Investigations in to the physiological and metabolic demands of elite rugby players: understanding how best to fuel the athlete

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Abstract:

Rugby is a complex, high-intensity, intermittent, collision sport with emphasis placed on players possessing high lean body-mass and low body-fat. After an 8-12 week pre-season focused on physiological adaptations, emphasis shifts towards optimizing competitive performance and recovery through periodising player's diets and training.

In Chapter 4 the physiological demands and nutritional intakes of 45 elite rugby players were assessed during a pre-season through a battery of strength and conditioning tests, quantification of training demands using global positioning system (GPS), and two 24-hour diet recalls. Mean weekly distance covered during training was 9774 ± 1404 and 11585 ± 1810 m with a total mean weekly session RPE (sRPE) of 3398 ± 335 and 2944 ± 410 arbitrary units (AU) for forwards and backs respectively. Mean daily energy intake was 14.8 ± 1.9 and 13.3 ± 1.9 MJ, carbohydrate (CHO) intake was 3.3 ± 0.7 and 4.14 ± 0.4 $g \cdot kg^{-1}$ body mass, protein intake was 2.52 ± 0.3 and 2.59 ± 0.6 $g \cdot kg^{-1}$ body mass, and fat intake was 1.0 ± 0.3 and 0.95 ± 0.3 $g \cdot kg^{-1}$ body mass for forwards and backs respectively. Markers of physical performance (1-RM strength, speed, and repeated sprint tests) and anthropometry (body fat, and estimated lean mass) significantly improved in all players, despite players' self-selecting a 'low' CHO 'high' protein diet. It may be speculated therefore that 'low' CHO 'high' protein intakes are appropriate to fuel the pre-season, although whether these intakes are sufficient to fuel the in-season is unknown.

Once the demands of the pre-season were established, the next aim of the thesis was to examine if requirements changed during the playing season, as well as quantifying energy expenditure. In Chapter 5 in-season training load using GPS and sRPE, alongside six-day assessments of energy intake (EI) and energy expenditure (EE) was measured in 44 elite Rugby Union players. Mean weekly distance covered was 7827 ± 954 m and 9572 ± 1233 m with a total mean weekly sRPE of 1776 ± 355 and 1523 ± 434 AU for forwards and backs, respectively. Mean daily EI was 16.6 ± 1.5 and 14.2 ± 1.2 MJ, and EE was 15.9 ± 0.5 and 14 ± 0.5 MJ for forwards and backs respectively. Mean CHO intake was 3.5 ± 0.8 and 3.4 ± 0.7 $g \cdot kg^{-1}$ body mass, protein intake was 2.7 ± 0.3 and 2.7 ± 0.5 $g \cdot kg^{-1}$ body mass, and fat intake was 1.4 ± 0.2 and 1.4 ± 0.3 $g \cdot kg^{-1}$ body mass for forwards and backs respectively. All players who completed the food diary self-selected a 'low' CHO 'high' protein diet during the early part of the week which increased in the days leading up to a match. EI and EE followed an inverse trend, with expenditure exceeding intake during the first four-days of the training week and then reversed in the day leading up to competition with intake exceeding expenditure. Despite this, mean EI exceeded EE which alongside no micronutrient deficiencies, suggest that the current dietary practices of these elite rugby players seem sufficient to fuel training during the in-season, providing energy intake and CHO are increased leading up to a match. Given that intakes reported in this study are still below recommended CHO intake for elite athletes (Burke et al 2011), however, it is still possible that such intakes are not optimal for match day performance.

Given that in Chapters 4 and 5 it was found that elite Rugby players appear to deliberately

select a low carbohydrate intake, it was deemed important to assess match-play glycogen demands following a low (the amount self selected in chapter 4) and higher (the amount self selected leading in to competition in chapter 5) carbohydrate diet. Therefore, in Chapter 6 the metabolic and physiological demands of rugby competition was assessed in 16 professional Rugby League players following either a 6g·kg (HCHO) or 3g·kg (LCHO) CHO diet for 36-hours. Muscle biopsy and blood was collected, alongside monitoring internal and external load through GPS and heart rate. Mean distance covered was 93.7 ± 12.4 and 89.4 ± 9.8 $m \cdot min^{-1}$ in the first, and 85.3 ± 13.1 and 86.9 ± 9.7 $m \cdot min^{-1}$ in the second half for HCHO and LCHO conditions respectively. Mean $\%HR_{peak}$ was 82.9 ± 6.1 and 81.9 ± 7.2 % in the first and 82.5 ± 7.5 and 78.4 ± 10.5 % in the second half for HCHO and LCHO conditions respectively. Mean muscle glycogen was 448.6 ± 50.8 and 444.2 ± 81.1 $mmol \cdot kg\ d \cdot w^{-1}$ pre-game, and 243.4 ± 42.5 and 297.7 ± 130.5 $mmol \cdot kg\ d \cdot w^{-1}$ post-game for HCHO and LCHO conditions respectively. Results demonstrate that a competitive RL match can result in ~40% muscle glycogen depletion and that match-day performance variables did not differ between conditions. It was postulated that an absolute amount of ~600 g CHO consumed 36-hours pre-match is a recommended strategy for rugby league players, although optimal dietary strategies to refuel after rugby competition are unknown.

The final aim of the thesis was to examine if the current post exercise CHO guidelines are appropriate for rugby players. In Chapter 7 the magnitude of muscle glycogen repletion after consuming an immediate, or delayed re-feed post Rugby League Match Simulation Protocol (RLMSP) was assessed in 16 university rugby league players using muscle biopsy and blood letting techniques. Muscle glycogen *very likely* increased 48-h post-simulation (272 ± 97 *cf.* 416 ± 162 $mmol \cdot kg^{-1}$d.w.) after an immediate re-feed, but changes were *unclear* (283 ± 68 *cf.* 361 ± 144 $mmol \cdot kg^{-1}$d.w.) after a delayed re-feed. Creatine Kinase (CK) *almost certainly* increased by 77.9 ± 25.4 % (0.75 ± 0.19) post-simulation for all players. Player Load (8 ± 0.7 AU) and $\%HR_{peak}$ (83 ± 4.9 %) were consistent with professional RL match-play. Time to exhaustion performance test revealed no difference between conditions. This study found that simulated RL match-play elicits lower muscle glycogen utilisation (21 *cf.* 40 %) despite similar player load and metabolic demands to a professional RL match. This may be attributed to the difficulties of replicating extensive structural damage and physical exertion from collisions during a simulation. It was also found that substantial muscle glycogen resynthesis was possible in the immediate dietary re-feed group despite evidence of muscle damage via increased blood proteins, indicating that with appropriate feeding strategies it is possible to replenish a damaged muscle.

Taken together, this thesis has characterized the training demands and energy balance of elite rugby players during the pre-season and in-season, alongside quantifying the metabolic demands of elite rugby match-play, and the most appropriate strategies to load and replenish muscle glycogen around such exercise. Future studies must now further titrate these studies and assess muscle glycogen utilisation over a number of games whilst assessing the glycogen content of individual muscle fibre types.

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‘Firstly, I would like to thank my Director of studies and mentor, Professor Graeme Close. Graeme has provided unwavering and continued support during these years of study and most importantly, has kindly afforded his time and guidance outside of these studies for which I cannot thank him enough. I have developed a considerable amount as a researcher, an applied practitioner and at a personal level. Simply put, without you I would not be where I am today. I am forever grateful for the opportunities you have provided me and I hope that we can continue to work together for years to come.’

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Dedication

'I dedicate this thesis to my parents Lawrie and Una for their continued support throughout my education and life without whom this would not have been possible. I know the "funny hat" will make you proud!'

'I also dedicate this thesis to my partner Claudia, who has offered encouragement, support, and patience throughout.'

Declaration

I declare that the work in this thesis, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own. Additionally, all attempts have been made to ensure that the work is original, and does not to the best of my knowledge breach any copyright laws, and has not been taken from the work of others, apart from work that has been fully acknowledged within the text of my work.

Publications of the work within this thesis are listed below.

Bradley, W., Cavanagh, B., Douglas, W., Donovan, T. F., Morton, J. P., & Close, G. L. (2015a). Quantification of training load, energy intake and physiological adaptations during a Rugby pre season: A case study from an Elite European Rugby Union Squad. *J Strength Cond Res*, *29*, 534-44. **(Chapter 4).**

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List of abbreviations

1-RM – 1 Repetition max

ACOD – Acyl-CoA oxidase

ACS – Acyl-CoA sunthetase

AH – Actiheart

AMP – Adenosine monophosphate

AMPK – Activated protein kinase

ANT – Adenine nucleotide translocator

ATP – Adenosine Tri-phosphate

AU – Arbitrary units

Back – Backs

BF% - Body fat %

BIA - Bioelectrical impedance analysis

BUCS - British Universities and College Sports

CHO - Carbohydrate

CHRM - commercial heart rate monitors

CK – Creatine Kinase

CL – Confidence limits

CMJ – Countermovement jump

CoA – Coenzyme A

CPT – carnitine palmitoyltransferase

DE – Delayed

DLW – Doubly Labelled Water

DXA – Dual Energy X-Ray Absorptiometry

EE – Energy expenditure

EI – Energy intake

EIMD – Exercise induced muscle damage

FABP – fatty acid binding protein

FAT – Fat

FAT/CD36 – Fatty acid translocase

FATP – Fatty acid transport protein

FFM – Fat free mass

FFQs – Food frequency questionnaires

FWD – Forwards

GD – Game day

GD-1 - Game day -1

GD-2 - Game day -2

GD-3 - Game day -3

GD-4 - Game day -4

GD-5 - Game day -5

GD+1 - Game day +1

GK – Glycerol Kinase

GLUTs – glucose transporters

GPA – glycogen phosphorylase A

GPO – Glycerol phosphate oxidase

GS/GP kinase - glycogen synthase/glycogen phosphorylase kinase

GSK3 - Glycogen synthase kinase-3

HCHO – High Carbohydrate

HCL – Hydrochloric acid

HEX – hexokinase

HR – heart rate

IM – Immediate

IRS1 – Insulin receptor substrate 1

ISAK - International Society for the Advancement of Kinanthropometry

KCl – Potassium Chloride

KOH – Potassium Hydroxide

LCHO – Low Carbohydrate

LDH – Lactate dehydrogenase

METs – Metabolic equivalent of task

MJ – Megajoules

mTOR - Mammalian target of rapamycin

NEFA – Non-esterified Fatty Acid

PDK1 – 3-Phosphoinostide-dependent protein kinase-1

PGM – Phosphoglucomutase

PI3K - Phosphatidylinositol-3-kinase

PIP2 - Phosphatidylinositol 3,4-disphosphate

PIP3 – Phosphatidylinositol 3,4,5-triphosphate

PKA – Protein kinase A

PKG/AKT - Protein kinase G/Protein kinase B

POD – Peroxide

PP1 - Protein phosphatase 1

PP_i - Pyrophospate

PPO – Peak Power output

PRO - Protein

PTG - Protein targeting to glycogen

RDA – Recommended daily allowance

RDL – Romanian Deadlift

RHIE – Repeated high intensity efforts

RL – Rugby League

RLMSP – Rugby League match simulation protocol

RMR – Resting metabolic rate

RPE - Rating of perceived exertion

RU – Rugby Union

SD – Standard Deviation

SENr - Sport and Exercise Nutrition Register

SO7 – sum of seven skinfolds

sRPE – Session rating of perceived exertion

SWA – SenseWear armband

TCA – Tricarboxylic acid

TEE – Total energy expenditure

TEI – Total energy intake

TTE – Time to Exhaustion

Tyr- - Tyrosine residue of Glycogenin

VL – Vastus Lateralis

CHAPTER 1

GENERAL INTRODUCTION

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

1.1 Background

Following the 1895 split in rugby football, Rugby Union (RU) and Rugby League (RL) differed in administration only. Modifications were made to the rules of RL soon after which resulted in two distinctly different forms of rugby. Since the professionalism of RU in 1995 and the advent of the RL Super League in 1996, pressures from the media and public for teams to succeed have increased, accelerating research in to rugby for optimal performance. Given that both games are now professional there are many similarities between the 2 games with an increasing numbers of players transferring between codes. For the purpose of this literature review the term rugby will be used for generic purposes with RL or RU used when it is specific to the individual code.

