012). Alternatively, coaches may wish to consider revising the structure of the pre-season period to give more time to gain lean mass if this is the priority of a given player.

It is also worthy of consideration that, rather than the pre-season being used for senior players to continually increase lean mass, this period may be better used to develop technical skills and resilience given that meaningful changes in body composition are rarely achieved. Studies suggest that both technical and tactical differences combined with minimal errors when in possession of the ball clearly distinguish between successful and less successful teams (Hulin, Gabbett, Kearney, & Corvo, 2015; Kempton, Kennedy, & Coutts, 2016; Kempton, Sirotic, & Coutts, 2017). Research should now assess what would be a minimal effective dose of resistance training to maintain lean mass and improve strength/power whilst allowing more time in the pre-season to be spent on technical skills along with exercise-based interventions for injury prevention. For example, both active stretching and water based activities are shown to improve mobility, agility and psychological stress in rugby players (Herman, Barton, Malliaras, & Morrissey, 2012; Suzuki et al., 2004) and coaches may therefore choose to focus more attention on these activities over hypertrophy based gym work.

In academy players, after the first pre-season, we report average total mass and lean mass values that were lower than senior grade players. Whilst we report no meaningful changes in lean mass over a single pre-season, the lean mass of the academy players by the end of the third pre-season was in line with that expected of a senior players reported in chapter 4. Taken together, these data support the notion that academy players need time to develop and achieve body compositions required to withstand the physicality of the modern game, rather than unrealistic expectations for youth players to achieve significant body composition changes in an individual pre-season period.

5.6. Practical Applications

The present study has several practical applications, which have immediate translational potential. Firstly, contrary to popular belief, in both academy and senior players, meaningful changes in lean mass are rarely seen during the pre-season period. Although the precise reason for this was not tested, this likely reflects the increased training loads during this period, including extensive high-speed running. Additionally, it is important to note that a meaningful change in body composition characteristic is anything above twice the CV error of the DXA scanner machine during the testing period (CV of whole body fat = 1.9 % and 0.37 kg lean mass 1.0 % and 0.44 kg, and percent body fat 1.9 % and 0.41 %). If gains in lean mass are essential, the pre-season training period should be tailored to the individual ensuring sufficient time is given for growth and repair. Secondly, it should be noted that in academy players, it took almost three-years for players to achieve a body composition commensurate with senior players and therefore players must be given sufficient time to develop. Finally, given that meaningful changes in body composition are rarely achieved during the pre-season in senior players, it could therefore be questioned if this crucial time period could be used more effectively rather than focussing on hypertrophy-based training. To this end, it would be interesting to investigate if teams that dedicate greater attention to the fitness and skill components of RL during the pre-season period, rather than striving to achieve increased lean mass, go on to achieve greater success during the competitive season.

5.7. Conclusion

In conclusion, the present study has for the first time assessed the body composition of academy European Super League players over three successive seasons using the gold standard DXA scan technology. We report *trivial* changes during an individual pre-season with *trivial* to *small* changes in body composition over the three-year period. We also show, for the first time in a large group of senior European Super League players using DXA scan technology, *trivial* changes in body composition during a single pre-season period. Given the heterogeneous sample of players from a variety of different clubs (with different coaches)

these data question the assumption that meaningful changes in body composition can occur in a single pre-season period in both academy and senior players.

To ensure players maintain optimal body composition profiles, an understanding of the typical in-season total EE alongside EI is essential. Considering that RL training and match-play demands differ (Gabbett, Jenkins, & Abernethy, 2012), it follows that EE may vary accordingly and hence, EI may also be adjusted to account for the goals of each particular day. To date, EE and EI have only been reported in isolation of each other (Lundy et al., 2006) or with academy players (Smith et al., 2018). Therefore, data within senior professional players during a typical in-season training week is currently not available, making it difficult to prescribe accurate nutritional guidelines for players to support training and match play demands.

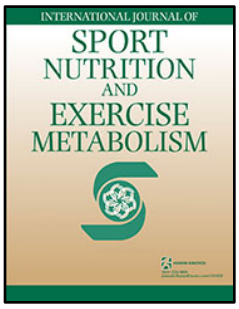
CHAPTER 6.

THE ASSESSMENT OF ENERGY EXPENDITURE DURING A 14-DAY IN-SEASON PERIOD OF PROFESSIONAL RUGBY LEAGUE PLAYERS USING THE DOUBLY LABELLED WATER METHOD

This chapter investigates the energy expenditure and intake of professional players from a single European Super League side. Findings suggest that total energy expenditure reported via the DLW method is higher than previously reported which may be represented by DLW accounting for all training and match related activities.

This study was published in the International Journal of Sports Nutrition and Exercise Metabolism in 2016.

Morehen, J. C., Bradley, W. J., Clarke, J., Twist, C., Hambly, C., Speakman, J. R. Morton, J. P., & Close, G. L. (2016). The assessment of total energy expenditure during a 14-day in-season period of professional rugby league players using the doubly labelled water method. *Int J Sport Nutr Exerc Metab*, 26(5), 464-472.



6.1. Abstract

RL is a high-intensity collision sport competed over 80 minutes of match-play. Training loads are monitored to maximize recovery and assist in the design of nutritional strategies although no data are available on the TEE of players. We therefore assessed the RMR and TEE from six Super League players over 2 consecutive weeks in-season including one game per week. Fasted RMR was assessed followed by a baseline urine sample before oral administration of a bolus dose of hydrogen (deuterium ^{2}H) and oxygen (^{18}O) stable isotopes in the form of water ($^{2}H^{18}O$). Every 24 hr thereafter, players provided urine for analysis of TEE via DLW method. Individual training load was quantified using session rating of perceived exertion (sRPE) and data were analysed using magnitude-based inferences. There were *unclear* differences in RMR between forwards and backs (7.7 ± 0.5 *cf.* 8.0 ± 0.3 MJ, respectively). Indirect calorimetry produced RMR values *most likely* lower than predictive equations (7.9 ± 0.4 *cf.* 9.2 ± 0.4 MJ, respectively). A *most likely* increase in TEE from Week 1 to 2 was observed (17.9 ± 2.1 *cf.* 24.2 ± 3.4 MJ) explained by a *most likely* increase in weekly sRPE (432 ± 19 *cf.* 555 ± 22 AU, respectively). The difference in TEE between forwards and backs was *unclear* (21.6 ± 4.2 *cf.* 20.5 ± 4.9 MJ, respectively). We report greater TEE than previously reported in rugby that could be explained by the ability of DLW to account for all match and training-related activities that contributes to TEE.

6.2. Introduction

In chapter 4 and 5, anthropometric characteristics from professional academy and senior RL player's were reported showing large intra- and inter-position variations for total mass, lean mass, fat mass and body fat percentage. Given the physical demands of the sport, these data provide greater insights into the body compositions that players strive to achieve to competitively play RL. To allow optimal nutritional strategies to be devised that help achieve these goals, it is important to assess players TEE from in-season training and match-play. TEE must also be reported alongside EI, which to date has only been reported with academy players (Smith et al., 2018). Such data would clearly inform appropriate training loads to maximise performance (Fowles, 2006) and potentially improve recovery from accumulated weekly muscle soreness (Fletcher et al., 2016) by ensuring adequate post-game nutrition. However, data within senior professional RL players during a typical training week is currently not available.

Typically the internal training loads imposed on RL players are monitored using heart rate and session-RPE (Lovell et al., 2013; Waldron et al., 2011; Weaving et al., 2014). Additionally, the growing use of micro technology incorporating GPS and accelerometers has attempted to quantify external training loads in the form of running (Evans et al., 2015; Gabbett, Jenkins, Abernethy, 2012; Twist et al., 2014), collisions (Oxendale et al., 2016) and, more recently, metabolic power (Kempton, Sirotic, & Coutts, 2015). Although some studies have attempted to quantify TEE in elite Rugby Union (RU) players (Bradley, Cavanagh, Douglas, Donovan, Twist, et al., 2015; Bradley, Cavanagh, Douglas, Donovan, Morton, et al., 2015) and elite RL players (Coutts, Reaburn, & Abt, 2003), these studies are somewhat limited by the methods employed. For example, Bradley et al (2015a) utilised Sensewear armbands that cannot be worn during game or physical collisions and therefore these data fail to account for the demands of match day competition that could contribute to a significant amount of the TEE. Kempton et al., (2015) have also used microtechnology to quantify EE based on the cost of accelerated running (di Prampero, 2005). However, Buchheit et al., (2015) has questioned the validity of this microtechnology derived metric, suggesting that it underestimates energy expenditure because of an inability to detect non-ambulatory related

activities. In particular the inability to account for increases seen in EE during rest periods (Highton et al., 2017) One technique that could assess all aspects of TEE in elite rugby players during training and matches, is the doubly labelled water (DLW) method (Schoeller et al., 1986). Despite the high validity associated with such measures, studies employing this approach are limited to other team sports (Anderson et al., 2017). Therefore, studies using DLW to identify TEE in senior players are yet to be performed.

Typically, resting metabolic rate is one of the largest components which contributes to TEE in humans (Speakman & Selman, 2003) which is often estimated using prediction equation (Cunningham, 1980), some of which have been validated in athletic populations (Cunningham, 1991; Ten Haaf & Weijs, 2014; Thompson & Manore, 1996). It is noteworthy, however, that the mean lean mass of athlete's in the original validation studies was ~46-63 kg (Cunningham, 1991) and therefore the appropriateness of the Cunningham equation for athletes with a larger body mass could be questioned. To date, no study has reported the typical RMR of senior elite rugby players measured using indirect calorimetry and consequently, estimates of RMR using standard prediction equations that are typically used in elite rugby practice might be flawed.

To help estimate an athletes TEE is it common to report the Physical Activity Level (PAL) of the sport, defined as any bodily movement produced by skeletal muscle that results in energy expenditure (Westerterp, 2013). The PAL score is expressesed as a magnitude of the RMR and is a useful tool for comparing between sports as well as estimating an athletes TEE. Whilst the PAL value of a vigorous lifestyle is approximately 2.4 (Westerterp, 2013), there has yet been no attempt to quantify the PAL of elite senior RL players. As a consequence of this lack of basic metabolic data, it is extremely difficult to prescribe science-informed rugby specific nutrition plans to help players achieve ideal body compositions and promote adaptations to training. As such, the aims of this study were to: 1) assess the TEE and EI of professional RL players during two competitive in-season weeks using the DLW method, food diaries, and calculate the PAL of the sport and 2) measure and compare the RMR of these players to current prediction equations.

6.3. Methods

6.3.1. Participants

Six professional first team RL players from the same European Super League club volunteered for the study. Body composition and metabolic characteristics are presented in Table 6.1. Based on playing position, three forwards and three backs were selected to represent typical RL positions (prop, hooker, wide-running forward, and stand-off, halfback, winger, respectively). All players remained injury free for the duration of the study. The local ethics committee of Liverpool John Moores University granted approval for the study with players providing written consent.

Table 6.1. *Body composition and metabolic characteristics for all six players.*

Player	Height (cm)	Body Mass (kg)	Lean Mass (kg)	Fat Mass (kg)	Body Fat (%)	RMR (MJ)
1	180.6	91.3	75	10	11.3	8.11
2	183	95.5	79.2	10.3	11.1	7.17
3	185.5	100.2	80.5	12.9	13.4	7.97
4	182.4	85	69	10	12.2	8.27
5	179	92.3	74.7	10.5	12	8.00
6	186	103.9	82	14.2	14.3	7.64
Mean	182.8	94.7	76.7	11.3	12.4	7.86
(SD)	(2.7)	(6.7)	(4.8)	(1.8)	(1.2)	(0.40)

6.3.2. Study Design

Data collection was performed during the first two weeks of the 2015 competitive ESL season during the month of February. The specific period of the season was chosen since week-1 and week-2 of the club's fixture schedules mirrored each other, consequently each training week begun on a Monday with a competitive match on each respective Sunday.

Throughout the two-weeks, players continued with in-season training as prescribed by the club coaches. TEE via the DLW method, RMR, body composition profiles and EI was assessed from all players. During training, session rating of perceived exertion (sRPE) and time were used to quantify training load (see section 3.4.2). All players completed two six-day food diaries to assess EI. The fortnightly training schedule is depicted in Table 6.2.