A RU team is comprised of 15 players split in to forwards (props, 2nd row, flankers, and number 8) and backs (scrum-half, fly-half, centres, wingers and fullback), whereas RL is comprised of 13 players split in to forwards (props, 2nd row and loose forward), adjustables (scrum-half, stand-off, hooker) and outside backs (fullback, wingers and centres). In-season, rugby players will typically train 4-5 days a week and, if selected, play in one 80-minute competitive match, split into two forty-minute halves with a 10-minute half-time break. Players are required to perform repeated bouts of relatively short, high-intensity efforts such as sprints, high-impact collisions, and sudden changes of direction, interspersed with low-intensity activities such as standing, walking, and jogging. RU match-play also involves set-pieces such as scrums and line-outs, alongside aggressive contests for the ball during mauling and rucking (Roberts et al., 2008) not seen in RL (although RL does involve scrums which are largely non-contested).

The rugby season is split in to two components; pre-season, and in-season. A typical pre-season in rugby lasts between 8-12 weeks and usually follows 3-6 weeks of rest time. A

player's ability to acquire high levels of muscular power is considered an essential component of success in collision sports such as rugby (Bevan et al., 2010, Kilduff et al., 2007). Many rugby players therefore use the pre-season to optimize their body composition, either gaining lean body mass, or reducing body fat ready to begin the competitive-season as physically fit as possible. To optimize physiological adaptation and body composition during the pre-season, it is vital to understand the day-to-day training of the player in order to prescribe an appropriate dietary programme. Research on the internal and external loads experienced, alongside the assessment of energy intakes of elite rugby players during the pre-season is currently lacking and therefore warrants investigation. Following the pre-season, a typical rugby in-season lasts approximately 34-36 weeks followed by 3-6 weeks of rest time depending on whether play off stages are reached. The central focus of the in-season is to prepare players for peak performance during competition. Strategies to prepare for and optimally recover from competition are therefore the objectives of this period with emphasis also placed on the maintenance of body composition to values attained at the end of pre-season. An understanding of players' daily energy expenditure and energy intake is therefore essential to avoid residual fatigue (Gamble, 2006), in the identification of appropriate recovery strategies, and to maximise performance (Fowles, 2006). Furthermore, monitoring training load throughout the rugby season may be crucial to determine whether an athlete is adapting to their training program, for the assessment of fatigue, and minimizing the risk of injury and illness (Quarrie et al., 2016, Foster, 2017). To date, no study has assessed internal load in rugby players combined with a measure of energy expenditure (EE), which in the authors opinion would provide a much more detailed picture of training loads experienced by players and potentially aid in the identification of injury risk. Moreover, information relating to the energy intakes of rugby players alongside measures of internal load would further improve our understanding of the appropriatness of dietary intakes for performance and recovery.

While researchers have investigated the movement patterns and physiological demands of rugby competition (Gabbett et al., 2012, Cunniffe et al., 2009, Duthie et al., 2003, Waldron et al., 2011, Austin et al., 2011, Jones et al., 2015, McLellan et al., 2011c, Roberts et al., 2008, Sirotic et al., 2009, Twist et al., 2014), the metabolic demands of rugby match-play are still unknown. Although these data have been reported after soccer match-play (Bangsbo et al., 2006, Krustrup et al., 2006, Krustrup et al., 2011), given distinct differences in match-play activities such as greater distances covered in football and numerous collision events in rugby, these data may not be transferable. Without this information, it is difficult to ascertain precise nutritional requirements of rugby competition, although current guidelines suggest consuming 6-10g.kg carbohydrate (CHO) for team-sport competition (Burke et al., 2011). For large athletes such as rugby players however, these quantities would be difficult to consume (~1.3 kg CHO for some) and may increase body fat. Whilst clearly warranted, no study to date has assessed the metabolic demands of rugby match-play, and only one has attempted to extract muscle samples from rugby players for glycogen analysis around match-play (Jardine et al, 1988). However, due to the invasive nature of a muscle biopsy, 'pre-match' samples in this study were extracted on a day with no match, and dietary intakes were very poorly controlled, resulting in inaccurate and unreliable data. It is still therefore unclear how dietary manipulation may affect baseline muscle glycogen concentrations, the magnitude of utilisation during a rugby match, or the effect on match-play activity and fatigue.

Strategies to recover after rugby competition are currently based on suggestions informed by studies in soccer and endurance exercise (Burke et al., 2011), but with numerous collision events experienced by rugby players (Austin et al., 2011, Cunniffe et al., 2009, Gabbett et al., 2013, Jones et al., 2015) which are known to cause tissue trauma (Cummins et al., 2013, Smart et al., 2008, McLellan et al., 2011a, Jones et al., 2014, Twist et al., 2012,

Takarada, 2003), it is currently unknown how the associated muscle damage might influence player recovery or indeed whether these suggestions are appropriate. A study by Costill et al, (1990) revealed impaired muscle glycogen resynthesis after eccentrically damaging the quadriceps on a bike ergometer, indicated by a large increase in serum creatine kinase (CK) and were similar to findings in rugby union (Jones et al., 2014) and rugby league (Twist et al., 2012) after match-play. Despite similar observations in all of these studies, the exercise demands of rugby are vastly different to cycling. Whilst it could be speculated that muscle glycogen replenishment may be impaired, it is currently unknown how such muscle damage responds to CHO provision post-rugby match-play, or indeed what refeeding strategy is necessary to optimize recovery.

1.2 Aims, objectives and structure of thesis

The overall aim of this thesis is to assess the nutritional requirements of elite rugby players during pre-season, in-season, and competition, to identify the most appropriate feeding strategies to fuel these demands. It is envisaged that the data achieved will assist players and coaching staff to further understand the demands of rugby training and competition allowing more informed decisions regarding nutritional intakes for adaptation, performance and recovery. This will be realised by the following objectives:

1) Characterise the training demands of a typical twelve-week rugby pre-season using GPS technology as well as reporting the changes in anthropometry, markers of physical performance, and typical macronutrient intakes including supplement use.
2) Characterise the training demands of a thirty-six week rugby in-season using GPS technology whilst establishing the typical macronutrient and micronutrient intakes, and energy expenditure using SenseWear armband technology.

3) Establish the metabolic requirements of a competitive rugby match by assessing muscle glycogen and blood metabolites prior to and post game using muscle biopsy and blood collecting techniques.
4) Determine the metabolic demands of a simulated RL match protocol compared with those established during a competitive match.
5) To assess the magnitude of muscle glycogen resynthesis after consuming either an immediate or delayed re-feed, using muscle biopsy and blood collecting techniques.

It is the author's hypothesis that:

1) Rugby players consume less than the recommended CHO intake for team-sport athletes (Burke et al., 2011) whilst consuming high protein intakes throughout the season.
2) Mean energy expenditure for rugby players will match energy intake, and energy intake (CHO) will surpass energy expenditure in the days leading up to rugby competition.
3) Rugby match-play will elicit similar muscle glycogen utilisation rates to those seen in soccer, although lower distance covered, and greater repeated high intensity efforts due to multiple contacts experienced.
4) Simulated RL match-play will elicit similar muscle glycogen utilisation rates to competitive match-play.
5) Immediately refeeding with carbohydrates after simulated RL match-play will result in a greater magnitude of muscle glycogen repletion.

CHAPTER 2

LITERATURE REVIEW

This section introduces key theoretical concepts and provides a critical review of relevant literature

2.1 Movement demands of rugby

The following section aims to outline the movement demands of two codes of rugby; rugby union (RU) and rugby league (RL). Playing position heavily dictates the predominance of activity performed during rugby match-play which will be discussed for each code in turn (see figures 2.1a and 2.1b for a visual representation).

2.1.1 Rugby Union

The main role of the forwards in RU is to gain and retain possession of the ball, whereas the backs control possession of the ball to gain territory and score points (Duthie et al., 2003, Quarrie et al., 2013). Research has shown that RU players cover total distances between ~4600 and ~7200 m during match-play, with significant differences reported between forward and back positional groups (Roberts et al., 2008, Cunniffe et al., 2009, Quarrie et al., 2013, Jones et al., 2015). Cahill et al. (2013) investigated the movement characteristics of 120 rugby players from eight professional English Premiership clubs and analysed differences between individual playing position and forward and back positional groups. Backs were shown to cover greater total distances (~6500 m) at higher average running speeds (71.1 $m \cdot min^{-1}$) than forwards (~5900 m and 64.6 $m \cdot min^{-1}$, respectively). More recent research by Jones et al. (2015) revealed similar findings for relative distances covered (67.3 $m \cdot min^{-1}$ and 60.7 $m \cdot min^{-1}$; backs and forwards respectively), although reported lower total distances (~6000 m and ~5000 m; backs and forwards respectively) due to reduced playing time. Interestingly, in this study backs covered greater distances walking compared to forwards, (30 $m \cdot min^{-1}$ *cf.* 26 $m \cdot min^{-1}$ respectively), while covering greater distances at high-speed (6.1 $m \cdot min^{-1}$ *cf.* 2.7 $m \cdot min^{-1}$ respectively). Given differences in sprinting ability between the backs and forwards and the absolute speed thresholds used

in these studies however, these data must be interpreted with a degree of caution (see section 2.3.1).

Studies have shown that very distinctive physical collision demands are experienced by RU players based on playing position (Deutsch et al., 2007, Jones et al., 2015). Forwards spend approximately 2.5 times longer in high-intensity activity than backs (Duthie et al., 2005, Roberts et al., 2008, Austin et al., 2011), up to 90% of which is in static exertion (Deutsch et al., 2007, Roberts et al., 2008). This is attributable to increased time involved in set-piece plays (scrums and line-outs) and contests for the ball (rucks and mauls; Duthie et al., 2005, Roberts et al., 2008, Austin et al., 2011), important elements of RU match-play that can account for up to 11 ± 4 % of match-time for forwards, with the same activity accounting for only ~2 % of match time for backs (Duthie et al., 2005, Roberts et al., 2008). Recent studies using GPS have reported forwards to be involved in a considerably higher number of heavy physical collisions (Cunniffe et al., 2009, Jones et al., 2015), performing a significantly greater number of contacts (~32-38) compared to the backs (~16-21 respectively; Jones et al., 2015). Furthermore, due to large amounts of time spent rucking and mauling, forwards spend substantially less time performing high-intensity running or sprint efforts, and more time running at a moderate-intensity (Cahill et al., 2013). Similarly, backs who may be further from the breakdown experience considerably less static exertions and collisions, but much greater high-intensity running loads and longer recovery periods (Cahill et al., 2013). Interestingly, despite variability in match-play characteristics between positional groups as described by Cahill et al, (2013), player work:rest ratio's evaluated by McLellan et al. (2011c) indicate that overall work was similar between forwards (1:5.8) and backs (1:5.7).