Table 6.2 *Table 6.2 shows a typical in-season training week. This was mirrored for both week-1 and -2 of the study. Training days are shown in relation to game day rather than days of the week. Number in parentheses indicates the duration in minutes of the particular activity. Swimming was performed off site whilst all other activities were performed on site at the rugby club.*

	Game Day-5	**Game Day-4**	**Game Day-3**	**Game Day-2**	**Game Day-1**	**Game Day**	**Game Day+1**
AM	Swim (30) Weights (40)	Weights (40)	Rest	Mobility (15)	Captains Run (30)	Game	Recovery
Mid-AM	Skills (40)	Skills (30)	Rest	Power Weights (30)	Rest	Game	Recovery
PM	Rest	Rugby (45)	Rest	Rugby (45)	Rest	Game	Recovery

6.3.3. Measurement of TEE via Doubly Labelled Water

On Monday morning of week-1, players were weighed to the nearest 0.1 kg (SECA, Birmingham, UK) wearing shorts only. A single baseline urine sample was then provided in a 50ml tube, after which players were administered orally with a single bolus dose of hydrogen (deuterium ^{2}H) and oxygen (^{18}O) stable isotopes in the form of water ($^{2}H_2{}^{18}O$) before training commenced. Isotopes were purchased from Cortecnet (Voisins-Le-Bretonneux – France) and the desired dose of 10% ^{18}O and 5% Deuterium was calculated

according to each participant's body mass measured to the nearest decimal place at the start of the study, using the following calculation:

$$^{18}O \text{ dose} = [0.65 \text{ (body mass, g)} \times \text{DIE}]/\text{IE}$$

Where DIE is the desired injection enrichment (DIE = 618.923 x body mass (kg)-0.305) and IE is the initial enrichment (10%) 100,000 parts per million.

To ensure the whole dose was administered, the glass vials were refilled with additional water which player's consumed. Approximately every subsequent 24-hour (between 0900-1000 before training commenced) player's provided body mass and the second urine pass of the day, with the first acting as a void pass. Urine samples were initially collected in 50ml tubes then allocated, stored and frozen -80°C in airtight 1.8 ml cyrotube vials for later analysis.

For DLW analysis, urine was encapsulated into capillaries, which were then vacuum distilled (Nagy, 1983) and water from the resulting distillate was used. This water was analysed using a liquid water analyser [Los Gatos Research; (Berman et al., 2012)]. Samples were run alongside three laboratory standards for each isotope and three International standards (Standard Light Artic Precipitate, Standard Mean Ocean Water and Greenland Ice Sheet Precipitation; (Craig, 1961; Speakman, 1997) to correct delta values to parts per million. Isotope enrichments were converted to daily energy expenditure using a two-pool model equation (Schoeller et al., 1986) as modified by (Schoeller, 1988) and assuming food quotient of 0.85.

6.3.4. Body Composition and Resting Metabolic Rate

All players underwent a whole-body fan bean DXA measurement scan (see chapter 3.3.2) to quantify players lean mass which is required to predict RMR using prediction equations. Players RMR was assessed using a Moxus Modular Metabolic System (AEI Technologies, IL, USA) following DXA measurements at the university laboratories. The automated gas analyser was calibrated according to manufacture's guidelines (Beltrami, Froyd, Mamen, &

Noakes, 2014). Before assessment players were laid supine and asked to relax in a dark room for 15-minutes. Following this, the Moxus Ventilation hood was placed over the head and shoulders to measure players RMR (Roffey, Byrne, & Hills, 2006) for a 15-minute period and data collected was converted using the MAX II Metabolic System software (version 1.2.14, Physio-Dyne Instrument Corp, Quoque) using the Harris and Benedict equation (Harris & Benedict, 1918).

6.3.5. Total Energy Intake

Macro- and micro-nutrient intakes were analysed from two (week-1 and week-2) individual 6-day food diaries for all players and reported in mega joules (MJ) as days away from a match (match day -5, -4, -3, -2, -1 and +1). The period of 6 days is considered to provide reasonably accurate and precise estimations of habitual energy and nutrient consumptions whilst reducing variability in coding error (Braakhuis et al., 2003) and has previously been used in RU (Bradley et al., 2015). Food diaries were explained to players by the club's sport nutritionist who is a graduate Sport and Exercise Nutrition Register (SENr) accredited practitioner and both player and nutritionist performed 24-hour recalls and diet history each morning for the previous days intake and to confirm player quantifications of food (Shim, Oh, & Kim, 2014). The club nutritionist provided daily sport specific supplements and on three occasions (match day -5, -4 and -2) in both weeks, lunch was provided for all players. To obtain energy and macro- and micro-nutrient compositions the Nutritics professional diet analysis software (Nutritics LTD, Ireland) was used.

6.3.6. Quantification of Weekly Training Load

Training loads were implemented by the clubs strength and conditioning coach who has played and worked in professional RL for over 10 years. In line, quantification of gym and pitch training loads were assessed using sRPE as previously described in section 3.4.2.

6.4. Results

6.4.1. Energy Intake and Expenditure

TEE and EI data are presented in Figure 6.1. DLW revealed that there was a combined fortnightly TEE of 22.5 ± 2.7 MJ and EI of 14.0 ± 0.7 MJ. There was a *most likely* increase in mean TEE from week-1 to week-2 (35.3%; ES 1.8 ± 0.71). Over the same period, there was also a *likely* increase in mean EI (5.6%; ES 0.74 ± 0.78). Differences in TEE between forwards and backs were *unclear* in both week-1 (12.4%; ES 0.44 ± 1.07) and week-2 (1.4%; ES 0.05 ± 1.03). Differences in EI between forwards and backs were *unclear* in week-1 (5.3%; ES 0.85 ± 2.23) but *very likely* higher for forwards in week-2 (9.1%; ES 3.2 ± 2.19). Forwards TEE was *very likely* and *most likely* higher than EI in week-1 (21.4%; ES 1.43 ± 0.73) and week-2 (38.7%; ES 2.87 ± 0.72), respectively whilst back's TEE was *unclear* and *very likely* higher than EI in week-1 (18.3%; ES 1.4 ± 1.58) and week-2 (42%; ES 2.1 ± 1.07).

6.4.2. Resting Metabolic Rate and sRPE

RMR data are presented as an average per day in Figure 6.2. Mean RMR was *most likely* lower (16.5%; ES 2.5 ± 0.87) when assessed using direct calorimetry (7.9 ± 0.4 MJ) compared with predicted RMR using the Cunningham equation (9.2 ± 0.4 MJ). A difference in RMR between forwards and backs was *unclear* (2.9%; ES 0.25 ± 0.9) when measured using direct calorimetry. Mean sRPE (Figure 6.3) was *most likely* higher in week-2 compared to week-1 (29%; ES 4.61 ± 0.24). Differences in weekly sRPE between forwards and backs were *unclear* in both week-1 (4.4%; ES 0.86 ± 1.57) and week-2 (4.9%; ES 1.26 ± 1.62).

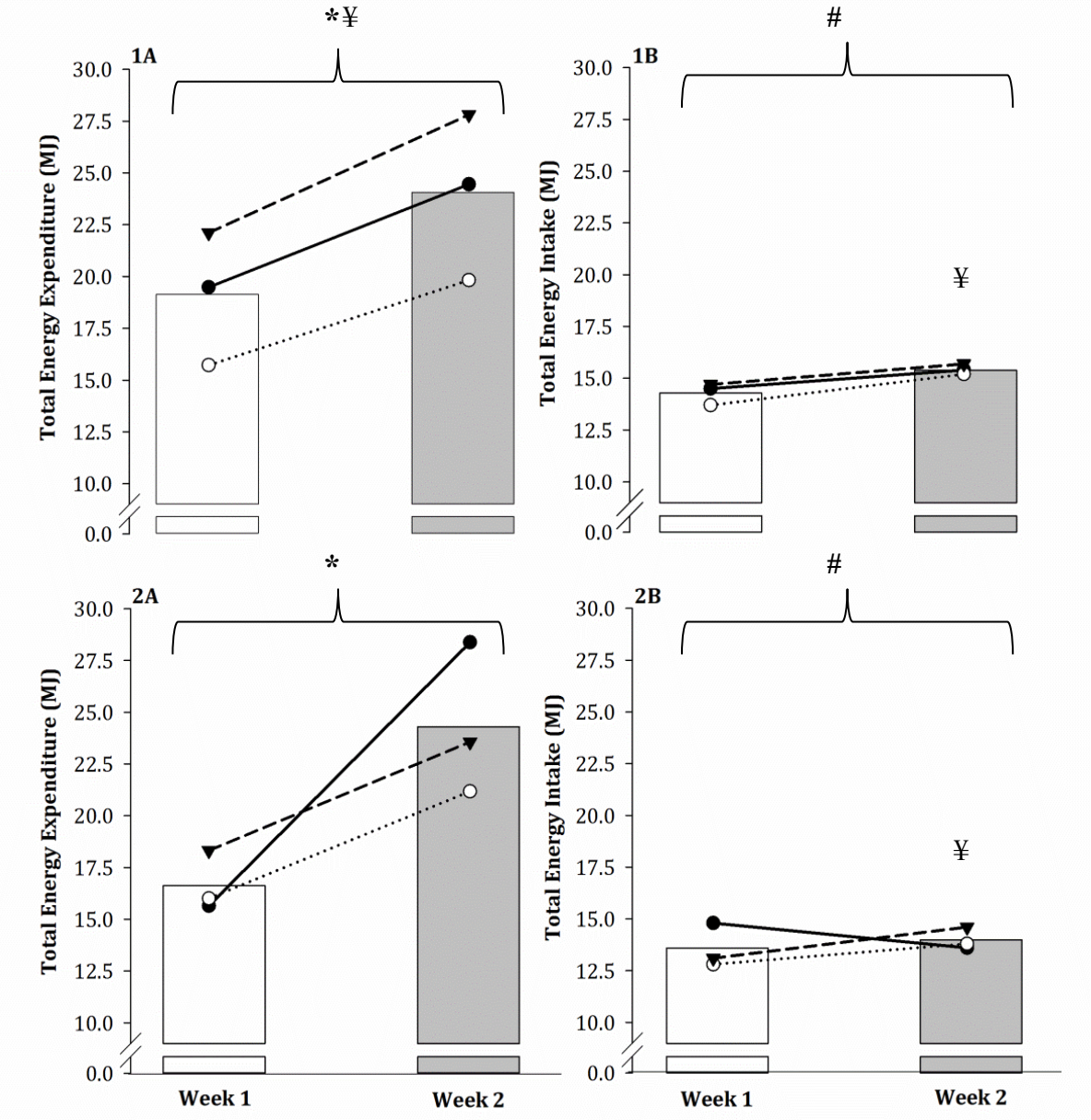


Figure 6.1. *TEE and EI of 6 ESL players over a two-week period taken from DLW data and food diary analysis. 1 = forwards, 2 = backs, A = TEE, B = EI. Lines represent individual player response whilst bars represent-playing position grouped mean either forwards or backs.* ***** *Denotes a most likely difference; # denotes a likely difference; ¥ denotes a very likely difference.*

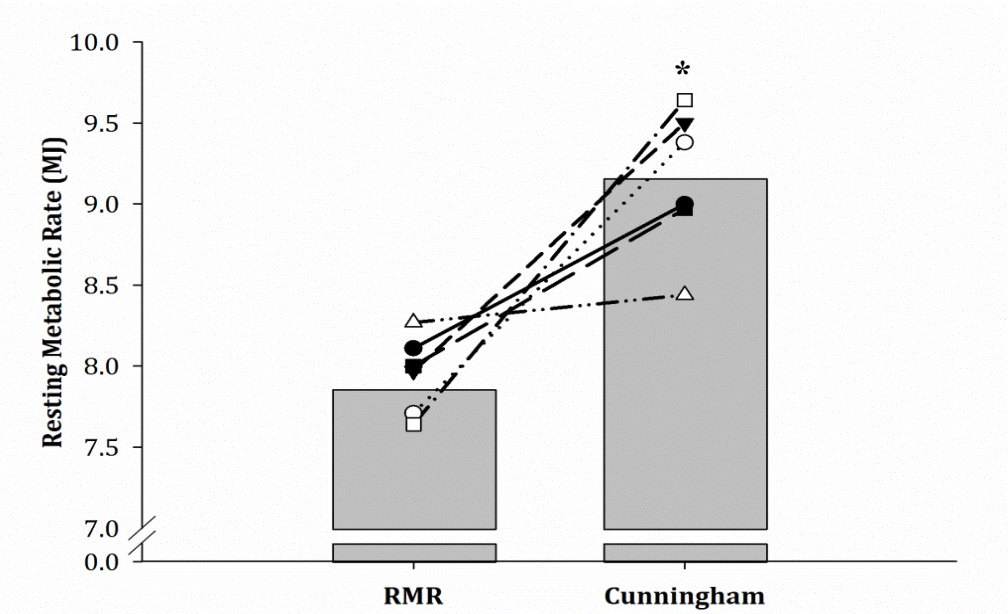


Figure 6.2. *A comparison of the RMR data for six ESL players and the commonly used predictive Cunningham equation. Lines represent individual player differences whilst bars represent the grouped mean of all players. * Denotes a most likely difference.*

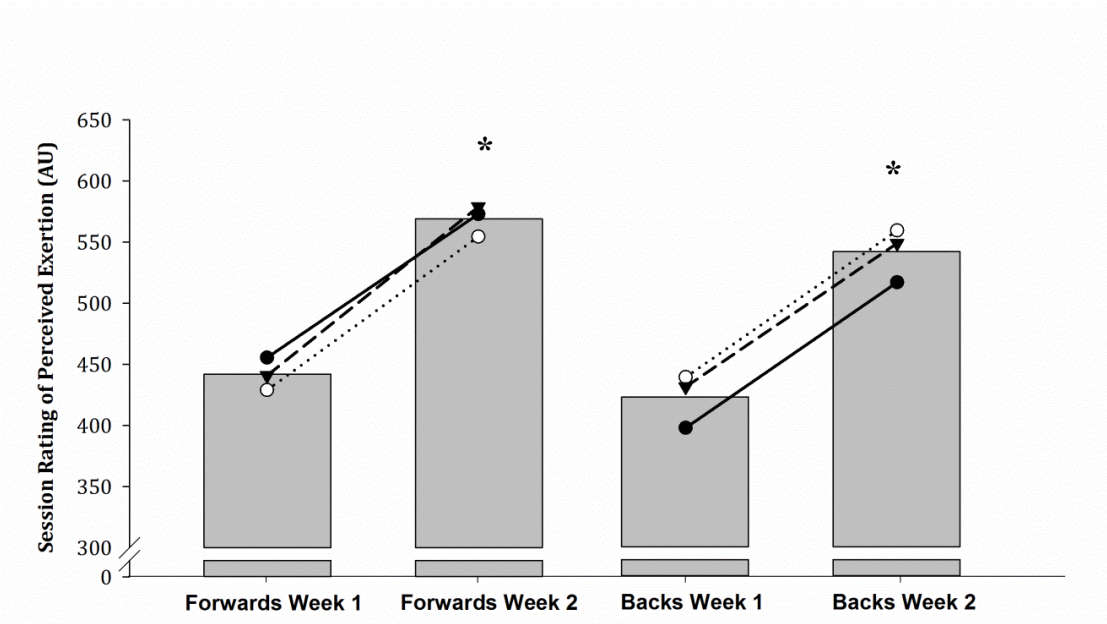


Figure 6.3. *Training demands of six ESL players over the two-week study period expressed as session rating of perceived exertions (sRPE). Lines represent individual player data whilst bars represent grouped mean for playing positions. A = Forwards, B = Backs. * Denotes a most likely difference.*

6.5. Discussion

The aims of the present study were to: (1) determine the TEE and EI of professional RL players during a competitive fortnight (including competitive matches) using the DLW technique and food diaries and (2) measure and compare the RMR of these players to a current predictive equation. We report for the first time that average TEE of all players using the gold standard DLW method was 22.5 MJ per day with clear differences between weeks supported by average daily EI of 14 MJ. We also report that RMR was 16.5% lower than values derived from commonly used predictive equations. Despite within group variations, there were no differences between forwards and backs in RMR. These data have immediate translational potential by informing applied practitioners working with professional RL players about the high TEE from the training and match demands of in-season RL. We also report caution when using a predictive equation to estimate RL players' RMR.

In this chapter we employed the DLW technique to quantify the TEE associated with RL training and match play, which incorporated running, physical collisions and recovery periods. Interestingly, the high TEE in both forwards (19.1 and 24.0 MJ) and backs (16.6 and 24.3 MJ) reported for week-1 and week-2, respectively, are higher than those values reported in-season using accelerometery for RU forwards (15.9 ± 0.5 MJ) and backs (14.0 ± 0.4 MJ) (Bradley et al., 2015). Differences in TEE between rugby codes could be because of differences in training and playing demands. However, weekly training loads (sRPE) were similar between studies, meaning the higher TEE reported in this study probably reflects: (1) the inability of previous studies to quantify physical contact and/or (2) that anaerobic contributions to training are difficult to quantify using wearable technology (Buchheit et al., 2015).

There were no differences in the TEE between the forwards and backs. Backs typically have longer playing times and perform more running whereas forwards are involved in more physical collisions (Twist et al., 2014; Waldron et al., 2011). We propose that the greater internal load caused by collisions in forwards (Mullen, Highton, & Twist, 2015) matches the

greater running volumes in backs (Gabbett, Jenkins, Abernethy, 2012), the outcome of which is the similar TEE observed between positional groups. There was no significant difference in RMR between forwards and backs, although there were inter individual variations. Despite the widespread use of prediction equations to estimate RMR (Cunningham, 1980), we report a difference of ~16.5% (~310 kcal) between this equation and indirect calorimetry. While RMR is a less important component of TEE in highly active rugby players compared to sedentary individuals (Speakman & Selman, 2003), it remains a fundamental measure to accurately prescribe nutritional advice. The Cunningham equation was originally validated on runners (~46-63 kg), so is likely to over-estimate RMR in our study because of the higher lean mass observed in elite rugby players (Harley et al., 2011). Interestingly, lean mass did not predict RMR in the six players tested in this study, with the highest RMR reported in the players with the lowest lean mass. Estimations of RMR in rugby players using existing predictive equations should be avoided, with future studies seeking to develop predictive RMR equations for athletes with higher lean body mass.

There was a large variation (as much as 7.5 MJ or 1800 Kcal) in the TEE between players that could not be explained by the RMR. This variation in TEE suggests that NEAT is a major contributor to the TEE in rugby players, despite the present study being unable to quantify these activities. Given that every aspect of a player's training day is carefully monitored and this information is then used to prescribe training loads (Weaving et al., 2014), it is essential that support staff understand and attempt to quantify the significant contribution of NEAT to TEE which might include players using wearable technology away from clubs. Similar observations have been reported in the Australian Football League, where a significant amount or TEE was from NEAT and suggests the habitual lifestyle of players outside of training is meaningful (Walker et al., 2016). The present study also attempted to define the PAL of professional rugby players. The players in this study had an average PAL value of 2.9, which is considerably higher than the 2.4 value suggested for people with vigorously active lifestyles but lower than 4.0 expressed by professional endurance athletes (Westerterp, 2013). Knowing an approximate PAL might provide a starting point for the prescription of nutritional plans as well as being a useful tool to compare between sports.

The reported EI was lower than the TEE in both the forwards and backs. Although some of the meals consumed by the players were provided and therefore monitored, the large discrepancy between TEE and EI probably reflects inaccuracies in self-reporting dietary intake (Bingham et al., 1994). This is further supported by the players' body mass remaining unchanged during the study (94.7-94.8 kg). Previous research has suggested that the self-reported EI bias can be as high as 34% (Ebine, Feng, Homma, Saitoh, & Jones, 2000; Fudge et al., 2006; Hill & Davies, 2002), which appears likely in the present study. These data confirm that caution should be taken when interpreting food diaries from athletes, even when considerable care has been taken by the athlete and the practitioner to complete them accurately.

6.6. Conclusion

The average weekly TEE values of ~22.5 MJ in professional RL players are higher than reported previously in RU players (Bradley, Cavanagh, Douglas, Donovan, Twist, et al., 2015; Bradley, Cavanagh, Douglas, Donovan, Morton, et al., 2015). We speculate that this high TEE reflects the ability of DLW to assess all aspects of rugby activity, including collisions. Recently this has been confirmed with higher TEE reported following collision activity during training in young RL players (Costello, Deighton, Preston, Matu, Rowe, Sawczuk, et al., 2018), which provides a strong rationale for players to consider specific post-match nutrition plans to aid recovery from the demands of match-play.

Considering players have high levels of lean mass, combined with the intense physical demands of RL and blunt force trauma, it follows that players are regularly exposed to muscle damage and soreness (Johnston, Gabbett, Seibold, & Jenkins, 2014; Oxendale, Twist, Daniels, & Highton, 2016; Takarada, 2003; Twist & Sykes, 2011) which may explain why rugby players have higher TEE values that other non-contact team sport athletes (Anderson et al., 2017). To date, although studies have used CK to report muscle damage in RL players (Oxendale et al., 2016; Twist, Waldron, Highton, Burt, & Daniels, 2012), CK is binary in nature and provides no indication to the magnitude of damage sustained. Therefore, to ensure players can implement the best recovery strategies, more functional (Twist & Highton, 2013)

and quantitative measures are required to further understand the amount of inflammation caused following match play.

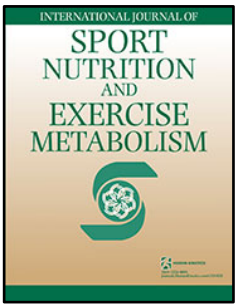
CHAPTER 7.

MONTMORENCY TART CHERRY JUICE DOES NOT REDUCE MARKERS OF MUSCLE SORENESS, FUNCTION OR INFLAMMATION FOLLOWING RUGBY MATCH PLAY

This chapter investigates the changes in IL-6, IL-8 and IL-10 following RL match play and the efficacy of consuming Montmorency tart cherries on reducing muscle soreness and function in academy RL players. Findings report increases in all three IL's at half-time and full-time compared to baseline and show no beneficial effects of consuming Montmorency cherries compared to a control beverage.

This study is currently being prepared for in the International Journal of Sports Nutrition and Exercise Metabolism.

Morehen, J. C., Clarke, J., Batsford, J., Morton, J. P., & Close, G. L. Does Montmorency tart cherry juice reduce markers of inflammation during professional rugby league match play. *Int J Sport Nutr Exerc Metab*, (in review).



7.1. Abstract

Given the unique demands of RL match play, muscle damage, inflammation and immediate and prolonged symptoms of fatigue are inevitable outcomes. Alterations in the structural integrity of muscle tissue through blunt force trauma results in a cascade of events, eventually releasing circulating cytokines, in particular interleukin-6 which signals the initiation of skeletal muscle recovery. In an attempt to alleviate markers of muscle soreness and facilitate recovery, common nutritional interventions, such as ingestion of Montmorency tart cherry juice, are employed pre- and post-match in team sport athletes, however to date, the efficacy of such supplementation in contact sport athletes has not been investigated. Eleven professional RL players competed in 2 competitive RL matches. During both matches, a randomised cross-over design was implemented with Montmorency cherry juice (MC) or a taste and colour matched placebo (PLB) supplemented for 7 consecutive days. Measures of match-play demands including total minutes (min), relative match intensity ($m.min^{-1}$), total contacts (n) and relative contacts ($con.min^{-1}$), interleukin concentration (IL-6, -8, -10), muscle soreness and sleep markers (using self-reported subjective wellness), and muscle function (using jump performance) were recorded during both matches. Mean IL-6, -8 and -10 concentrations all increased post-match compared with 48 h pre-match values (IL-6 = 2.95 ± 2.62 $pg.mL^{-1}$ Vs 0.66 ± 0.74 $pg.mL^{-1}$, IL-8 = 3.56 ± 1.55 $pg.mL^{-1}$ Vs 1.96 ± 1.09 $pg.mL^{-1}$, IL-10 = 2.37 ± 2.06 $pg.mL^{-1}$ Vs 0.52 ± 0.49 $pg.mL^{-1}$). Mean total contacts across both matches were 28 ± 11 with 0.4 ± 0.2 con.min-^{1}. There were no significant effects of MC on muscle soreness, muscle function and self-reported sleep, fatigue, soreness, mood and stress, or IL 6, 8 or 10 compared with PLB at any of the time points. We report novel data showing no effects of MC on all measured markers of recovery post-match in elite rugby players and therefore question the efficacy of such supplementation in contact sports when players have followed a non-polyphenol depleted diet.