Studies investigating the repeated high intensity effort (RHIE) demands of RU show that on average, forwards perform significantly more RHIE bouts than backs (~12 *cf.* ~6

respectively; Jones et al., 2015). Furthermore, research has shown that mean RHIE bout duration is significantly longer for forwards (~45-52 s) compared with backs (26-28 s), with great variability in the relative contributions of high-intensity running and physical collisions to RHIE demands between positional groups (Austin et al., 2011). Position specific conditioning of RHIE and running load is therefore crucial for the preparation of the professional rugby player for competition, and although numerous studies have quantified these during match-play, these data are currently unknown for RU training.

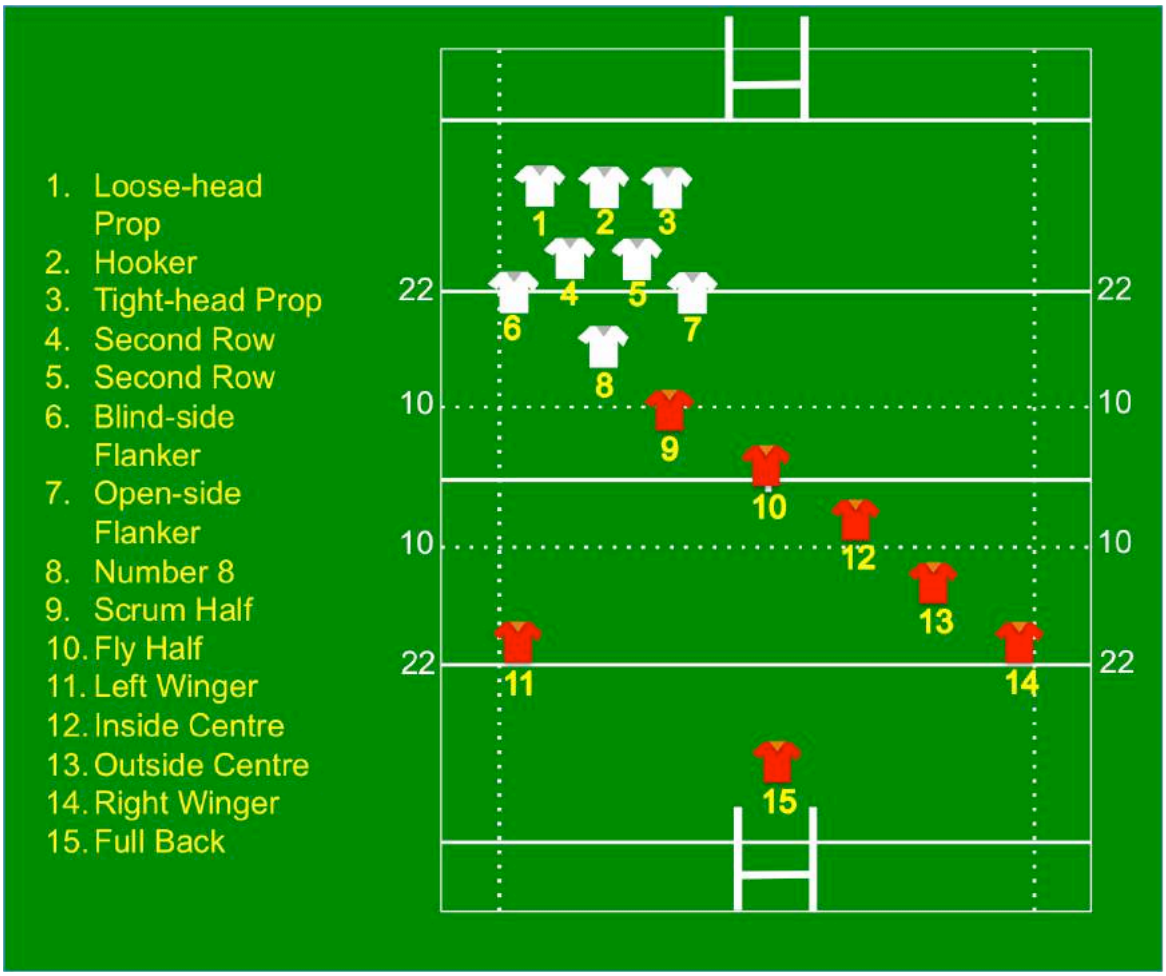


Figure 2.1a – Schematic representation of a Rugby Union team formation.

2.1.2 Rugby league

The main role of the forwards in RL is to carry the ball forward into collisions making positive ground, and the adjustables and outside backs travel greater distances, run more into open spaces and support offensive plays (Twist et al., 2014). Research has shown that outside backs cover greater absolute (~7100 m) and high-speed (~550 m) distances than adjustables (~6800 m; ~410 m) and forwards (~5700 m; ~320 m; Twist et al., 2014), although other studies have shown considerable variance in total distances covered ranging between ~3600 – 9700 m, which may be due in part to the methodological differences with video-based studies (4300-9700 m; Sirotic et al., 2009, Sykes et al., 2009), compared with GPS analysys (~3600-8000 m; Sykes et al., 2009, Waldron et al., 2011, McLellan et al., 2011c, Gabbett et al., 2012, Cummins et al., 2013, Twist et al., 2014). Varied playing times, grouping of players, and playing standard may all contribute to this large variability, and data must therefore be interpreted with a degree of caution. Similar mean running velocities have however been reported between positional groups (hit-up forwards ~94 $m \cdot min^{-1}$, wide-running forwards ~96 $m \cdot min^{-1}$, adjustables ~101 $m \cdot min^{-1}$ and outside backs ~93 $m \cdot min^{-1}$; (Cunniffe et al., 2009, Waldron et al., 2011, Gabbett et al., 2012). This data is consistent with other research suggesting that mean running intensities are indeed similar between playing positions in elite RL (Sirotic et al., 2009, Gabbett et al., 2012, Austin and Kelly, 2013). This being said, the intensity and frequency of collisions and RHIE demands of RL have been shown to vary significantly between positions (Austin et al., 2011, McLellan et al., 2011, Gabbett et al., 2012). Furthermore, player work:rest ratios evaluated by McLellan et al, (2011c) indicate that forwards (1:6) perform more work than backs (1:7), but less than RU players (1:5.7) which is likely due to the lack of physically exerting set-pieces seen in RU and not in RL.

Observations by Austin et al, (2011) revealed greater numbers of RHIE bouts (12 bouts)

and shortest mean recovery time (367 s) for hit-up forwards, compared with adjustables (6 and 442 s respectively) and outside backs (5 and 820 s respectively). Further analysis of RHIE bouts revealed a greater percentage of tackling for hit-up forwards compared with outside backs (55 % *cf.* 40 % respectively) although a lower percentage of sprinting (45 % *cf.* 60 % respectively). Research has previously shown that sprinting and tackling (RHIE) is associated with greater physiological cost (i.e. perceived exertion and heart rate) than repeated-sprints alone (Johnston and Gabbett, 2011, Johnston et al., 2015), suggesting that the forwards who perform more frequent bouts of RHIE comprised of a higher percentage of collisions, experience greater RHIE demands than backs. Although locomotor activity has been shown to maintain over both halves of a RL match, reductions in heavy physical collisions and RHIE ability have been observed in the second half of match-play suggesting fatigue (Gabbett, 2013). A player's ability to cope with RHIE demands is crucial for performance and can easily influence the outcome of a game especially when occurring near the try line during defensive or offensive play (King et al., 2009, Austin et al., 2011). Strategies to improve a player's RHIE capability should therefore be explored. One candidate may be the provision of energy (muscle glycogen) to help support RHIE efforts during the latter stages of a match, yet despite the importance of energy availability for performance, the energy demands of elite RL match-play are currently unknown and warrant investigation. A summary outlining the differences in match-play running demands between rugby codes can be seen in table 2.1.

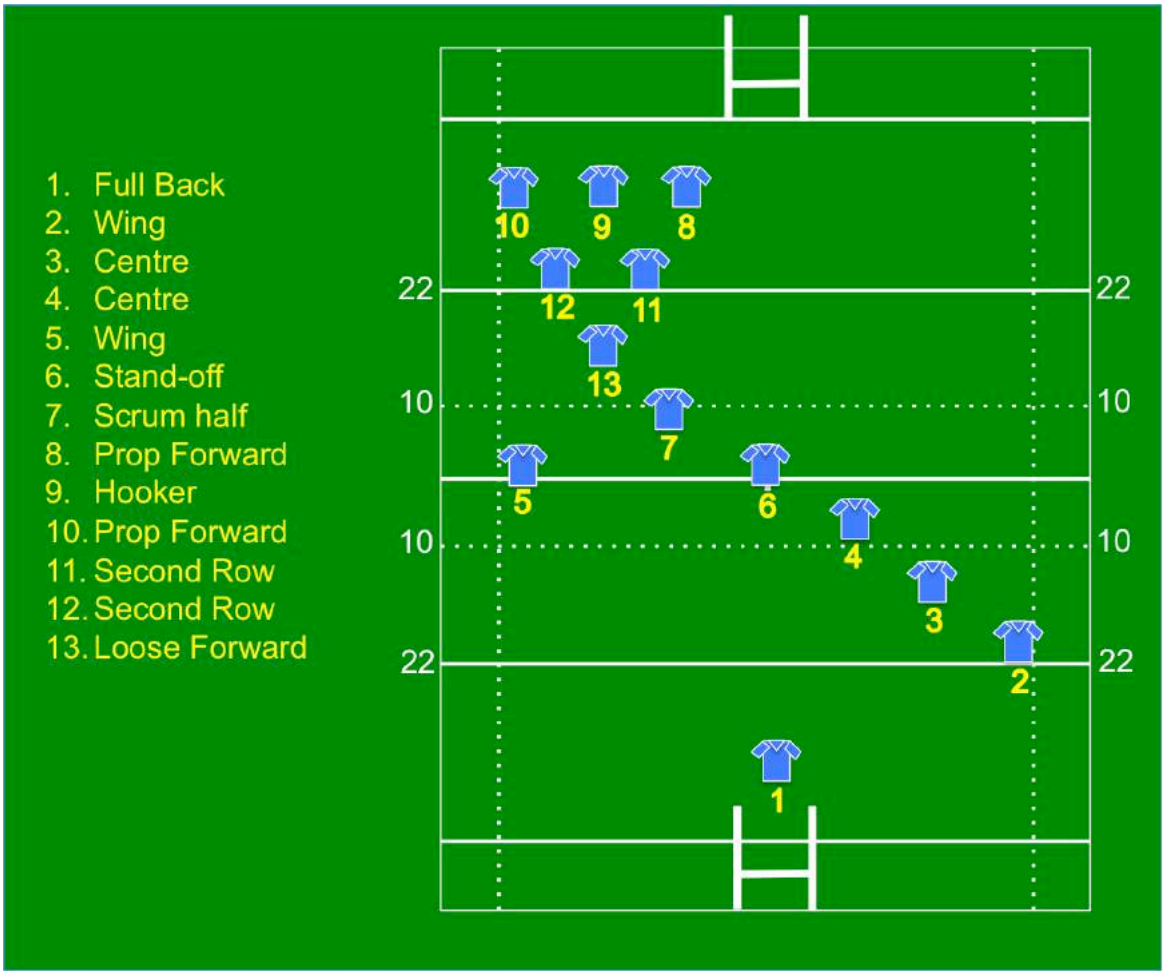


Figure 2.1b – Schematic representation of Rugby League team formation.