7.2. Introduction

In chapter 4 we reported than the mean mass of RL players was 89.5 kg, which is much larger than other team sport athletes and as such the physical collisions in matches often results in muscle soreness and damage (Fletcher et al., 2016). We then demonstrated in chapter 5 that players strive to increase lean mass throughout their professional careers with varying degrees of success possibly as a results of inadequate energy intakes as reported in chapter 6. The TEE of senior RL players was reported over two competitive in-season weeks via the DLW technique. The ability for DLW to pick up all aspects of RL match play demands resulted in high TEE values (5374 ± 644 vs. 3566 ± 585 kcal) reported in players when compared to other non-contact sports (Anderson et al., 2017) and higher than previously reported for rugby players (Bradley et al., 2015). One of the main challenges faced by support staff working with RL players is to assist with recovery strategies and to reduce muscle soreness using multiple recovery modalities such as ice baths, compression wear, active recovery and nutrition.

Given that players possess high levels of lean muscle mass as reported in chapter 4, combined with the intense physical demands including collisions, tackling, running into contact with the ball and contacts with the playing surface (Lindsay et al., 2016; Takarada, 2003; Twist & Sykes, 2011), players are regularly exposed to muscle damage with limited recovery days between matches (McLean, Coutts, Kelly, McGuigan, & Cormack, 2010). Reported feelings of soreness peak at 24 h post-match, which remain elevated for up to four days post-match (McLean et al., 2010; Twist, Waldron, Highton, Burt, & Daniels, 2012), persisting throughout the entire playing season (Fletcher et al., 2016). Routine exposure to both exercise- and impact-induced muscle damage results in losses of strength and power (Cheung & Maxwell, 2003) ultimately resulting in reduced physical performance (Johnston, Gibson, et al., 2013; Johnston, Gabbett, & Jenkins, 2013). Given that players strive to commence each match as physically ready as possible, it is crucial to identify strategies that may help to alleviate post-match muscle damage and soreness.

To allow the best recovery strategies to be identified, markers of damage and inflammation from match play must be assessed. CK has previously been used to assess muscle damage in RL players (Cunniffe et al., 2010; McLellan, Lovell, & Gass, 2011; Oxendale, Twist, Daniels, & Highton, 2016; Smart, Gill, Beaven, Cook, & Blazevich, 2008; Takarada, 2003; Twist, Waldron, Highton, Burt, & Daniels, 2012), however the limitations of this approach have now been well documented (Fridén & Lieber, 2001). Briefly, CK can, at best, be used as a binary measure to assess if muscle damage has occurred or not, although it has no ability to quantify the magnitude of damage, despite it often being used in the literature to do so. The reason for this is that CK appears in the muscle following exercise, as a direct consequence of disturbances to the muscle energy process, and therefore does not necessarily represent muscle damage. Rather, circulating CK reflects relative amounts of CK released, degree of enzyme activity of released CK and the rate of clearance of CK from the serum (Baird et al., 2012). Furthermore, CK has been shown to have large individual responsiveness, inter-player variability (Hody et al., 2011; Kim & Lee, 2015; Koch, Pereira, & Machado, 2014; Twist et al., 2012), and often does not correlate at all with either direct measures of muscle damage (Baird et al., 2012) or the loss of muscle function following exercise (Oxendale et al., 2016a; Twist et al., 2012). In this context of assessing muscle damage post RL match play, it is more important to quantify the magnitude muscle damage, rather than if damage has occurred or not *per se*, and as such more quantitative measures are required.

In recent years, the relationship between the production of pro- and anti-inflammatory agents, produced in skeletal muscle (myokines) and in the circulation (cytokines), has received great interest following exercise and tissue injury (Hennigar & Pasiakos, 2017). Following the commencement of exercise, the presence of IL-6, precedes by the increase of IL-10, provides a marker of inflammation that consistently increases more than any other cytokine (Hennigar & Pasiakos, 2017). After exercise, if injury to skeletal muscle has occurred, signifcant elevations in circulating IL-6 are regarded as a signal to the recovery process (Chan McGee, Watt, Hargreaves, & Fabbraio, 2004; Fischer, 2006). Despite the importance of assessing interleukins in response to disruptions sustained to muscle, to date studies have only assessed IL-6 and not IL-8 or IL-10 following rugby match play. Furthermore, IL-6 was only assessed

at pre- and post-match play time points in elite RU players, with no assessment at half-time or on recovery days (Cunniffe et al., 2011; Cunniffe et al., 2010), and to date is yet to be performed following RL match play. Therefore, such data may help to identify the magnitude of damage caused from RL match play and help practitioners who want to implement best practice strategies to accelerate post-match recovery.

Considering players experience both exercise- and impact-induced muscle damage following match play (Naughton et al., 2017), the development of individual recovery strategies are crucial. Post exercise nutrition has received considerable attention, in particular foods or food components thought to possess anti-inflammatory properties such as polyphenols (Owens et al., 2018). One of the most researched polyphenols in terms of its effects on exercise-induced soreness are Montmorency cherries (*Prunus cerasus*), which has shown promising evidence in laboratory-based trials (Bell et al., 2016; Bowtell et al., 2011), and following marathon running (Howatson et al., 2010). Montmorency cherries reduce muscle soreness, possibly through its anti-inflammatory properties with data demonstrating attenuated increases in IL-6 post elbow contraction and marathon running (Connolly, McHugh, et al., 2006; Howatson et al., 2010). However, it is important to consider that such observations have been made following restrictions of polyphenols in the participants habitual diets prior to the intervention and therefore do not reflect nutritional intakes that are typically consumed from team sport athletes (Anderson et al., 2017) and rugby players (Bradley et al., 2015; Morehen et al., 2016). Moreover, to date there are no data assessing the effects of Montmorency cherries on contact sports like rugby despite the supplement being regularly consumed in such environments in an attempt to reduce post-exercise muscle soreness. It is therefore crucial that real world studies with appropriate dietary controls are now performed to assess the true benefit of Montmorency cherry juice supplementation on muscle damage (Close et al., 2019). Such data would therefore be useful for practitioners working with rugby players to identify if the ingestion of Montmorency cherries is indeed beneficial in aiding recovery from match play.

To this end, the aim of this study was to assess, for the first time in elite team sport athletes, the effectiveness of ingesting Montmorency cherry juices prior to and following competitive

match play on markers of muscle soreness, markers of inflammation and functional measures when players have consumed their regular diets leading into the game.

7.3. Methods

7.3.1. Study Design

A schematic representation of the overall study design can be seen in Figure 7.1. A familiarisation week was initially implemented to ensure players and coaching staff were comfortable with researchers collecting data, including venous bloods, during normal training and match day time points including the half-time period. Following this, a single blind randomised cross over design study was conducted for two weeks (termed weeks 1 and 2), including two consecutive scheduled matches during the 2016/2017 season. Players continued with their in-season training throughout the study period, as prescribed by the club's coaching staff. During week one players consumed either Montmorency tart cherries or a placebo beverage which was then reversed in week two. Muscle soreness, subjective wellness including sleep, fatigue, stress and countermovement jump and drop jump performance were measured before and after matches in weeks 1 and 2. Match play demands were recorded via GPS in all players during each match. Blood samples were collected 48 h pre-match (the most suitable timepoint for a baseline sample), at half time of the matches, full time (within 30 minutes of the match finishing) and 48 h post-match.

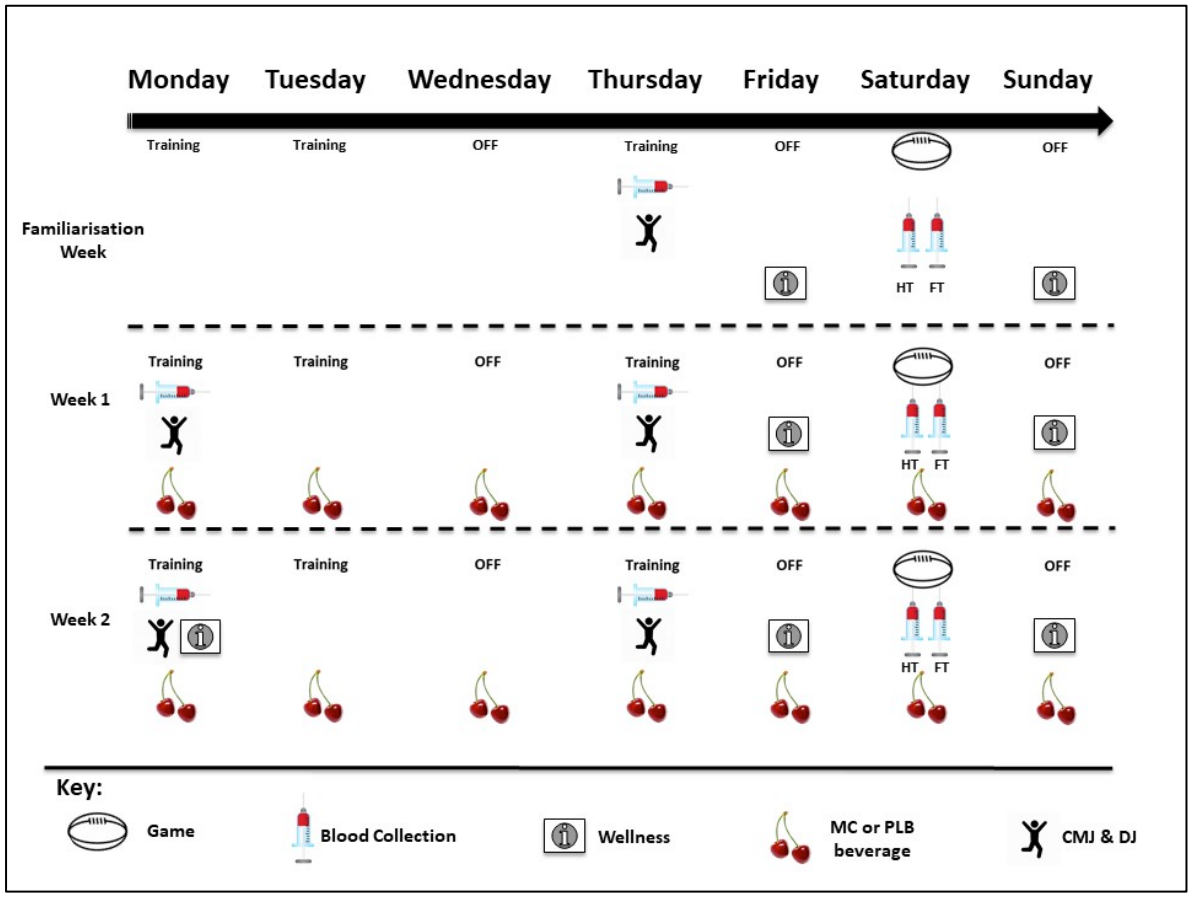


Figure 7.1. *Schematic showing the overall study design. A familiarisation week is followed by a two-week intervention period which included two matches, blood collection, subjective wellness scores and jump performance. HT = Half-time, FT = Full-time, MC = Montmorency tart cherries, PLB = Placebo, CMJ = Countermovement Jump, DJ = Drop jump.*

7.3.2. Participants

Eleven professional academy RL players (mean ± SD; age 18 ± 1 years, body mass 92.2 ± 8.6 kg, height 182 ± 0.04 cm) from a ESL rugby club volunteered to take part in this study. Eleven players were selected as these were the prominent starting players that were most likely to play in both matches and represented all positional groups. Players were from the same club and were injury and illness free during the study period. Ethical approval was granted from the university ethics committee (H17/SPS/020).

7.3.3. Supplementation

Using an online random number generator, players were randomly assigned to either a Montmorency cherry (MC) or placebo (PLB) group. The MC supplements were prepared by mixing two 30 mL dosages (Per 30 mL: 102 kcal, 25 g carbohydrate, 0 g fat, 1 g protein, 320 mg anthocyanins) with two 100 mL bottles of water prior to consumption. The MC was a commercially available Montmorency cherry concentrate (CherryActive™, Sunbury, UK). The PLB supplement was a commercially available fruit cordial, mixed with water and maltodextrin (Science in Sport, London, UK) into two separate bottles, to match for energy and carbohydrate content with the MC (102 kcal, 25g carbohydrate). Two MC or two PLB supplements were prepared for each player, each day, into separate bottles and then sealed by the club's sports nutritionist off-site in order to maintain the single blind design and followed previously published administration protocols (Bell et al., 2016; Howatson et al., 2010). The MC or PLB supplementation was provided to the players at the training facility along with instructions detailing the dosing schedule (one bottle in the morning and one bottle in the evening, for seven consecutive days, five days before and two days after the match). Each day the players were reminded to consume each bottle by using cellular contact (WhatsApp messages) from the club nutritionist.

7.3.4. Match Analysis

All players were fitted with a micro-technology device (Optimeye S5, Catapult Innovation, Melbourne Australia) to allow measurement of movement demands of the matches to be recorded. These were simultaneously activated at pitch side before kick-off, to enable acquisition of satellite signals. Match duration, relative and absolute number of collisions, and distances covered were recorded. Collisions experienced were determined via accelerometer and gyroscope data provided in G force (outlined in section 3.4.1). For a collision to be registered, the player maintained a nonvertical position classified as leaning forward by more than 60°, backward by more than 30° or leaning left or right by more than

45° for 1 second. During each match, players either played the full duration of the game or were substituted, as a result of tactics, by the head coach given that these were competitive in-season league fixtures.

7.3.5. Whole Blood Sampling

Whole blood samples (10ml) were drawn from a superficial vein in the antecubital fossa of the forearm using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson). Blood samples were collected at the training facility 48 h before and 48 h after each fixture as players arrived for normal training commitments and stored on ice for ~2 hrs before being transported back to the laboratory for serum separation. On match day, blood samples were collected in the changing room at each respective rugby club. This occurred during the normal half-time interval (~10 min) and within 30 min of each match finishing. All blood samples were successfully obtained within the allocated time frame, enabled by six researchers being present in the changing room at each match. Blood samples were stored on ice and transported back to the laboratory. All blood samples were centrifuged at 1500 g for 15 min at 4°C before duplicate aliquots of serum were stored at -80°C for later analyses.

7.3.6. Preparation of Human Soluble Protein Flex Set Assay

Commercially available Cytometric Bead Array (CBA) Human Soluble Protein Master Buffer Kits and individual Human Flex Sets for IL -6, -8 and -10 (BD Biosciences™, San Diego, CA) were used, according to manufacturer's instructions. A series of double diluted standards ranging from 0 - 2,500 $pg.mL^{-1}$ were prepared by serial dilution using fresh assay diluent. Known detection limits from the manufacture for each interleukin were as follows: IL-6; 1.6, IL-8; 1.2 and IL-10; 0.13 $pg.mL^{-1}$. The capture beads for the proteins of interest (provided in BD CBA Flex sets) were mixed together in a single Eppendorf tube to which 500 µl of wash buffer was added before being vortex mixed for 15 s. Capture beads were then centrifuged at 2000g for 5 minutes. The supernatant was aspirated whilst avoiding the bead pellet. The beads were resuspended with Capture Bead Diluent according to

manufacturer's instructions, before being vortex mixed and incubated for 15 min at room temperature (RT).

Standards and samples were aliquoted according to manufacturer's instructions and incubated with pooled, resuspended capture beads for 1 hr at RT with the orbital shaker at 225 rpm. Subsequently, PE-detection reagents for all analytes were pooled, resuspended in assay diluent and added to the standards and samples plus capture beads for 2 h at RT with shaking (in the dark) on an orbital shaker at 225 RPM. On completion of this incubation, wash buffer was added to all samples and standards prior to vortex mixing at 2000g for 5 min at RT. Following centrifugation, the supernatant was aspirated whilst avoiding the bead pellet. The beads were resuspended with wash buffer and analysed using the BD FACSCalibur™ (Becton Dickinson, Franklin Lakes, NJ, USA), supported by Cell Quest Pro Software (Becton Dickenson, Franklin Lakes, NJ, USA). Data were uploaded from Cell Quest Pro and Filtered using FCS Filter™ and analysed using FCAP array software (Hungary Software Ltd, for BD Biosciences, San Jose CA, USA). According to manufacture's instructions, 200 events were captured per analyte per sample.

7.3.7. Countermovement Jump and Drop Jump Performance

At 48 h before and 48 h after each match players performed a series of CMJ's and DJ's wearing trainers. Jump height was estimated from an individual's flight times (the difference between take-off and landing time) using two photoelectric parallel bars (Optojump™, Microgate, Bolzano, Italy). All players were accustomed to jump procedures as part of the clubs regular monitoring process. Following a standardised warm-up prescribed by the club strength and conditioning coach, during the CMJ protocol players began in an upright position and were instructed to place and keep both hands on their hips throughout testing. Participants then flexed the knees rapidly to ~90° before jumping for maximal height and landing back onto the mat.

During the DJ protocol, a 30 cm box was placed in front of the Optojump™ bars and players were instructed step onto the box and to keep both hands on their hips throughout testing. Players were then encouraged to step off the box without lifting their centre of gravity and land on the floor in front of them and to jump for maximal height following minimal contact with the ground. Take-off and landing position of the lower limbs was assumed to be the same, with any jumps deviated from the described procedures repeated. Players performed three jumps with the maximum flight time (Twist et al., 2012) and minimal contact time used as an index of the maximal rate of force development and reactive strength index (RSI = jump height divided by contact time) (Twist & Eston, 2007) used for analysis.

7.3.8. Self-reported Subjective Wellness

At 24 h pre-match, 24 h and 48 h post-match, participants provided a rating of perceived sleep quality, fatigue, muscle soreness, mood and stress using a 1-5 Likert scale which has been adapted from others (McLean et al., 2010) and previously used with RL players (Twist et al., 2012). Higher values were indicative of a positive response to the question, while lower values reflected a negative outcome. Similar scales have been shown to possess good reliability and validity (De Vries & Van Heck, 2003). Participants were familiar with this procedure as part of their habitual club monitoring processes and were instructed to complete on their own to avoid any influence from other players or coaching staff.

7.4. Results

7.4.1. Match Characteristics

Comparisons between match one and match two for playing duration, absolute and relative distance covered, and collisions are shown in Table 7.1. Match characteristics were similar between match one and match two, with *likely trivial* or *unclear* differences, although relative distance covered in match two was *most likely* greater compared to match one.

7.4.2. Interleukin-6

Mean IL-6 concentration was greater in both conditions at full-time when compared with 48 h pre-match (448 %, ES 1.27 ± 0.33, *most likely positive*), 48 h post-match (284 %, ES 0.94 ± 0.44, *very likely positive*) and half-time (62.4 %, ES 0.44 ± 0.39, *likely positive*). There was, however, no meaningful effects of MC supplementation on IL-6 at any time point compared with PLB (48 h pre-match: 3.2 %, ES 0.02 ± 0.65, *unclear*; half-time: -19 %, ES -0.30 ± 0.49, *possibly negative*; full-time: -40.1 %, ES -0.40 ± 0.42, *possibly negative*; 48 h post-match: 52.7 %, ES 0.32 ± 0.57, *unclear*), see Figure 7.2A.

7.4.3. Interleukin-8

Mean IL-8 concentration was greater in both conditions at full-time when compared with 48 h pre-match (92.6 %, ES 1.03 ± 0.21, *most likely positive*), 48 h post-match (57 %, ES 0.81 ± 0.28, *most likely positive*) and half-time (52.7 %, ES 0.65 ± 0.24, *most likely*). There was, however, no meaningful effects of MC supplementation on IL-8 at any time point compared with PLB (48 h pre-match: 10.6 %, ES 0.13 ± 0.70, *unclear*; half-time: -0.8 %, ES -0.01 ± 0.57, *unclear*; full-time: -5.5 %, ES -0.10 ± 0.56, *unclear*; 48 h post-match: 11.8 %, ES 0.20 ± 0.34, *possibly positive*), see Figure 7.2B.

7.4.4. Interleukin-10

Mean IL-10 concentration was greater in both conditions at full-time when compared with 48 h pre-match (259.5 %, ES 1.25 ± 0.48, *most likely positive*), 48 h post-match (194.9 %, ES 1.15 ± 0.41, *most likely positive)* and half-time (245.6 %, ES 1.25 ± 0.38, *most likely positive).* There was, however, no meaningful effects of MC supplementation on IL-10 at any time point compared with PLB (48 h pre-match: -7.5 %, ES -0.07 ± 0.83, *unclear*; half-time: 7.9 %, ES -0.06 ± 0.43, *unclear*; full-time: 5.9 %, ES 0.06 ± 0.41, *unclear*; 48 h post-match: -27.2 %, ES -0.28 ± 0.52, *unclear*), see Figure 7.2C.

Table 7.1. *Match comparison duration played, absolute and relative distance covered, and collisions performed from two rugby league competitive matches, Mean ± SD.*

	Match 1	**Match 2**	**Change in mean (%)**	**Effect Size ± Confidence Interval**	**Qualitative Interpretation**
Playing duration	67:10 ± 19:7	67:10 ± 19:3	0.0	0.05 ± 0.17	Likely trivial
Total distance (m)	6334 ± 1924	6596 ± 1776	2.1	0.05 ± 0.47	Unclear
m·min^{-1}	72.6 ± 4.8	79.3 ± 5.5	7.9	0.81 ± 0.27	Most likely ↑
Total collisions (n)	28.2 ± 11.1	28.9 ± 13.2	1.0	-0.02 ± 0.33	Unclear
n·min^{-1}	0.4 ± 0.2	0.4 ± 0.3	0.9	0.01 ± 0.26	Unclear

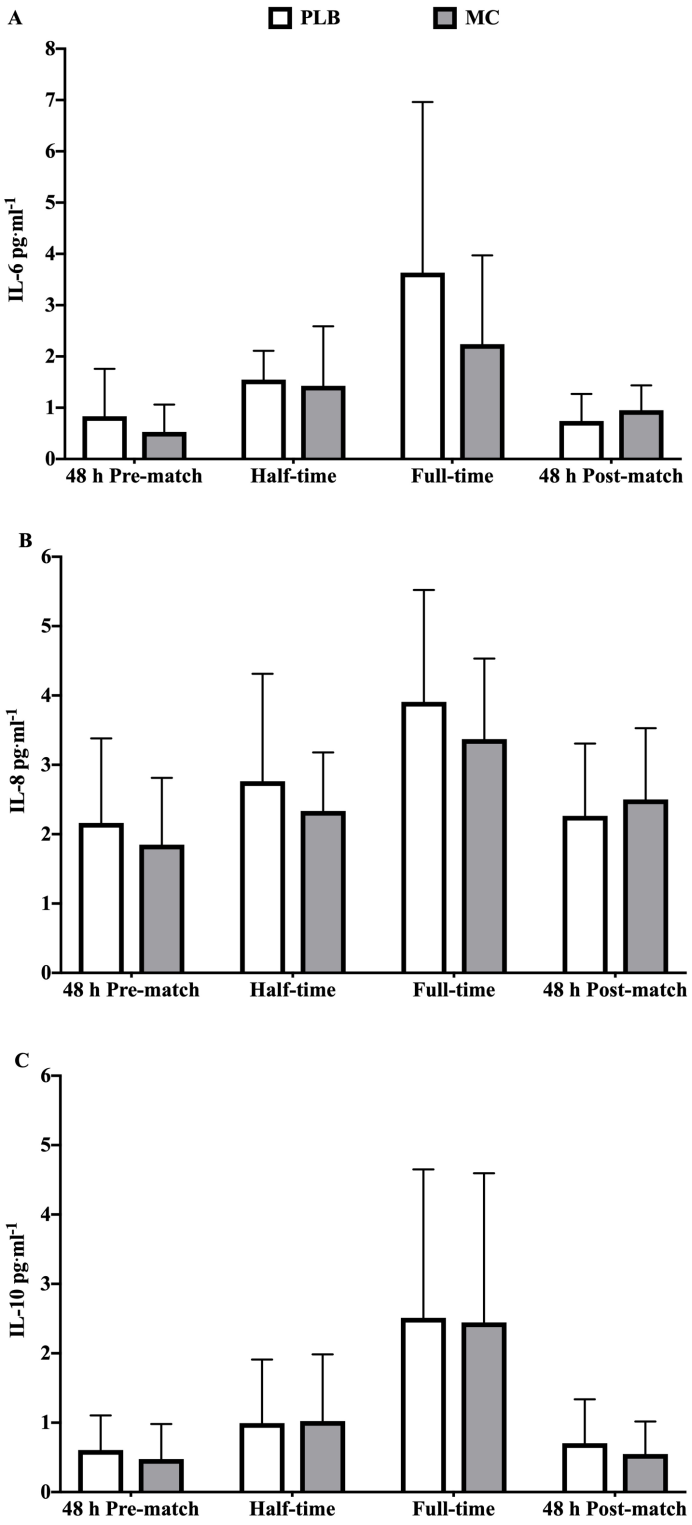


Figure 7.2. *Interleukin-6 (2A), -8 (2B) and -10 (2C) concentration at 48 h pre-match, half-time, full-time and 48 h post-match. Light bars represent placebo group, black bars represent Montmorency cherries group.*

7.4.5. Self-reported Subjective Wellness

Magnitude based inferences report *unclear* differences between baseline and 24 h post-match and 48 h post-match for sleep, mood and stress. An increase in player fatigue and muscle soreness (as indicated by a lower score) was reported at 24 h post-match in both MC and PLB groups. A decrease in muscle soreness was reported in both MC and PLB groups at 48 h post-match compared with 24 h post-match. There was, however, no meaningful effects of MC supplementation on player fatigue (2.6 %, ES 0.07 ± 0.23, *likely trivial*) or muscle soreness (-5.1 %, ES -0.12 ± 0.52, *unclear*) compared with PLB group (see Table 7.2).