Table 2.1. Match-play movement demands of RU and RL. Back – RU Backs/RL Outside Backs, Fwd – RU forwards/RL forwards, Adj – RL adjustables. [U] indicates studies conducted in rugby union, [L] indicates studies conducted

	Total Distance (m)			**Relative Total distance ($m^{\cdot}min^{-1}$)**			**High Intensity (HI) Distance (m)**			**Relative HI distance ($m^{\cdot}min^{-1}$)**		
Study	Back	Fwd	Adj	Back	Fwd	Adj	Back	Fwd	Adj	Back	Fwd	Adj
Austin et al., (2011)[U]	5434	4962		67.1	56.4		738	524		9.1	6.0	
Cahill et al., (2013)[U]	6545	5850		71.1	64.6							
Cunniffe et al., (2009)[U]	7227	6680		72.9	66.7		524	313		6.2	3.7	
Gabbett et al., 2012)[L]	6819	4565	6411	93	96	101						
Gabbett et al., (2015)[L]	6633	5216	7154	79.1	87.3	86.9	485	251	453	5.8	5.2	5.5
Jones et al., (2015)[U]	6029	4995		67.3	60.7		528	225		6.1	2.7	
McLellan et al., (2011c)[U]	5573	4982					321	153				
Roberts et al., (2008)[U]	6127	5581					448	298				
Twist et al., (2014)[L]	7133	5733	6766	85.6	101.7	104.4	550	320	410	6.6	5.6	5.9
Waldron et al., (2013)[L]	6917	4184	6093	89	95	94	316	119	196	4.1	2.7	3.0

2.2 Physical Characteristics of Rugby Players

Body composition of rugby players naturally fluctuates throughout the season due to periodisation in the training programme, and none moreso than during the pre-season, a time which players use to optimize their body composition. It has previously been found that rugby players can reduce their sum of 8 skinfold thickness by ~11 mm during a pre-season (Argus et al., 2010), although nutritional intake was not recorded during this study. Strategies to prepare for, and recover opimally from competition whilst maintaining body composition attained during the pre-season also see body composition fluctuate during the in-season. Positional demands dictate player body composition, with some anthropometrical attributes more suited to certain playing positions where it is advantageous to fulfill positional requirements (Sayers, 2011). The marked differences in physical characteristics across RU playing positions appear less pronounced in RL. To accommodate a faster style of play, RL players across all positions require a high level of mobility. Simultaneously, the reduced incidence of set-piece plays such as line-outs and scrums during RL match-play further increases the homogeneity of anthropometric characteristics among positions. Despite the apparent importance of body composition to success in rugby, appropriate nutritional strategies to compliment a periodised training programme and elicit body composition/physiological adaptation, have yet to be defined in either code of the game.

2.2.1 Body Mass

Due to the physical nature of rugby, a large emphasis is placed upon body-mass, and specifically lean body-mass (LBM) in all positions, compared with other team-based sports such as soccer (Drust et al., 2000). Higher body-mass has been shown to positively impact many aspects rugby performance such as collisions and impacts (Barr et al., 2014), which

since the professionalism of the sport and increasing pressures to succeed, has lead to a progressive linear shift in the size and physical profile of rugby players exceeding that of the general population (Olds, 2001, Quarrie and Hopkins, 2007, Smart et al., 2014). While overall body-mass is important to perform at the elite level, the actual composition of body-mass is becoming crucial for optimal performance. Players possess increasingly higher lean mass and lower body fat than ever before (Morehen et al., 2015), significantly improving players power:weight ratio. Interestingly however, relative to body mass, centres and back 3 elicit higher peak power output (PPO) than forwards (59.8 *cf.* 49.4 W) which may reflect a necessity for these playing positions to possess higher muscle power relative to body mass (Heffernan et al., 2017). Variation in positional anthropometry illustrates the heterogeneity of contact sport such as rugby, with each position requiring a unique set of physical qualities (Gabbett and Seibold, 2013, Meir et al., 2001, Holway and Garavaglia, 2009). Anthropometric and physiological variations are evident between forwards and backs, with forwards tending to be heavier and stronger compared with backs who tend to be leaner and faster (Duthie et al., 2006, Morehen et al., 2015). These differences are further pronounced in distinct sub-groups of playing positions in RU, due to the requirement for forwards to carry extra weight to compete during RU specific match-play demands such as scrummaging, line-outs, rucking and mauling (Duthie et al., 2006). For example; in the 2011 Rugby World Cup, the tight five forwards (front row, 113.3 ± 7.9 kg; second row, 114.2 ± 6.1 kg; mean ± SD) were heavier than the back-row forwards (107.3 ± 5.7 kg), who were heavier than backs (92.8 ± 8.2 kg; Fuller et al., 2013). Within the backs, halves were the lightest (87.8 ± 6.7 kg) and centres the heaviest (97.2 ± 6.9 kg). Due to a slightly more uniform style of play, RL players possess a more homogenous body mass across positions, the heaviest being the forwards (Prop, 102.2 ± 8.5 kg; Back row forwards 93.3 ± 5.5 kg) followed by the adjustables (Halfback, 81.1 ±

8.0 kg, Hooker, 83.7 ± 9.5 kg) and outside backs are the lightest (Fullback and Wingers, 85.9 ± 8.2 kg, Centre, 91.2 ± 6.6 kg; Morehen et al., 2015).

2.2.2 Height

With progressing levels of competition there is also a linear increase in the height of rugby players (Duthie et al., 2003, Gabbett et al., 2008). A positive correlation between mean team height and final ranking in RU Rugby World Cup tournaments is evident, with taller teams performing better (Olds, 2001). Marked differences in the height of forwards and backs are also displayed due to distinct positional demands. For example, greater height is advantageous for RU second row players who are required to compete for the ball up to 3.5m from the ground during line-outs (Sayers, 2011) and have been shown to be the tallest playing position (1.98 ± 0.03 m; Fuller et al., 2013). The remaining forwards are all shorter than the second row players, with back-row forwards (1.90 ± 0.04 m) taller than the front-row forwards (1.84 ± 0.04 m; Fuller et al., 2013). Differences in height amongst the backs are more homogenous (1.83 ± 0.06 m; Fuller et al., 2013). Similar to body mass, RL players are generally shorter than their RU counterparts due to the lack of necessity for any specific height advantage over their opponents, and height is more uniform across positions with the forwards (Prop, 1.87 ± 0.04 m; Back row forwards 1.86 ± 0.04. m) taller than the outside backs (Fullback and Wingers, 1.81 ± 0.06 m, Centre, 1.85 ± 0.06 m) who are taller than the adjustables (Halfback, 1.77 ± 0.7 m, Hooker, 1.76 ± 0.05 m; Morehen et al., 2015).

2.2.3 Body Composition

Forwards from both codes are typically comprised of higher lean mass (~8 %) and body fat (~25 %) than backs (Brewer and Davis, 1995, Quarrie et al., 1995, Quarrie et al., 1996, Gabbett, 2000, Meir et al., 2001, Scott et al., 2003, Gabbett, 2005b, Duthie et al., 2006, Morehen et al., 2015) which probably highlights a larger strength demand placed upon forwards during contacts (Bell, 1973, Quarrie et al., 1996, Lundy et al., 2006). It has been proposed that the higher body fat observed in forwards may be considered advantageous when withstanding the impact forces associated with tackles and collisions (Bell, 1973, Gabbett et al., 2008), although it is now believed that excess body fat has a detrimental effect upon performance due to a reduced heat dissipating ability, and increased metabolic demands (Meir et al., 2001). It has also been reported that lower lean mass and higher skinfold thicknesses are associated with reduced tackling ability (Gabbett, Jenkins, & Abernethy, 2011a). Higher lean mass contributing to total mass is therefore considered a more appropriate physical attribute for the modern rugby player, translating to increased strength and power during competition (Duthie et al., 2006, Gabbett et al., 2008). Given the greater running volume and increased high-intensity running of RL competition compared with RU (Suarez-Arrones et al., 2012), it is unsurprising RL players have comparatively lower body-fat levels.

Studies assessing body composition in rugby players (Table 2.2) have typically utilised measures of skinfold thickness (as a proxy marker of body fat) and predictive equations, which have obvious limitations (Reilly et al., 1995, Doran et al., 2014). Measurements of skinfold thickness have been shown to differentiate between playing standards (Gabbett et al., 2011a, Till et al., 2011) and are often utilised as a selection tool in senior elite National Rugby League (NRL) players (Gabbett, 2009, Gabbett et al., 2011b) although this selection process has yet to be reported in RU. Dual Energy X-Ray Absorptiometry (DXA)

has also been used to assess the body composition of rugby players (Harley et al., 2011, Morehen et al., 2015), however this method comes with many limitations including repeated exposure to radiation, time required to conduct and assess a scan, and financial burden incurred. Another method commonly used to assess body composition is bioelectrical impedance analysis (BIA). This method sends a low intensity electric current through the body and measures the magnitude of the body's resistance (opposition to the electric current flow) and reactance (opposition to the electric current flow caused by the capacitance produced by cell membrane) to the current. There are many limitations with this method however which reduce the ability to replicate testing conditions including and not limited to; hydration status, physical activity, use of diuretics, fasting, age, ethnicity, menstrual period, body shape, health and nutritional status (Pinheiro Volp et al., 2011, Barbosa et al., 2001). Skinfold thickness measurements may therefore provide a cheaper and more efficient means of body composition analysis that can be repeated regularly.

Table 2.2 - Body composition of professional rugby players; Sum of Sevens skinfolds, Sum of Eight Skinfolds, mean ± SD. [u] indicates studies conducted in Rugby Union (n = 191), [L] indicates studies conducted in Rugby League (n = 132).

Source	Position (# of players)	Body Composition			
		Skinfold		DXA	
		Sum of Seven (mm)	Sum of Eight (mm)	Lean mass (kg)	Body Fat (%)
Appleby et al, 2012[u]	Forwards (n = 12) Backs (n = 8)	66 ± 16	-	-	-
Argus et al, 2010[u]	Unspecified (n = 33)	-	82 ± 23	-	-
Duthie et al, 2006[u]	Forwards (n = 40) Backs (n = 32)	84 ± 19 60 ± 13	-	-	-
Gill et al, 2006[u]	Unspecified (n = 23)	-	88 ± 22	-	-
Harley et al, 2011[L]	Unspecified (n = 20)	-	-	77.4 ± 9.4	15.2 ± 3.6
Morehen et al, 2015[L]	Forwards (n = 60) Backs (n = 52)	-	-	77.1 ± 5.3 71.5 ± 5.5	15.2 ± 2.2 12. 4 ± 2.6
Slater et al, 2006[u]	Forwards (n = 9) Backs (n = 11)	74 ± 22	-	-	-
Smart et al, 2008[u]	Forwards (n = 12) Backs (n = 11)	-	88 ± 22	-	-

2.3 Match-play demands of rugby

2.3.1 Global Positioning System

At the time of data collection for Chapter's 4 and 5 of this thesis, absolute speed thresholds were well documented in the literature and therefore utilised. In recent years however,

studies have begun to favour the use of relative rather than absolute thresholds. The literature will now be reviewed and limitations discussed in this section and Chapter 8.3.

Technological advancements over the last decade have seen the gradual introduction of micro-technologies (global positioning systems), enabling data collection from numerous players simultaneously whilst instantaneously providing feedback. This has lead to a decline in the use of the time consuming methods such as time motion analysis (TMA) post-match or training (Roberts et al., 2006). Given that the accuracy of this technology hinges greatly on the positioning and number of satellites interacting with the receiver however, information relating to the measurements recorded by the receiver should be reported for clearer interpretation (Witte and Wilson 2004). Quantification of the satellites distribution across the horizon is normally presented as the horizontal dilution of precision (HDOP), with a HDOP figure of 1 representing the ideal distribution of satellite in the sky and those above the receiver in a tight cluster approaching the maximum value of 50 (Witte and Wilson 2004).