7.4.6. CMJ and DJ Performance

There was a *likely* decrease (-4%, ES -0.58 ± 0.45, *likely negative*) in mean countermovement jump performance from 48 h pre-match (0.53 ± 0.03 s) to 48 h post-match (0.51 ± 0.03 s). There was, however, no meaningful differences between both MC and PLB (-1.2 %, ES -0.17 ± 0.71, *unclear*). Similarly, there was a *possible* decrease in drop jump performance from 48 h pre-match (2.15 ± 0.34 $m \cdot s^{-1}$) to 48 h post-match (2.06 ± 0.40 $m \cdot s^{-1}$). There was, however, no meaningful differences between MC and PLB (5.9 %, ES 0.23 ± 0.72, *unclear*), see Table 7.2.

7.4.7. Relationship Between Match Demands and Recovery

Correlations between selected match demands, markers of fatigue and interleukin concentrations at full time and 48 h post-match in both conditions are shown in Table 3. *Moderate* to *large* correlations were observed between IL concentrations and running based match demands. Playing duration displayed a *moderate* correlation with IL-10 (r = .33) at 48 h post-match. Total distance covered displayed a *moderate* correlation with IL-6 (r = .32) and a *large* correlation with IL-10 (r = .53) at 48 h post-match. Relative distance covered displayed a *moderate* correlation with IL-6 (r = .33) and IL-8 (r = .30) at full-time and IL-6 at 48 h post-match (r = .39). Similarly, total collisions displayed a *moderate* correlation with

IL-6 (r = .30) and IL-8 (r = .31) at full-time and IL-6 at 48 h post-match (r = .34). Relative collisions displayed a *moderate* correlation with all IL's at the full-time time point (IL-6 = r = .30, IL-8 = r = .30, IL-10 = r = .49). Finally, muscle soreness displayed moderate to large correlations with all IL's at all time points (Table 7.3).

Table 7.2. *Magnitude based inferences for subjective markers at 24 h and 48 h post-match and CMJ and DJ performance 48 h post-match in comparison with baseline, Mean ± SD.*

	Post-Match							
	24 h post-match	**Change in mean (%)**	**Effect size ± CI**	**Qualitative interpretation**	**48 h post-match**	**Change in mean (%)**	**Effect size ± CI**	**Qualitative interpretation**
Sleep quality (AU)	3.5 ± 0.5	0.6	0.03 ± 0.65	*Unclear*	3.5 ± 0.8	-1.1	-0.04 ± 0.56	*Unclear*
Fatigue (AU)	*2.7 ± 1.0	-21.1	-0.49 ± 0.60	*Likely ↓*	3.2 ± 0.8	-3.2	-0.11 ± 0.42	*Unclear*
Muscle soreness (AU)	#2.4 ± 0.9	-37	-1.07 ± 0.63	*Very likely ↓*	#2.8 ± 1.0	-23.6	-0.74 ± 0.61	*Likely ↓*
Mood (AU)	3.3 ± 0.5	-7	-0.37 ± 0.77	*Unclear*	3.3 ± 0.5	-5.1	-0.22 ± 0.53	*Unclear*
Stress (AU)	3.3 ± 0.5	-5.1	-0.28 ± 0.75	*Unclear*	3.4 ± 0.7	-3.2	-0.14 ± 0.55	*Unclear*
CMJ (s)	-	-	-	-	0.51 ± 0.03	-4	-0.58 ± 0.45	*Likely negative*
DJ (AU)	-	-	-	-	2.06 ± 0.40	-5.7	-0.26 ± 0.46	*Possibly negative*

* indicates small effect size from 24h pre-match value. # indicates moderate effect size from 24h pre-match value. CMJ = Countermovement jump, DJ = Drop jump.

Table 7.3. *Correlations between IL-6, -8 and -10, and match demands and markers of fatigue.*

	IL-6						IL-8						IL-10					
	Full-time			48 h post-match			Full-time			48 h post-match			Full-time			48 h post-match		
	r	**90% CI**	***R^2***	***r***	**90% CI**	***R^2***	***r***	**90% CI**	***R^2***	***r***	**90% CI**	***R^2***	***r***	**90% CI**	***R^2***	***r***	**90% CI**	***R^2***
Playing duration (min)	-.09	-.59 to .46	.00	.26	-.31 to .69	.06	-.17	-.64 to .39	.02	-.18	-.64 to .38	.03	-.33	-.73 to .23	.10	.33#	-.23 to .73	.10
Distance (m)	-.18	-.64 to .38	.03	.32#	-.24 to .72	.10	.04	-.49 to .55	.00	-.19	-.65 to .37	.03	-.69	-.89 to -.26	.47	.53*	.01 to .82	.28
Distance ($m \cdot min^{-1}$)	.33#	-.23 to .73	.10	.39#	-.17 to .76	.15	.30#	-.27 to .71	.09	-.34	-.73 to .22	.11	.14	-.41 to .62	.01	.25	-.32 to .68	.06
Collisions (n)	.30#	-.27 to .71	.09	.34#	-.22 to .73	.11	.31#	-.26 to .72	.09	-.20	-.66 to .36	.04	.15	-.41 to .62	.01	.01	-.52 to .53	.00
Collisions ($n \cdot min^{-1}$)	.30#	-.27 to .71	.09	-.23	-.67 to .33	.05	.30#	-.27 to .71	.09	.10	-.45 to .59	.00	.49#	-.05 to .81	.24	-.20	-.66 to .36	.04
Muscle soreness (AU)	.56*	-.05 to .84	.31	.37#	-.19 to .75	.13	.39#	-.17 to .76	.15	.31#	-.26 to .72	.09	.38#	-.18 to .75	.14	.64*	.17 to 87	.40

#moderate correlation. *large correlation.

7.5. Discussion

The aim of the present study was to assess the effects of Montmorency tart cherries on markers of muscle soreness and recovery following RL match play. To this end, using flow cytometry, we measured concentrations of IL-6, IL-8 and IL-10 collected at 48 h pre-match, half-time, full-time and 48 h post-match from eleven players over two successive in-season rugby matches. We report for the first time in academy RL players, increases in IL-6, IL-8 and IL-10 at half-time and full-time when compared with 48 h pre-match, as well as subsequent reductions in markers of recovery 48 h post-match. Furthermore, we show for the first time, that supplementation with Montmorency tart cherry juice resulted in *unclear* and *trivial* difference in IL-6, IL-10, jump performance and subjective markers of fatigue compared with a visually identical placebo. Collectively, these data contradict the assumption that meaningful reductions in markers of inflammation and improvements in recovery are possible following the ingestion of cherry juice and highlights the importance of looking at the efficacy of polyphenol supplementation following impact-induced muscle damage.

Previous research has shown increases in IL-6 concentration following match play in professional RU players (Cunniffe et al., 2011; Cunniffe et al., 2010). We confirm and extend these findings by showing increases in IL-6, IL-8 and IL-10 concentrations following competitive RL match play at half-time and full-time in RL players. The increases in IL-6 at the full-time period in the present study (3.0 ± 2.6 $pg \cdot ml^{-1}$) are lower to those reported from senior level RU (3.7-6.9 $pg \cdot ml^{-1}$) (Cunniffe et al., 2011; Cunniffe et al., 2010). These differences in post-match IL-6 may reflect that the mean body mass of the RU players was 102 kg compared with 92 kg in the academy RL players thus, increasing the force of the physical collisions, although this suggestion is purely speculative. Interestingly, although previous research has shown increases in IL-8 (4.7 ± 1.7 $pg \cdot ml^{-1}$) from other team sport athletes (Bell et al., 2016), this was performed in a controlled laboratory environment and following a simulated football exercise test, and as such, we show for the first time in academy RL players, *most likely* elevations in IL-8 following in-season RL match play (3.6 ± 1.6 $pg \cdot ml^{-1}$). Similarly, we provide novel data in academy RL players showing *most likely*

increases in the anti-inflammatory cytokine IL-10 (2.4 ± 2.0 pg·ml^{-1}), which has previously only been shown following laboratory-controlled resistance training and yet to be investigated in team sport athletes (Nieman et al., 2004). To this end, measurement of IL-6, -8 and -10 may provide a marker of inflammation following match-play that involves impact-induced muscle damage and routine measurements should be considered. Although these data are promising, more work is needed to further understand the relationship between inflammation and varied match play demands (Gabbett, Jenkins, & Abernethy, 2011; Johnston, Gabbett, & Jenkins, 2014).

In the present study *moderate* to *large* correlations are shown with IL's and match demands of absolute and relative distance covered and absolute collisions, subjective measures of muscle soreness at full-time and 48 h post-match (Table 7.3). These data confirm and extend previous research, which has used CK and muscle soreness as markers of fatigue, showing significant correlations with distance covered and collisions performed from senior ESL RL players (Oxendale et al., 2016; Twist et al., 2012). The correlations between the ILs and match demands suggest that the markers of inflammation reported following RL match play are dependent upon the physical demands that players perform. Specifically, if players spend more time on the field, then they are more likely to cover greater distances, be involved in more high-impact physical collisions and therefore, experience inflammation, increased muscle soreness, and reductions in muscle function post-match. Consequently, there were *moderate* correlations with IL-6 and playing duration, and relative distance covered with *moderate* to *large* correlations with muscle soreness and absolute and relative collisions. Although these correlations disagree with others (Fletcher et al., 2016) this may reflect the possibility that IL's are a more sensitive marker of RL match play then alternative measurements. With this in mind, IL-6 is well cited to be a sensitive energy sensor whereby increases presented in the circulation correlate to the duration of the exercise rather than the amount of damage caused, thus highlighting the link between energy metabolism and exercise duration (Hennigar & Pasiakos, 2017).

Similar to IL-6, there were *moderate* correlations between IL-8 and relative distance covered and collisions, absolute collisions and muscle soreness. To the author's knowledge, this is

the first study to examine the relationship between RL match characteristics on changes in IL-8 concentration and muscle soreness. As with IL-6, it appears the increases reported in IL-8 are dependent of the physical demands that players perform and agrees with others who have found comparable correlations with total contacts and CK following rugby demands (Cunniffe et al., 2010; McLellan, Lovell, & Gass, 2011; Takarada, 2003). Finally, IL-10 shows *moderate* correlations with relative collisions and *moderate* to *large* correlations with total distance covered and muscle soreness. Taken together, it appears that IL's have the potential to detect EIMD, however more importantly for rugby practitioners, also the potential to detect IIMD which is unique to contacts sports like RL. It is plausible that quantifiable markers of inflammation like IL's may be able to give an idea into how fatigued and sore players may present in the days following match play, however this remains speculative and requires further investigation.