The use of global positioning systems (GPS) have now become commonplace in elite rugby, helping practitioners understand the objective movement demands of training and competitive match-play. Developments in technology have seen the incorporation of tri-axial accelerometers (motion sensors) embedded in the latest versions of GPS devices (GPSport - SPI Elite, Wi SPi, SPI Pro; Catapult – MinimaxX; VXsports). 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 ($m \cdot s^{-1}$) measured in reference to gravitational forces ("G" forces equating to 9.81 $m \cdot s$ to 1G; McLellan and Lovell, 2012). These advancements in GPS technology have enabled practitioners to establish impacts in team sports (Cunniffe et al., 2009, Austin et al., 2011, McLellan et al., 2011a, McLellan et al., 2011b, Dwyer and Gabbett, 2012, McLellan

and Lovell, 2012, Boyd et al., 2013), with a high level of reliability (r=0.96, P< 0.01) when compared with expert video coding (Gabbett et al., 2010), particularly indoors where GPS cannot connect to satellites. However, a more recent study by Reardon et al, (2016) revealed that manipulating the G force thresholds required for detection of an impact does not provide a valid tool for the accurate coding of impacts in RU. The use of gyroscopic data in addition to accelerometer data seems to have mitigated the research trend of over-reporting collisions in the case of RL, explaining the reliability reported by Gabbett et al, (2010). Importantly, types of impacts were not separated in this research (tackle, ruck, maul, carry) meaning that it is unknown whether the GPS and associated software is better able to accurately code certain types of contacts. Therefore, due to limited research existing regarding the validity or reliability of these devices (Gabbett et al., 2010, Tran et al., 2010, Boyd et al., 2011, Waldron et al., 2011, Kelly et al., 2012, Cummins et al., 2013, Rearden et al., 2016), data should be interpreted with caution. A recent review has demonstrated however that 10 Hz units provide more accurate and reliable data compared with lower sampling frequency devices (Cummins et al., 2013). Indeed, the 10 Hz units used in this thesis are two to three times more accurate at detecting changes in velocity, and up to six-fold more reliable than devices sampling at 5 Hz (Varley et al., 2012). 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., 2012).

The measurement of different movement intensities during training or match-play requires specification of speed zones, enabling the practitioner to analyse running volumes at different intensities. Pre-determined speed zones are typically assigned to different locomotive movement classifications, however, many practitioners have created their own ‘in house’ categories, reporting an array of different speed zones causing inconsistencies in

the literature. There have been attempts in recent years to standardise these speed categories for different sports (Dwyer and Gabbett, 2012), however, these are still based on absolute speed thresholds, and moreover, rugby was not amongst the team-sports assessed in this study. Furthermore, it is important to consider the arbitrary use of the 'sprint' threshold used in studies examining the match demands of professional rugby. The amount of sprinting performed by an individual player may not be accurately reflected using a predefined sprint threshold e.g. a player with a peak running speed of 7.1 $m \cdot s^{-1}$ (such as a forward) may never reach a predefined sprint threshold of 7 $m \cdot s^{-1}$, they may however perform substantial amounts of sprinting at velocities <7 $m \cdot s^{-1}$. Any difference observed in relative distances covered at a predefined sprinting velocity could therefore be explained in part by differing sprint abilities of players (Gabbett et al., 2013), or the opportunity to reach sprinting velocity. For example, a forward who typically has little ground to cover before colliding with the opposition, would rarely be exposed to a situation where maximum locomotive speeds are possible. Rather, a forward would usually maintain a low to moderate running intensity in-between performing high volumes of RHIE and static exertions (Roberts et al., 2008, Austin et al., 2011, Jones et al., 2015). Moreover, backs would typically have a larger distance to produce locomotion before reaching their opposing player compared with the forwards, impacting speeds attained (Deutsch et al., 2007). Given the highly individualized nature of the exercise intensity continuum (Whaley, 2006), there may be potential for errors in the measurement of the distance run at high-intensity based on absolute speed thresholds, and it could be speculated therefore that players should be considered on an individual basis (Reardon et al., 2015).

2.3.2 Training load

Due to the highly intermittent nature of the training activities in rugby, it is difficult to accurately monitor training loads experienced. Moreover, due to multiple physical contacts and static exertions experienced by rugby players (Cummins et al, 2013, Jones et al., 2014, Jones et al., 2015, Smart et al., 2008, Twist et al.. 2012) and other conatct sports, quantification of these actions is harder still. There are currently a number of methods utilised to quantify training loads by athletes (Borresen and Lambert, 2009) which can be categorized as internal or external. Internal training loads can be defined as subjective markers of physiological or psychological stress imposed on an athlete during competition or training, with measures commonly used such as blood lactate, heart rate, ratings of perceived exertion (RPE) and oxygen consumption (Foster, 2017). External training loads can be defined as objective measures of the work performed by the athlete during competition or training, with measures commonly used including GPS (velocity, distance covered, accelerations/decelerations), time motion analysis, power output and speed (Foster, 2017). Theory suggests that internal load may be the most appropriate measure for monitoring training load, whereas external load is generally considered important for the prescription and periodisation of training (Scott et al., 2012). It was proposed in a review of aerobic training in football players (Impellizzeri et al., 2004) that although internal load may provide a more acute marker of training load, it is more effective and gives a much greater insight in to training load and injury risk when combined with external measures. For example; an athlete completing the exact same work load as a previous exercise session may elicit quite different measures of internal load due to a number of variables and not limited to; recent training history, energy expenditure, state of fatigue, dietary intake, or illness, which may lead to increased injury risk.

Meeuwissee et al, (2007) identified a model of injury causation such that interactions between activity-related (external) and athlete-related (internal) risk factors modify the likelihood of injury. High competition and training loads as a risk factor for injury have previously been identified in RU (Brooks et al., 2008) RL, (Gabbett, 2004b, Gabbett and Jenkins 2011, Killen et al., 2010), football, (Dupont et al., 2010) and Australian Rules Football (Rogalski et al., 2013). It was recently postulated by Gabbett (2016) that the relationship between acute (recent) and chronic (longer term) training load is important in the determination of injuries. Higher injury risk is associated with players who have had minimal exposure to match-play due to a lack of conditioning, whilst high levels of exposure to match-play and training also increase the likelihood of both acute and gradual onset injuries due to factors such peripheral and transient fatigue.

Alongside the more commonly utilised measures of external load such as GPS, power output, and speed, it may be pertinent for athletes to keep a log of foods consumed and where possible, energy expended, resulting in a far greater insight into the causality of fatigue. This information may be valuable for the coach and nutrition support staff enabling a more informed decision when planning or adapting a training or nutrition programme. For example; HR and RPE data may reveal greater values for an identical exercise session, analysis of the players food diary reveals a restricted energy intake and energy expended is high away from the club. Armed with this information, appropriate nutritional guidance can be provided alongside a potential manipulation of training load enabling the athlete to recover, reduce the risk of injury/illness, and aid in preparation for competition.

2.3.3 Physiological and metabolic demands of rugby

The underlying mechanism behind a reduced exercise performance at the end of a rugby game is unclear. Numerous well-controlled lab based studies have reported that early fatigue can also result from dehydration and concomitant hyperthermia through reduced muscle blood flow, reductions in cardiac output and perfusion pressure, and by reducing oxygen supply and oxidative metabolism (Gonzalez-Alonso et al., 1999, Hargreaves et al., 1996, Montain & Coyle, 1992, Sawka et al., 1979). The deleterious effects of mental fatigue on cognitive performance have also been reported in football players after performing the stroop task (mental fatigue) followed by a football specific decision making task (Smith et al., 2016). It is the authors opinion however that the depletion of muscle glycogen stores is the principle contributor to fatigue, since development of fatigue during prolonged intermittent exercise has a strong association with reduced muscle glycogen (Bangsbo, 1994, Hawley et al., 1997). Peripheral and cognitive fatigue resulting from low muscle glycogen concentration leads to reductions in both physical and technical performance (Sykes et al., 2011, Kempton et al., 2014). It is thought that a decline in calcium ($Ca2^{+}$) release from the sarcoplasmic reticulum reduces muscular force production (Ortenblad et al., 2011, MacLaren and Morton, 2012, Gejl et al., 2014), which may attenuate maximal high intensity efforts such as single and repeated sprints, accelerations, contacts, and sudden change of direction. Furthermore, it has been demonstrated that by elevating muscle glycogen before prolonged intermittent exercise using a high CHO diet improves performance (Bangsbo et al., 1992, Balsom et al., 1999a). Observations by Jardine et al. (1988) in club level RU players showed that a 3-day CHO load increased 'pre-match' muscle glycogen content (non-loaded - 420 mmol·kg d·w^{-1} *cf.* CHO-loaded - 580 mmol·kg d·w^{-1}), but was not necessary since severe muscle glycogen depletion did not occur during a match. It is important to highlight however, that since it was believed

players could not participate in a rugby match after such invasive measures, the 'pre-match' muscle biopsies in this study were extracted on a day on which no match was scheduled. Post-match biopsies were then collected completely independently of this control and no time scale reported between measures. There is an incorrect assumption therefore that muscle glycogen would increase to relative concentrations after a separate 3-day CHO load or 3-day habitual diet (non-loaded). Moreover, limited specification of dietary intakes (~70% CHO or habitual intake for 3 days preceding muscle biopsy; CHO loaded and non-loaded respectively) offer little insight in to the dietary conditions under which participants were subjected, and as such, these findings must be interpreted with caution.

Saltin (1973) observed in soccer that muscle glycogen stores were almost depleted at half-time when the pre-match muscle glycogen concentration was low, and those starting the game with normal muscle glycogen concentrations had rather high values at half-time but still below 215 mmol·kg d·w^{-1} at the end of the game. Others have reported concentrations of 200 mmol·kg d·w^{-1} after a match (Jacobs et al., 1982, Krustrup et al., 2006), indicating that muscle glycogen stores are not always depleted in a soccer game, which is in line with work by Jardine et al. (1988). The extent to which muscle glycogen is depleted heavily influences the magnitude of resynthesis (Zachwieja et al., 1991). Moreover, numerous eccentric muscular contractions and physical collisions experienced during rugby match-play can cause significant skeletal muscle membrane damage, and the extent to which this affects muscle glycogen repletion may impact CHO feeding strategies around competitive rugby match-play.

2.4 Exercise-induced muscle damage (EIMD)

EIMD is a common condition following (unaccustomed) exercise activities involving repeated muscular contraction or highly eccentric movements (Del Coso et al., 2012, Kyrolainen et al., 1998) and is characterized by myofibrillar disruption, followed by the inflammatory response and alterations in excitation-contraction coupling. This can present as tender or aching muscles (Cheung et al., 2003), muscle swelling and increases in blood myofibre proteins (e.g. creatine kinase and myoglobin). Moreover, low frequency neuromuscular fatigue which can be characterized by a relative loss of force at low frequencies of muscle stimulation is also evident. This is believed to be most significant for the athlete (Byrne et al., 2004). Evidence from intracellular measurements suggest that low-frequency fatigue is due to a reduction in Ca2+ release, and may inhibit recovery for up to days after exercise. Plasma Creatine Kinase (CK) is regularly used as an indirect marker of skeletal muscle damage following contact sport competition (Takarada, 2003, Kraemer et al., 2009). During homeostatic conditions, CK is located within the myofibrils and it is thought exercise induces varying degrees of mechanical muscle damage resulting in the release of CK into the extracellular fluid (Baird et al., 2012). Circulating CK levels are known to increase to different extents, depending on exercise type and intensity, muscles involved, as well as individual factors, with some authors defining subjects as 'high responders' or 'low responders' to CK activity following muscular exercise (Hody et al., 2011).