The main aim of the present study, however, was to assess the effects of MC supplementation on markers of recovery and inflammation following competitive RL match play. There was no meaningful effect of consuming Montmorency cherries compared with an energy matched placebo beverage on IL-6, -8 and -10, muscle soreness and jump performance (Figure 7.2 and Table 7.2). Our findings conflict others who have shown beneficial effects of Montmorency cherries on reducing markers of inflammation and soreness following laboratory trials (Bell et al., 2016; Bowtell et al., 2011), and marathon running (Howatson et al., 2010). A possible explanation as to why we found no difference between treatment groups could be because the analgesic effects of polyphenols may only exert beneficial effects with exercise modalities that involve metabolic inflammation and soreness from EIMD, without the additional IIMD that is experienced from contacts sports like RL. Indeed, we report large correlations with IL-6 and collisions at 48 h post-match (r = .52) and muscle soreness at full-time (r = .54), which is consistent with others who have observed associations between CK as a marker of muscle damage and collisions experienced (Oxendale, Twist, Daniels, & Highton, 2016; Twist et al., 2012). Another possible explanation for the disagreement between this study and previous literature, may be the fact that players in our study were not asked to restrict any polyphenol rich foods or beverages prior to, or during, the intervention period unlike previous studies (Bell, Walshe, Davison, Stevenson, & Howatson, 2015; Bell

et al., 2016; Bowtell et al., 2011). It is possible that the benefits of Montmorency cherries are only reported when repleating deficient concentrations of polyphenols following a restriction period, rather than the added benefit of enhanced polyphenol concentrations. To this end, if players follow a good diet including fruits and vegetables then the use of such supplements may not be needed, however there still may be a benefit for those players who do not have a polyphenol rich diet.

A limitation of the present study was that we did not measure players dietary polyphenol intakes due to the known limitations of collecting weighed food dietary intakes (Hill & Davies, 2001; Magkos & Yannakoulia, 2003). However, all players were educated on the benefit of a regular polyphenol rich diet through daily consumption of fruit and vegetables and this was monitored by the clubs registered sports nutritionist throughout the study. We feel allowing players to consume habitual intakes throughout the study period was indeed a better reflection of what elite rugby diets consist of (Bradley et al., 2015) and therefore, when consuming a typical rugby diet pre and post-match play, we show no beneficial effects of Montmorency cherry juice supplementation on muscle soreness, function and markers of inflammation.

7.6. Practical Applications

This study has several practical applications which could be used by applied practitioners for immediate translation as well as suggestions for further studies. Firstly, IL's appear to be a promising marker for match play demands, in particular those activities that result in IIMD that are unique to contact sports like RL. Future studies should now further explore this relationship looking specifically at positional differences, the magnitude of collisions involved in and in senior RL players during congested fixture weeks. Secondly, it should be noted that when consuming Montmorency tart cherry juice, alongside habitual nutritional intakes, no beneficial effects are reported for reducing markers of inflammation, muscle soreness and functional performance following RL match play.

7.7. Conclusion

In conclusion, the present study has for the first time assessed inflammatory markers following RL match play whilst assessing the effectiveness of Montmorency cherries consumption in professional academy RL players. We report *most likely* increases in IL-6, -8 and -10 at half-time and full-time compared to 48 h pre-match. We also show, for the first time in team sport athletes, no beneficial effects of consuming Montmorency tart cherry juice on markers of inflammation, muscle soreness, muscle function, or sleep following both EIMD and IIMD. The data in this study therefore questions the use of such supplements in a sport like RL where budgets are often a key consideration for the wider performance department, and when, typically the majority of athletes, of whom most work with a nutritionist, normally consume a rich polyphenol-based diet in the first place.

CHAPTER 8.

THESIS SYNTHESIS

This chapter provides a synthesis of the main aims and outcomes of the studies described in this thesis. The findings are then outlined in relation to the aims and objectives outlined in Chapter 1. The chapter then progresses to examining the limitations and practical applications of the findings. Finally, future research directions are presented. A schematic of the thesis is presented in Figure 8.1.

8.1. Realisation of Aims

8.1.1. Aim 1 – To assess the position specific body composition profiles in senior professional players

This aim was addressed in Chapter 4. Body composition profiles were assessed from 112 professional senior players across 5 different ESL clubs using DXA scan technology. Body composition profiles reported that, despite *small* to *very large* inter-positional differences in all anthropometric variables across 6 positional groups, in particular the prop forwards and other positions, there was large intra-position variation in body fat, lean mass and total mass. Therefore, in a large group of professional senior RL players from different clubs, a standard position specific body composition profile is difficult to establish. As such, these data provide a base for talent identification and player profiling, although caution must be taken given the prevalence for deviations from the standard position profile.

8.1.2. Aim 2 – To assess the body composition changes of professional academy players over three successive seasons with a particular focus given to the pre-season period

This aim was addressed in Chapter 5. Changes in body composition profiles were tracked over three successive pre-season periods in 11 academy RL players, from two separate ESL clubs, using DXA scan technology. Body composition profiles reported no meaningful changes in lean mass during any of the pre-season periods, with only *small* changes occurring over the three-year study period with similarities reported for total mass and fat mass levels. Therefore, in a small group of academy players, these data suggest young players need time to develop towards player profiles congruent with those seen from senior players. As such, coaches should use these data to help plan more appropriate and timely increases in lean muscle mass, appreciating that for some players, meaningful changes can take one to three years to achieve.

8.1.3. Aim 3 – To assess the body composition changes in professional senior players following a pre-season period

This aim was addressed in Chapter 5. Changes in body composition profiles were assessed in 99 elite senior players, from three different ESL clubs, at the beginning and end of the same pre-season period, using DXA scan technology. Across all body composition characteristics, in all positional groups, we report *trivial* changes prior to and following a pre-season training period. These data contradict the assumption that significant gains in lean mass are made during a RL pre-season and highlights the importance of coaches looking at changes in body composition across an entire season, along with the need for player specific training and nutrition strategies to maximise training adaptation.

8.1.4. Aim 4 – To assess the TEE and EI during a 14 day in-season period in senior professional players using the gold standard technique of DLW

This aim was addressed in Chapter 6. Resting metabolic rate, EI and total TEE, using the gold standard technique of doubly labelled water, was assessed in 6 ESL players over two consecutive in-season weeks. RMR, via indirect calorimetry, showed unclear differences between forwards and backs and values *likely* lower (16.5 %) than predictive equations. This questions the use of traditional predictive equations for players with a large muscle mass with future research now needing to develop more specific equations. TEE reported differences between the two competitive weeks and was greater than previously suggested in rugby (mean of 22.5 MJ), which likely represents the ability of DLW to account for all aspects of TEE including the energetic demands of contact situations. In particular, the high NEAT reported in this study, suggests support staff should try and quantify activities that players are performing away from the club. We believe the data to have immediate translational potential to help support staff at rugby clubs to evaluate the energy cost of training sessions and aiding player specific diet plans.

8.1.5. Aim 5 – To assess the inflammation caused from RL match play and the effects of Montmorency tart cherry juice supplementation on the recovery period of professional rugby players

This aim was addressed in Chapter 8. Changes in interleukin-6, -8 and -10 concentrations following RL match play was assessed in 11 elite academy players following two in-season matches. We also assessed for the first time in elite team sport athletes, the effectiveness of ingesting Montmorency tart cherries following competitive match play on reducing markers of inflammation and functional measures when players have consumed their regular diets leading into the match. Concentrations of IL-6, -8 and -10 were all *likely* elevated at half-time and full-time when compared with baseline, and furthermore *moderate* to *large* correlations were reported with both EIMD and IIMD, resulting from tackles and collisions. These data suggest that IL's may be a suitable candidate to assess both EIMD and IIMD from match play demands and this now requires further investigation. We also show, *unclear* and *trivial* effects of Montmorency tart cherry juice on IL-6 and IL-10 concentrations, jump performance and subjective markers of fatigue compared with a placebo. These data therefore contradict the assumption that meaningful reductions in markers of inflammation and improvements in recovery are possible following the ingestion of Montmorency tart cherry juice after IIMD.

Figure 8.1. *Schematic summary of study 1, 2 3 and 4 from the present PhD Thesis*

8.2. General Discussion

Many coaches working in RL clubs aim to develop both academy and senior player's body compositions profiles concomitant to that of the modern-day player. In doing so, players present at the start of pre-season with large total mass and lean mass profiles, resulting in repeated high impact physical collisions during training and match play. These demands result in high amounts of TEE reported from both training and match play which in a sport that involves both repeated high intensity sprints and collisions, makes it unsurprising that players experience elevations in markers of inflammation and muscle soreness. The developments in the measurement of biochemical markers of inflammation are providing a greater understanding to the magnitude of inflammation experienced. In an attempt to reduce inflammation and accelerate recovery, many athletes choose to consume popular nutritional supplements including Montmorency tart cherries. However, currently, the efficacy of such supplements in elite team sport athletes is limited. Current understandings, future directions and practical implications for this thesis will now be discussed.

8.2.1. Body Composition of RL Players

As would be expected, within the player performance pathway, total body mass increases with age (Till et al., 2013; Waldron, Worsfold, Twist, & Lamb, 2014c), with many academy players striving to reach body composition profiles concomitant with senior players in chapter 4. In an attempt to measure changes in body composition profiles of academy RL players, many studies have utilised skinfold measurements (Dobbin et al., 2018; Till, Jones, et al., 2015), however, with known limitations of predictive equations (Doran et al., 2014), monitoring body composition via skin fold assessments is questionable. With this in mind, others have attempted to assess academy player body compositions using DXA scan technology, although this has only been performed at one time point in the whole season (Till, Jones, O'Hara, et al., 2016) or from two time-points six years apart (Jones et al., 2018). Body composition data from just one time point in the season only provides a snap shot of what changes may occur over the whole season, and more importantly during the crucial

development years of academy player's early career. Furthermore, body composition data from the crucial pre-season period is currently limited. To this end, in Chapter 5, body composition from eleven academy players was tracked over three successive seasons. In particular, we tracked these changes during the pre-season period in an attempt to identify what changes are possible during this important period of the whole season. We provide novel body composition data from academy players showing no meaningful changes were possible during the same pre-season period in each year. Interestingly, players only showed meaningful changes between the start of pre-season in year 1 and the end of pre-season in year 3, highlighting the requirement of coaches to plan more appropriate and timely change in body composition profiles, appreciating that for some players, meaningful changes can take one to three years to achieve.

At the end of the study in Chapter 5, all eleven academy players were playing regular senior level RL. With many academy players striving to achieve position specific body composition profiles similar to senior players, it is important to therefore understand what body composition profiles look like at senior level. To this end, DXA scan technology has previously been used to measure body composition in elite RL players (Georgeson et al., 2012; Greene et al., 2017; Harley et al., 2011), however to date these studies have failed to split players into player positional groups or have been performed over the whole season, without taking into consideration the pre-season period. During Chapter 4, we therefore assessed body composition profiles from 112 professional senior RL players, from 5 different ESL clubs, allowing profiles of each positional group to be established. Interestingly, both inter- and intra-variability in body composition was reported in total mass, lean mass and body fat resulting in an optimal position specific body composition profile difficult to establish. Furthermore, only players from the Prop forward position were significantly different to other positions, possessing greater total mass, lean mass and fat mass profiles that all other positions. These data suggest that the modern demands of RL are beginning to change the body composition requirements of the varying positional groups with all players now expected to have high levels of lean mass and low body fat.

Given the emphasis on high levels of lean mass, it is crucial to establish the magnitude of change that is possible during a senior RL player pre-season. To realise this, we assessed body composition, using DXA scan technology, from 99 senior RL players from four different ESL clubs, at the start and end of the same pre-season period. Similarly, to academy players, body composition changes in senior players from the start to the end of the same pre-season period show no meaningful changes across all playing positions. These data contradict the assumption that significant gains in lean mass are made during a single RL pre-season and highlights the importance of looking at body composition over the whole season. Furthermore, collectively these data highlight the need for both academy and senior player specific training and nutrition strategies to maximise training adaptations. Alternatively, coaches may wish to amend the structure of pre-season periods to give more time for players to develop lean mass if this is a priority of a given player, or consider refocusing this time to develop other skills that determine success during match play for example, fitness, conditioning, and handling skills.

8.2.2. RL Demands and TEE

A RL pre-season lasts between 12-14 weeks and is followed by a 30-34 week in-season period. For most players, the in-season period involves 3-4 training days, 1 match day and 1-2 rest or recovery days. To allow specific diet plans to be provided to players during this time period it is important to understand the TEE in conjunction with EI of a typical in-season week although prior to this thesis such data were limited. For example, previous research has attempted to quantify TEE in elite RU players (Bradley et al., 2015) and elite RL players (Coutts et al., 2003) these studies were unable to assess the expenditure during the contact situations thus missing a major component of the training week. DLW (Schoeller et al., 1986), is one technique that can assess all aspects of TEE in elite rugby players, however due to financial implications studies in elite rugby players are limited. To this end, in an attempt to understand the TEE of a typical week, using the gold standard technique of DLW, 6 senior RL players were assessed over a 14 day in-season period along with RMR and EI in Chapter 5. We report for the first time in elite RL players, RMR values that are greater than commonly

used predictive equations (Cunningham, 1980) and TEE values significantly higher than previously shown for elite rugby players (Bradley et al., 2015; Kempton, Sirotic, Rampinini, et al., 2015). This likely reflects the ability for DLW to account for all aspects of RL demands, in particular the physical impacts that are sustained following RL training and match play. Additionally, our data shows large variation in the TEE of players that could not be explained by the RMR or monitored training sessions. These variations suggest that NEAT is a major contributor to the TEE in rugby players and therefore it is essential that support clubs understand and attempt to quantify this contribution away from club commitments. It is therefore crucial that clubs attempt to assess the TEE of their players to allow accurate dietary advice to be provided to players. It is possible that the lack of changes in the pre-season period discussed above reflects inappropriate fuelling of this key time period due to a lack of understanding of the TEE of the players.

8.2.3. Post-match Inflammation, Soreness and Recovery

Considering the physical demands and repeated high intensity periods of activity, it is commonly reported that RL players experience muscle damage and soreness following match play. Indeed, studies have shown increases in CK, following match play, with subsequent increases in player ratings or soreness and decrements in muscular function, as well as moderate to strong correlations with match duration and running demands, which has resulted in EIMD (Fletcher et al., 2016; Oxendale et al., 2016; Twist et al., 2012). However, there is agreement that rugby involves both EIMD and, uniquely compared with other team sports, IIMD. For example, high impact physical collisions that cause blunt force trauma have also been shown to correlate to increases in CK (Takarada, 2003). Recently, the use of CK as a marker of damage and inflammation has been questioned with known limitations, including individual clearance rates, individual responsiveness and providing no magnitude to the amount of damage sustained (Fridén & Lieber, 2001). To this end, an alternative marker of inflammation is the assessment of interleukins which has consistently been shown to increase at the onset of exercise (Fischer, 2006) with elevations reported following match play in RU players (Cunniffe et al., 2011; Cunniffe et al., 2010). However, studies investigating IL's following RL match play are yet to be performed, and as such in Chapter

7, we assessed the changes in IL-6, IL-8 and IL-10 following two competitive RL matches in 11 academy players. We confirm and extend previous work in rugby (Cunniffe et al., 2011; Oxendale et al., 2016; Twist et al., 2012), by reporting increases in all three IL's at half-time and full-time periods of RL match-play. Furthermore, we show increases in muscle soreness, and reductions in muscle function following both matches. In an attempt to enhance recovery following muscle damage and inflammation, many athletes utilise post-match nutritional strategies, one of which is the consumption of a popular supplement - Montmorency tart cherries. Although some studies have shown beneficial effects of consuming such supplements on recovery following muscle damage, they have done so following polyphenol elimination diets prior to intervention (Bell et al., 2015; Bowtell et al., 2011), laboratory controlled interventions (Bell et al., 2016; Bowtell et al., 2011), or exercise modalities that have caused damage from EIMD rather than IIMD, for example marathon running (Howatson et al., 2010). To this end, we assessed for the first time in elite team sport athletes, the efficacy of Montmorency tart cherry juice supplementation on markers of inflammation, reducing muscle soreness and assisting recovery post RL match play. We report no meaningful effects of Montmorency tart cherry juice on any IL concentration, or any beneficial effects on reducing soreness post-match. This data therefore questions the assumption that consumption of Montmorency tart cherries is beneficial, especially when athletes may be consuming diets that are contain various fruits and vegetables containing a high amount of natural polyphenols from food based sources. In summary, the present thesis has identified the body composition profiles of elite players, assessed the magnitude of the change possible in elite academy and senior players, quantified the TEE of elite players using gold standard techniques and evaluated the effectiveness of a nutritional intervention to alleviate muscle soreness. As such, the data has made meaningful contributions to the existing rugby literature and has immediate translational potential.

8.3. Thesis Limitations and Future Directions

8.3.1. Chapter 4

Whilst this study provides novel data for the literature, inherent limitations exist as a direct consequence of collecting data on elite full-time professional athletes. Although players were asked to arrive to the laboratory euhydrated, no formal hydration assessment was performed. Additionally, dietary intake was not controlled for 24 h before DXA assessment and this might have influenced results through food intake affecting lean mass estimates (Nana et al., 2014). Players were only measured once at the start of pre-season or at the beginning of the season and it is well documented that anthropometric profiles might change throughout the season (Georgeson et al., 2012; Harley et al., 2011), therefore data measured during the start and end of the same pre-season period would be valuable. Finally, future research should be performed on a large sample of Super League clubs and players in an attempt to establish:

1. If anthropometric profiles change between the start and end of the same pre-season period?
2. If anthropometric profiles change between playing level? For example, collecting data from academy players as they develop into senior squads, or from within a single pre-season period would more meaningful data for practitioners working within clubs.

8.3.2. Chapter 5

As with chapter 4, inherent limitations of assessing body composition from elite full-time athletes are also a possible limitation and have been outlined previously in section 8.3.1. During this study, we provide novel data showing no meaningful changes in each pre-season period, although this was only performed from a small cohort of players. One of the reasons for eleven players being tracked, and not more, was due to the difficulties of accessing players who were contracted to the same club, with the same coaching staff, during a 3-year study period. In addition, due to the small number of players assessed, differences between

positional groups were not reported. Finally, although pre-season changes were reported from 99 elite senior players, this was collected from within one single pre-season period and may not reflect the differences between each pre-season and changes that may have occurred over the whole season. With this in mind, future research should now look to explore if:

1. Meaningful changes can be detected in a larger sample of academy players?
2. Body composition changes differ between multiple academy squads.
3. Changes in senior player body composition profiles change over the whole season and across multiple pre-seasons?
4. Teams who physically unloaded players from running demands, allowing more time for resistance training, were able to elicit greater gains in lean mass?
5. Teams who dedicated more time to improving tactical training rather than changes in body composition, went on to achieve greater success in-season?

8.3.3. Chapter 6

Although the use of DLW is the gold standard method of TEE in free living humans, this technique is not without its limitations. The method uses several assumptions such as a constant rate of CO_2 production and constant size of body water pool throughout the measurement period. In addition, not all researchers use the same methods to calculate the isotope pool spaces, the constant elimination rate, the fractionation factors and the mode of CO_2 conversion to energy expenditure (Buchowski, 2014). Furthermore, the calculation of TEE via DLW is based on the elimination rate of each isotope from the body. To this end, when performing this technique in athletes who may present with body mass profiles greater than 100 kg, the elimination rate is approximately 14-days, making it difficult to establish day to day TEE. Day to day TEE can only be established by dividing the total TEE by the assessment period. Unfortunately, calculations via this method make it difficult to detect the different demands of training or match play independently of each other. The reported EI was lower than the TEE in both the forwards and backs. Although some of the meals consumed by the players were provided and therefore monitored, the large discrepancy between TEE and EI probably reflects inaccuracies in self-reporting dietary intake (Bingham

et al., 1994). This is further supported by the players' body mass remaining unchanged during the study (94.7-94.8 kg). Previous research has suggested that the self-reported EI bias can be as high as 34 % (Ebine, Feng, Homma, Saitoh, & Jones, 2000; Fudge et al., 2006; Hill & Davies, 2002), which appears likely in the present study. These data confirm that caution should be taken when interpreting food diaries from athletes, even when considerable care has been taken by the athlete and the practitioner to complete them accurately. Finally, this study was only performed on six players, due to known financial costs for each isotope (£1000 per isotope per player) and therefore, future studies might wish to:

1. Extend these data into a larger population of players
2. Collect TEE from two separate time points of the season
3. Attempt to validate more routine measures of TEE (e.g. Sensewear, Actigraph) against DLW

8.3.4. Chapter 7

Study 4 was performed on only eleven players due to the difficulty in collecting blood samples at 48 h pre-match, half-time, full-time, and 48 h post-match time points consistently across the starting playing squad, over the two-week intervention period, and therefore does not represent the full seventeen playing squad. A further limitation is that the data were collected from only one team, over two matches, and as such may not represent the varied demands of training and match play that are seen from different match outcomes (Johnston, Gabbett, & Jenkins, 2014). For example, a particular match may involve a higher amount of defensive sets, including a greater number of tackles compared with another match. Furthermore, considering body composition profiles of academy and senior RL players are different (Greene et al., 2017; Till, Jones, O'Hara, et al., 2016), it is feasible to suggest the extent of inflammation caused from repeated physical collisions from senior players, who are heavier and stronger than academy players (Comfort, Graham-Smith, Matthews, & Bamber, 2011; Till et al., 2014), would be greater than those reported in this study. Dietary intakes of polyphenols were not measured due to known limitations of collecting weighed food dietary intakes (Hill & Davies, 2001; Magkos & Yannakoulia, 2003) and as such it is

possible that some players were consuming higher polyphenolic diets than others. Finally, measures of muscle damage were taken from circulatory blood markers and without muscle biopsy data, it is difficult to ascertain actual structural damage to muscle. Taken together future research should now look to investigate changes of inflammatory markers and the efficacy of Montmorency cherries from:

1. Multiple matches during congested fixture windows
2. A full playing squad including substitutes
3. A senior RL playing squad
4. A polyphenolic deplete diet to test the hypothesis that it is indeed the removal of the polyphenols that supports protection

8.4. Conclusions and Practical Implications

In conclusion, the data from this thesis has contributed novel data to the rugby literature, most of which has immediate translational potential. We have reported that changes in body composition are limited within a single pre-season period in senior players, and it can take one to three years to see any meaningful differences in academy players. TEE of professional rugby players is greater than previously reported which directly impacts the nutritional strategies coaching staff may implement with players, particularly the importance of increasing total energy intake during congested training and match periods to account for the increased EE. Finally, we report increases in IL-6, -8 and -10 and muscle soreness following RL match play, with no beneficial effects on recovery markers when consuming Montmorency tart cherry juice compared with a placebo.

As described in the opening chapter of this thesis, and as described by the data presented, body composition differences and changes are varied between academy (Chapter 5) and senior RL players (Chapter 4). This is shown particularly during the pre-season period with no meaningful changes found in both academy and senior players (Chapter 5). Data presented in this thesis also shows in-season TEE, to be much greater than previously reported for

professional rugby players (Chapter 6). It has been shown that RL match play causes structural damage to muscles, resulting in increases in circulating CK, which have been correlated to specific match play demands. While the increase of this marker is important, a far better indication of the magnitude to inflammation caused from both EIMD and IIMD, is the assessment of interleukins (Chapter 7). Although many athletes consume nutritional aids in an attempt to try and reduce soreness following match play, we show for the first time in team sport athletes no meaningful benefit of such supplements when compared with a control beverage.

In summary, it is important that coaches appreciate body composition changes are individual and may take time to see any meaningful changes from between and within each positional group. Additionally, TEE from in-season rugby match play demands are greater than originally reported and this data should be used to inform nutritional strategies to help maintain body composition profiles obtained during the pre-season period. In particular, diets should be prescribed on an individual basis taking into consideration the body composition of each player as well as the position specific demands of match play and how this will effect levels of muscle soreness and as such nutritional interventions to aid with recovery. Finally, coaches should question the importance of purchasing such supplements, that can prove to be expensive, when at present, the efficacy of their use within elite team sport athletes is non-meaningful.

The most significant practical implications of the data are:

1. There is no one position specific anthropometric profile of elite rugby players and therefore generalised targets, like seen in other sports are not possible
2. Meaningful changes in body composition profiles are rarely seen from the start and end of the same pre-season period in senior players
3. Meaningful changes in lean mass in academy players takes time and coaches should appreciate that individual players develop at different rates

4. In senior players, TEE is much greater than previously reported and RMR is significantly different to predictive equations, both of which have direct implications for diets that may be prescribed to the professional player
5. Supplementing Montmorency tart cherry juice has no meaningful benefit, when compared with a placebo, on recovery in rugby players whilst they are consuming a normal habitual diet, and as such the use of such supplements in team sport athletes is questionable

CHAPTER 9.

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