Significant increases in CK have been observed 30-min (+56%) and 24-hr (+91%) post RL match-play and remained elevated up to 96-hr post-match (Twist et al., 2012, McLellan et al., 2011b), supporting previous findings in RU (Takarada, 2003, McLellan and Lovell, 2012) and soccer (Russell et al., 2015). A significant link has also been established between tissue trauma and number of collisions in RU (Cummins et al., 2013, Jones et al.,

2014, Smart et al., 2008, Takarada, 2003) and RL (McLellan et al., 2011a, Twist et al., 2012) match-play, suggesting these data reflect blunt trauma as well as mechanical damage. Additionally, larger increases in CK have been observed following small-sided games involving contact compared with non-contact small-sided games (Johnston et al., 2014). Multiple/repeated accelerations, decelerations and rapid changes in direction are known to result in structural damage of skeletal muscle (Howatson and Milak, 2009) and are fundamental components of rugby match-play (Evans et al., 2015a) and training (Gabbett et al., 2012). In addition to locomotive eccentric damage, large physical stress experienced during static exertions, (scrummaging/mauling/rucking in RU) and high-impact collisions associated with gravitational forces (G) from impact zone 4 (7.1 - 8.0 G) to zone 6 (>10.1 G) (McLellan and Lovell, 2012) cause extensive tissue trauma (Johnston et al., 2014) and associated inflammation (Cunniffe et al., 2010). Resulting muscle soreness peaked at 24-hr post-exercise and remained elevated from baseline for several days (Fletcher et al., 2016, Oxendale et al., 2016, Twist et al., 2012). Similar observations have been reported in AFL (Cormack et al., 2008) and soccer (Magalhaes et al., 2010) and provide strong evidence that such sports are characterized by tissue damage and low frequency fatigue in the days after a game. Despite the significance of optimal recovery from rugby match-play for subsequent performance, it is currently unknown how the resulting muscle membrane damage (Proske and Morgan, 2001) affects muscle glycogen repletion in the following days.

2.5 Substrate requirements for high-intensity exercise

During all-out exercise of 1-2 minutes in duration, the supply of ATP from PCr utilisation is greater than that from glycolysis, and contributes to ~70 % of the ATP formed during the first ~3 s of maximal muscle contraction (Hultman and Sjoholm, 1983). The rate of PCr breakdown then diminishes after a few seconds of maximal muscle contration, and thereafter, glycolysis and aerobic oxidation of carbohydrates (and fats at lower intensities) become the primary source of ATP provision. Most glycogen in humans is made and stored in cells of the muscles (~350 – 700 g; depending on training status, diet, muscle fibre type composition, sex and bodyweight) and also stored in the liver (~100 g), and can be reduced by low intake of dietary CHO, fasting, and/or by exercise. Distribution of glycogen is different across muscle fibres (subsarcolemmal ~5-15 %, intermyofibrillar ~75 % and intramyofibrillar ~5-15 %; Ortenblad et al., 2011), and it appears that subsarcolemmal, intermyofibrillar and intramyofibrillar glycogen fuels different mechanisms in muscle contractions. It is believed that intermyofibrillar glycogen fuels the release of Ca2+ stored in the sarcoplasmic reticulum activating tropomyosin active sites. High-intensity exercise seems to favour the use of intramyofibrillar glycogen (Nielsen et al., 2011) alongside lipid oxidation contributing to energy production (See figure 2.2 for an overview of fatty acid and glucose metabolism) during team-sports such as soccer (Bangsbo, 1994, Bangsbo et al., 2006, Krustrup et al., 2006). The intensity and duration of an exercise bout, alongside an athlete's training status will determine the relative use of energy source during exercise (Cermak and Van Loon, 2013). During moderate intensity exercise (30-65 % of VO_{2peak}), fat is the most dominant energy source, whereas the relative contribution of CHO oxidation to total energy expenditure increases simultaneously alongside exercise intensity, with muscle glycogen becoming the most important substrate (van Loon et al., 2001). It is important to understand that CHO alone can be mobilized and

oxidized rapidly enough to meet the energy requirements of high intensity exercise, despite relatively small stores within the body (muscle and liver) when compared with endogenously stored fat (Tsintzas and Williams, 1998).

A systemic release of amino acids from the muscle and simultaneous increase in fatty acids mobilization occurs whilst exercising in a state of low glycogen availability, resulting in a reduction in exercise intensity to a rate sustainable for lipid oxidation. This has been observed during the latter stages of a soccer match (Bangsbo, 1994, Krustrup et al., 2006), indicated by an increase in free fatty acids signifying an increased reliance on lipid oxidation as a fuel source with declining muscle glycogen concentrations. Because of the importance of muscle glycogen for sustaining prolonged intense exercise, maximising performance, and preparing athletes for subsequent activity, considerable research has been undertaken to establish the most efficient means to load (and replenish) glycogen stores (Ivy, 2004, Burke et al 2011), however, this research is lacking in contact sports such as rugby.

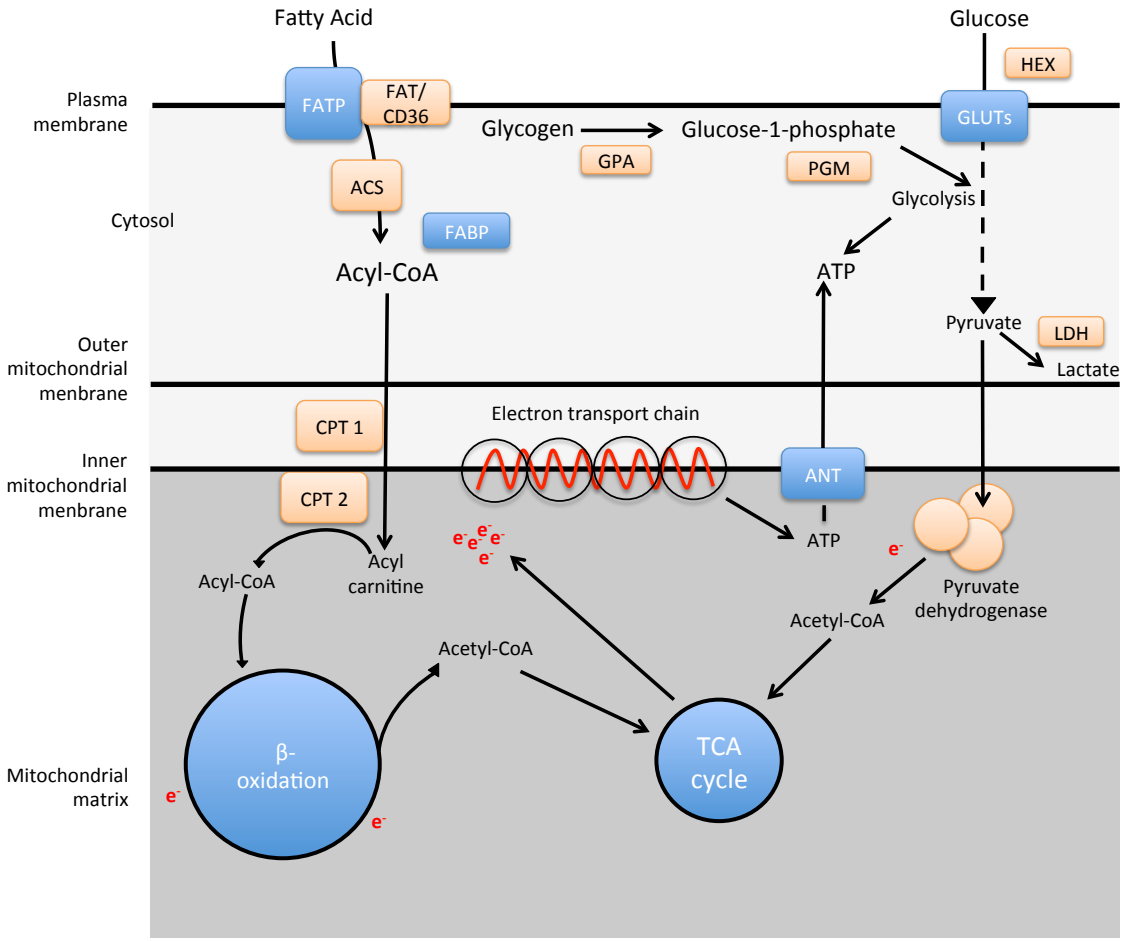


Figure 2.2 - Depicts the major routes for ATP production from catabolism of fatty acids and glucose. Abbreviations: FATP, fatty acid transport protein; FAT/CD36, fatty acid translocase; FABP, fatty acid binding protein; ACS, acyl-CoA synthetase; GLUTs, glucose transporters; CPT, carnitine palmitoyltransferase; TCA, tricarboxylic acid; ANT, adenine nucleotide translocator; GPA, glycogen phosphorlyase A; PGM, phophoglucomutase; HEX, hexokinase; LDH, lactate dehydrogenase.

2.5.1 Glycogen and fatigue

The proficiency of skeletal muscle to exercise at high intensities is impaired when muscle glycogen is reduced to low concentrations, even with sufficient quantities of other fuels available (Bergstrom and Hultman, 1967). Muscle glycogen concentrations below ~200 mmol·kg^{-1} dry weight (d.w.) have been associated with reductions in power output and impaired muscle function due to reductions in the release rate of Ca2+ from the sarcoplasmic reticulum (Dunhamel et al., 2006, Ortenblad et al., 2011). Moreover, skeletal

muscle fatigue correlates strongly with reductions in glycogen (Nielsen et al., 2010) through decreased Na, K-ATPase activity leading to decreases in ATP cleavage, and subsequently a lower energy production to fuel high-intensity exercise (Ortenblad et al., 2013). Furthermore, a paralleled perception of fatigue has been reported with declining muscle glycogen concentrations (Bergstrom and Hultman, 1967). Observations in competitive soccer have revealed such performance impairments with decreasing muscle glycogen concentrations (Krustrup et al., 2006), and subsequent repletion of muscle glycogen over the following 2-3 days (Krustrup et al., 2011). While no study has considered glycogen depletion after a rugby match, the evidence from other team sports with similar game durations (Krustrup et al., 2006, Krustrup et al., 2011), indicates that reductions in muscle glycogen is a potential mechanism of post-match fatigue. The study of the rate of glycogen utilisation and resynthesis, and the causal relationship between muscle glycogen concentration and fatigue during rugby competition is therefore warranted for the determination of appropriate nutritional strategies to fuel rugby competition.

2.5.2 Muscle glycogen synthesis

Glycogen synthesis (glycogenesis) is the formation of glycogen from glucose precursors in the cytosol (Jentjens and Jeukendrup, 2003b). To enable diffusion of glucose molecules into the muscle cell, glucose transporters (GLUT4) located intracellularly, translocate to the sarcolemma. This GLUT4 translocation is stimulated by skeletal muscle contraction, via activation of adenosine monophosphate (AMP) by activated protein kinase (AMPK) and/or increased levels of insulin (as a result of increased blood glucose levels following CHO consumption). This occurs via different intracellular signalling pathways, which have additive effects on translocation (Jensen and Richter, 2012, Richter and Hargreaves, 2013).

Once inside the muscle cell, the glucose molecule undergoes a series of reactions before being attached onto a glycogen molecule (see figure 2.3); a reaction catalysed by the enzyme glycogen synthase (Jentjens and Jeukendrup, 2003b, Bouskila et al., 2010).

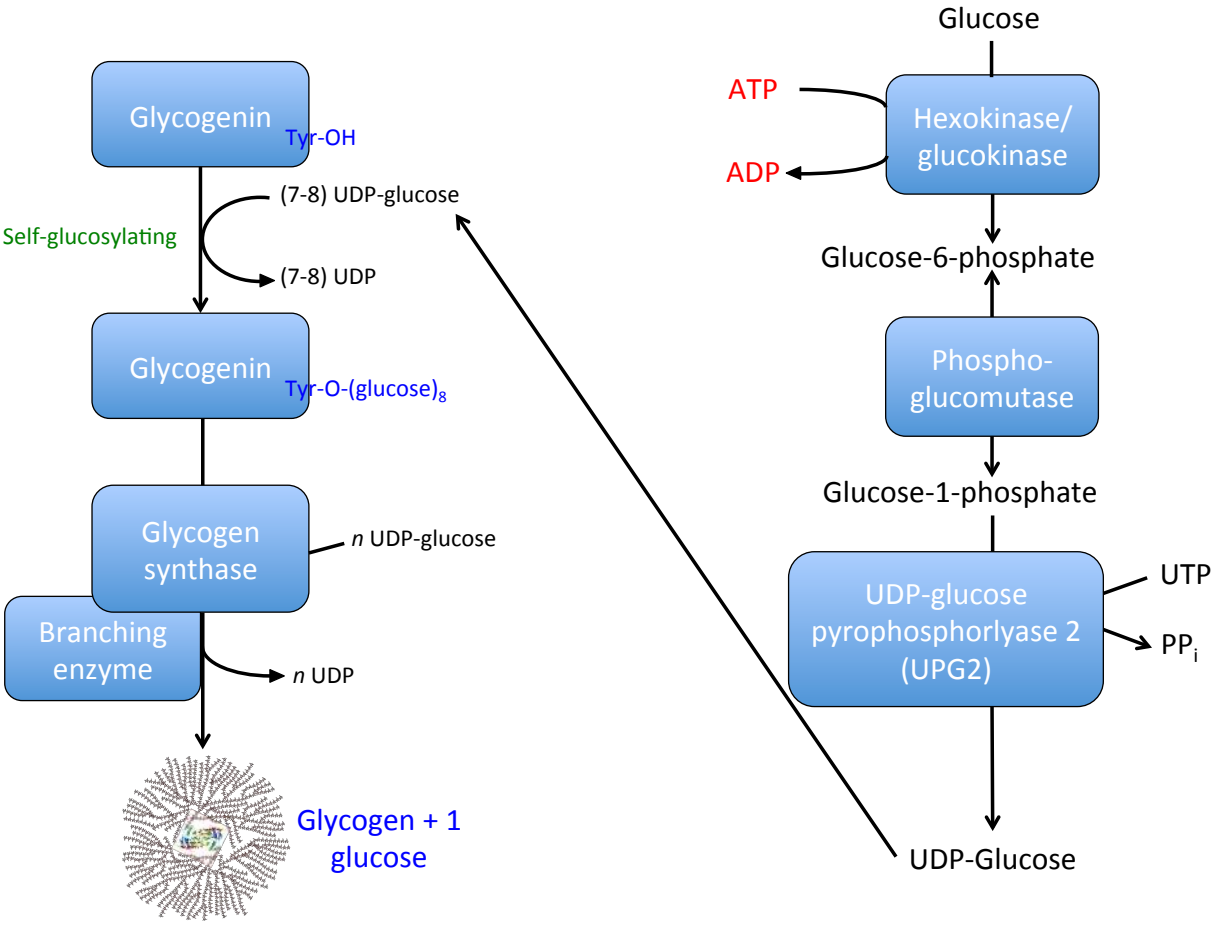


Figure 2.3 – Muscle glycogen synthesis: Glucose is phosphorylated by hexokinase or glucokinase to glucose-6-phosphate (G6P). G6P is then converted to glucose-1-phosphate (G1P) via phosphoglucomutase (PGM). G1P is then "activated" for glycogen synthesis via the addition of uridine nucleotide catalyzed by UDP-glucose pyrophosphorylase 2 (UGP2). The resultant UDP-glucose can then be use as a substrate for the self-glucosylating reaction of glycogenin, or if pre-existing glycogen polymers exist, the UDP-glucose is utilised as the substrate for glycogen synthase.

2.5.3 Muscle glycogen replenishment post-exercise

Post-exercise glycogen resynthesis occurs in a biphasic manner (Price et al., 1994). The initial rapid phase lasts up to 2-hr in undamaged skeletal muscle and occurs independently of insulin (Price et al., 1994, Jentjens and Jeukendrup, 2003b; see figure 2.4). Research has

suggested that this phase may only occur if muscle glycogen concentrations are <150 mmol·kg d·w^{-1} (Price et al., 2000), with lower concentrations resulting in greater rates of glycogen resynthesis post exercise (Price et al., 2000, Yeaman et al., 2001). The second insulin dependent phase can last up to 48-hr post exercise in undamaged skeletal muscle, and is characterized by an increase in insulin sensitivity in the muscle (Jentjens and Jeukendrup, 2003b, Beelen et al., 2010; see figure 2.4). In damaged skeletal muscle however, glycogen resynthesis may be reduced as glucose uptake into the muscle cell is impaired (Costill et al., 1990, Asp et al., 1995, Zehnder et al., 2004). This was speculated to be in relation to a reduction in GLUT 4 (Asp et al., 1995), however, a more recent study by Asp et al. (1996) measured a delay of muscle glycogen resynthesis after a muscle damage inducing marathon run in which GLUT 4 concentration was unaltered, meaning other factors must be involved. A potential explanation involves the cytokine-inducible enzyme (Kapur et al., 1997), one of three nitric oxide synthase isozymes most prevalent in fast-twitch extensor muscles (Reid, 1998). This activity causes impaired insulin-stimulated glucose uptake, reducing muscle glucose uptake and therefore glycogen resynthesis (Zehnder et al., 2004). Additionally, large increases in intramuscular inflammatory cells after exercise induced muscle damage (EIMD) have been found to reduce the amount of glucose available for glycogen synthesis due to an affinity for glucose oxidation (Costill et al., 1990). In this study by Costill et al., (1990), an eccentrically damaging cycling protocol was utilised and significant (~25 %) reductions in muscle glycogen repletion were observed when compared with a control. Studies in soccer have also shown that muscle glycogen concentrations did not replenish after 48-h of a high CHO diet following match-play (Jacobs et al., 1982, Bangsbo et al., 2006), which is in contrast to earlier observations after prolonged concentric exercise where supercompensation was possible with immediate ingestion of CHO post-exercise (Bergstrom and Hultman, 1967, Sherman et al., 1981, Tarnopolsky et al., 1997, Kiens and Richter, 1998, McInerney et al., 2005). This may be

explained in part by muscle damage from eccentric exercise during soccer match-play, which is known to impair post-exercise glycogen resynthesis (Costill et al., 1990, Pascoe and Gladden, 1996, Asp et al., 1998, Zehnder et al., 2004).

It is important to understand that a number of intrinsic factors such as training status (trained or untrained) or sporting event (endurance, power, team-sport etc) may heavily influence the ability of an athlete to synthesize muscle glycogen. For example, it has been reported that glucose uptake is augmented in trained *vs.* untrained individuals when working at the same high relative workload, which is speculated to be in relation to an increased GLUT-4 carrier protein content in the muscle (Kristiansen et al., 2000). This training adaptation also correlates with increased mitochondrial biogenesis, (Hawley and Morton, 2014) increased ability of insulin to increase glucose transport (Dela et al., 1993, Ebeling et al., 1982) and increased action of glycogen synthase (Christ-Roberts et al., 2004) which will vary on an individualised basis. Moreover, high intensity intermittent athletes (such as rugby players) require a phenotype that features a high oxidative capacity combined with large levels of strength/power to achieve maximal performance (Knuiman et al., 2015) resulting in a very specific 'conditioning' of energy systems and substrate metabolism unique to the given sport. Therefore, given that unique movement patterns and great physical loads are experienced during rugby match-play through repeated high speed collisions and static exertions, (Austin et al., 2011, Gabbett et al., 2015, Jones et al., 2015), how the associated skeletal muscle damage affects muscle glycogen repletion after rugby competition is curently unknown and demands investigation.

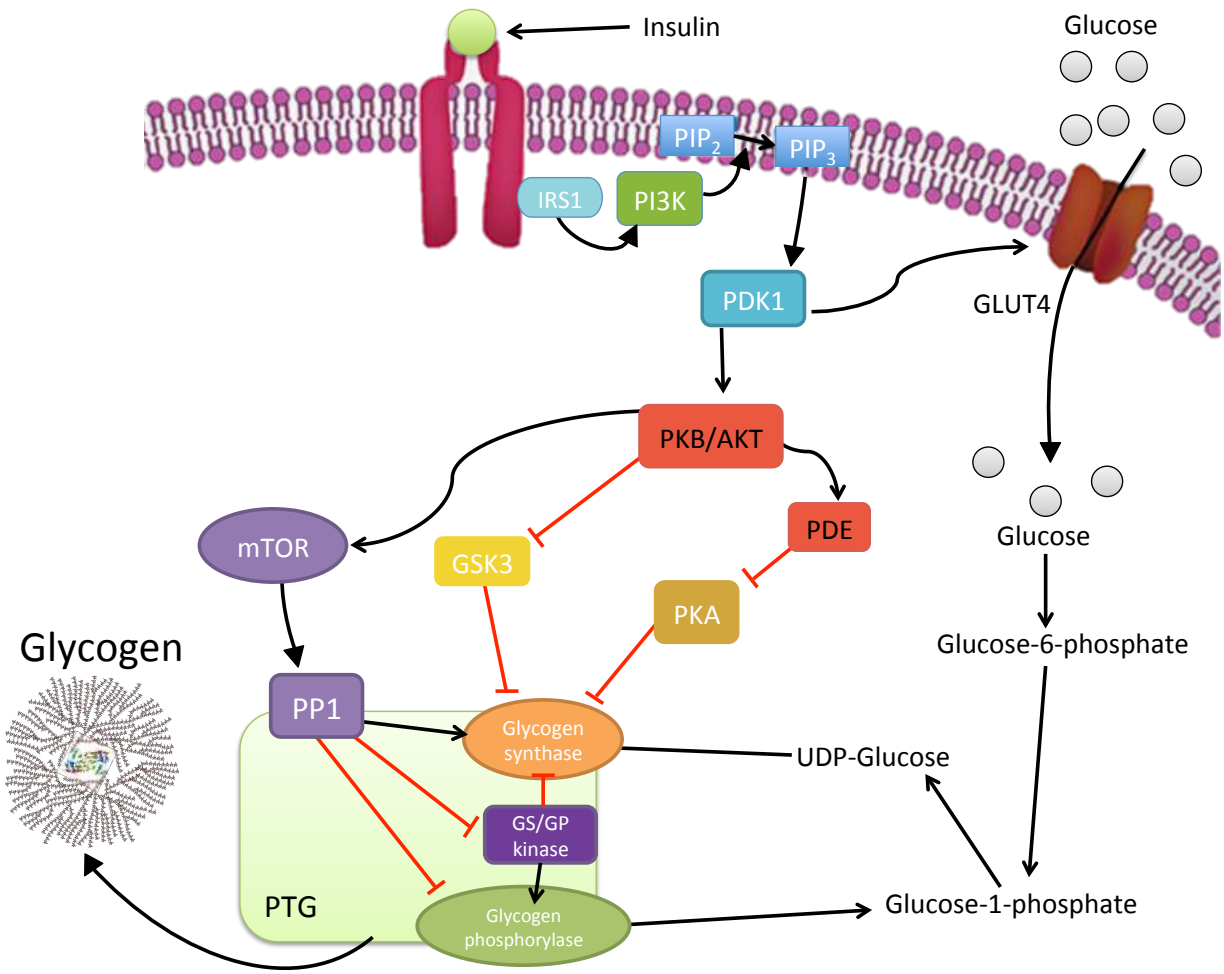


Figure 2.4 - Insulin-mediated effects on glycogen homeostasis: Insulin activates the synthesis of glycogen, while simultaneously inhibiting glycogenolysis, through the combined effects of several insulin receptor activated pathways. Shown in this Figure are the major insulin-regulated activities and how they can rapidly exert their effects since all the activities are closely associated through interactions with protein targeting to glycogen (PTG). As indicated above PTG is actually a regulatory subunit of the heterotetrameric PP1. There is a muscle-specific PTG (PPP1R3A) and a liver-specific PTG (PPP1R3B). Also illustrated is the response (to insulin) of glucose transport into cells via GLUT4 translocation to the plasma membrane. PDK1: PIP_3-dependent protein kinase 1. GS/GP kinase: glycogen synthase: glycogen phosphorylase kinase (PHK). PP1: protein phosphatase-1. PDE: phosphodiesterase. Arrows denote either direction of flow or positive effects, red T lines represent inhibitory effects.

2.5.4 Timing of carbohydrate intake on muscle glycogen replenishment

Resynthesis of muscle glycogen post-strenuous exercise is negligible until adequate CHO is made available (Ivy et al., 1988a, Ivy et al., 1988b, Zawadzki et al., 1992). Furthermore, muscle glycogen replenishment is known to be more efficient when CHO is consumed immediately following exercise, due to a rapid provision of substrate to the muscle whilst

simultaneously taking advantage of the increased insulin sensitivity and membrane permeability of the muscle to glucose (Ivy et al., 1988a). The rate of glycogen resynthesis when CHO is ingested immediately post-exercise averages between 26 – 34 mmol·kg d·w·h, whereas delaying by several hours reduces this by ~50 % (Maehlum et al., 1977, Blom et al., 1987, Ivy et al., 1988a) which may be detrimental for athletes with short recovery periods between competition. Levenhagen et al. (2001) reported that leg glucose uptake was found to increase 3-fold above basal with immediate supplementation of CHO post-exercise, whereas a smaller 44 % increase above basal was observed when CHO was ingested 3-h after exercise. This disparity occurred despite no differences in leg blood flow, or blood glucose and insulin concentrations between the two treatments suggesting that immediately consuming CHO is important for optimal muscle glycogen replenishment and subsequent exercise performance. Despite these findings, the aforementioned studies all reported findings in non-damaged muscle (steady state cycle ergometer utilised for all studies) which may not be applicable to contact sports such as soccer or rugby due to skeletal muscle damage incurred. Observations from elite soccer (Krustrup et al., 2011) showed that despite players consuming a diet comprising very high CHO (9.5 $g.kg^{-1}$) for 5-days post-match-play, and refeeding with CHO ~60 min post-match-play, muscle glycogen resynthesis was restricted (Asp et al., 1998, Zehnder et al., 2004), possibly through muscle damage incurred during the game. Moreover, rugby players who experience greater and more numerous muscle damaging collisions and impacts during match-play (Duthie et al., 2003, Cunniffe et al., 2009, Twist et al., 2014) may display an augmented reduction in muscle glycogen repletion compared with soccer, and investigation into the replenishment of muscle glycogen following rugby match-play is therefore warranted.

2.6 Nutrition and Rugby – Methods to assess energy balance

An athlete's daily nutrition should meet the fuel requirements of training in order to support high training intensities (Burke et al., 2004b), facilitate growth and repair (Tipton and Wolfe, 2001) and provide essential micronutrients for general health. To allow diets to be designed whilst accommodating these requirements, it is also important to understand the energy demands of the sport, and which substrates are utilised during competition for optimal performance. Although there have been many studies on the energy intakes of endurance sports such as cycling, running and swimming (Maughan, 1997), as well as a growing number studies in soccer (Maughan and Shirreffs, 2007, Russell and Pennock, 2011, Ono et al., 2012), there are few studies on the nutritional demands of non-soccer team sports (Mujika and Burke, 2010). With emphasis placed on body composition and physiological adaptation during the pre-season, transition to in-season shifts focus from physiological adaptation to competition preparation and recovery, with training programmes modified to reflect this transition. Consequently, nutritional intakes must also be modified to meet training and competition requirements. To the authors' knowledge, there is limited research evaluating the nutritional intake of elite rugby players and, as such, evidence based recommendations regarding the nutritional intake and composition required to fuel rugby players throughout the season are hard to produce.

In order to maximise performance (Fowles, 2006), improve body composition (Morehen et al., 2015), and potentially accelerate recovery from accumulative muscle soreness incurred during training and match-play (Fletcher et al., 2016), an appropriate nutrition programme must be implemented. It is therefore important to obtain accurate, valid and reliable methods of energy intake (EI) and energy expenditure (EE) to implement an effective nutritional plan. These data are of great importance to allow informed decisions to be made with regard to players' diets whilst complimenting a periodised training programme.

2.6.1 Energy Intake (EI)

The assessment of EI has been described as the most difficult of all physiological methods due to the difficulty of obtaining accurate and reliable data (Hackett, 2007). Given that there is no gold standard tool to assess EI (Hackett, 2009), the choice of method is dependent on the population being measured (Magkos and Yannakoulia, 2003). Furthermore, evaluating an athlete's diet requires special expertise and is often time-consuming and labour intensive (Magkos and Yannakoulia, 2003). The most common tools used to assess the dietary intake of an athlete can be roughly classified into two categories; retrospective and prospective. Retrospective methods (diet recall, food-frequency questionnaires [FFQs], and diet history) depend on the individual's memory and honesty to assess recent, or less recent food intakes. Prospective methods (duplicate portion and diet records) monitor current and ongoing food consumption, but due to the degree of subject cooperation required and burden incurred, players may under report their total EI (Bingham, 1987a, Deakin, 2000) which raises concerns over the accuracy of the data collected from professional athletes (Hackett, 2009). A major issue faced by practitioners is the underreporting of dietary intake (~30 % of total energy intake), which can be explained by intentionally or unintentionally omitting some of the food consumed and/or intentionally or unintentionally reducing food intake during the study period (Magkos & Yannakoulia, 2003, Hackett, 2009). Consequently, as suggested by Magkos and Yannakoulia (2003), the best option is to select the most appropriate method that suits the situation whilst clearly acknowledging the limitations of the chosen method alongside careful interpretation of the data. A summary outlining the advantages and disadvantages of each of these methods can be seen in table 2.3

Table 2.3. Dietary assessment methods: description, advantages, disadvantages, and major applications in clinical practice and research, adapted from Magkos and Yannakoulia, (2003)

Method	Description	Advantages	Disadvantages	Applications
Retrospective				
Diet history	Subject describes all food and drinks consumed on a typical day, completes a FFQ, and reports usual menus (originally it also incorporated a 3-day diet record, see below)	Provides information on both quantitative and qualitative aspects of usual diet. Captures day-to-day and seasonal variations inherent of professional sport.	Requires highly skilled interviewer. Time consuming and resource demanding. Depends on memory. Relatively expensive to conduct and analyse.	Mainly used to assess usual intakes of individuals in clinical practice. Good for longitudinal studies.
Diet Recall	Subject describes all food and drinks consumed over the past 24-hr, or in the preceding day.	Easy to administer. Fast to complete. Low respondent burden. Minimal distortion of food intake.	May not be representative of usual intake. Requires a trained interviewer. Relies on memory.	Mainly used to rank food or nutrient intakes of groups of people. May not be suitable for individual assessment. Useful to gather information on groups of athletes.
Food-frequency questionnaire (FFQ)	Subject describes the frequency of consumption of specific food items on a predetermined list.	May be self-administered. Inexpensive. Believed to assess usual diet. Can also provide quantitative information.	Respondent burden rises as the food list increases. Difficult quantification of portion sizes. Each questionnaire is population-specific and requires validation.	Mainly used to detect, measure or rank specific nutrients or food intakes in groups or individuals. Used for cross-checking data obtained from other methods.
Prospective				
Weighed food inventory	Subject weighs items on a scale and records (at the time of consumption) all food and drink consumed; plate waste is also weighed.	Increased accuracy	High respondent burden. Usual intake may be altered. Expensive and time-consuming. Possible underestimation of actual energy intake by 10-30%.	Mainly used to determine eating habits for 1-7 days. Used for validating other methods.
Estimated diet record	Subject estimates portion sizes and records (at the time of consumption) all food and drink consumed.	Acceptable accuracy. Increased compliance compared to weighed record.	Compliance decreases as period of diet recording increases. Possible underestimation of actual energy intake by 20-50%.	Mainly used to determine eating habits for 1-7 days.

2.6.2 Estimated Diet Record

Estimated diet record, or food diaries 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. The subject is asked to keep a detailed

record of all food and drinks, and the amount of each consumed on a daily basis or on specified days. Quantification is achieved by describing portions in terms of household measures (cups, spoons), in dimensions, or by reference to items of predetermined size (estimated or semi-weighed diet record). In general, increasing the recording period undeniably increases the reliability of collected data, but demands greater subject cooperation and may lead to reduced compliance, or to deliberate alteration of eating behaviour to simplify the recording process. The number of days needed to measure dietary intake reliably varies among subjects and for different nutrients, and also depends on the level of precision required. Group assessment requires considerably fewer days of data collection than individual assessment, as does the estimation of macronutrient intakes (protein, CHO, fat) in comparison with micronutrient (vitamins, minerals) intakes (Beaton et al., 1983, Marr and Heady, 1986, Basiotis et al., 1987, Basiotis et al., 1989, Nelson et al., 1989). Research has suggested that the self reported total EI bias can be as high as 34% (Ebine et al., 2000, Hill and Davies, 2002, Fudge et al., 2006), although a 7-day food diary has been shown to be 2-3 times less variable than a 1-day diary (Braakhuis et al., 2003). Furthermore, nutrients such as vitamin A, C, and cholesterol require a longer sampling period than macronutrients due to a three-fold increase in variability (Braakhuis et al., 2003). A 3–7-day diet monitoring period is believed to provide reasonably accurate and precise estimations of habitual energy and macronutrient consumption (Braakhuis et al., 2003), and additionally is less-onerous than the weighed food inventory method (Hackett, 2009).

2.6.3 24-hr diet recall

24-hour recalls involve low subject burden, minimal distortion of food intake and are easy to administer (Hackett, 2009), and as such are a useful tool when working with professional athletes. Furthermore, they can be scheduled around daily activities,

conducted by a single face-to-face short interview (15–30 min) or by telephone, meaning multiple recalls be collected, and a large number of athletes can be studied. One study using the multiple recall method found that 8 days of dietary records were required to minimize the effect of random error (day-to-day variation in dietary intake) in a cohort of overweight and obese men and women. Other similar studies found that a minimum of 6 days of dietary intake records were required for adult males and 11 days for adult women (Domas et al., 1997); 6 days for both women and men (Basiotis et al., 1987); and between 2 and 6 days for both women and men, depending upon the nutrient of interest (Palaniappan et al., 2003). Caution must be taken whilst comparing between these studies however given that different dietary intake methodologies were used for each, thereby presenting different sources and magnitudes of random error. Furthermore, the aforementioned studies utilised only non-athletic subjects, and limited studies exisit on athletic populations. One review by (Magkos and Yannakoulia, 2003) postulated that 24-hr diet recalls are effective in assessing the EI of a group of athletes given that increasing the number of subjects measured decreases the variability, and they have even been shown in some situations to be more accurate than food diaries (Sawaya et al., 1996). This is likely due to the ability of a practitioner to extract more thorough and finer details from an athlete, compared with an athlete working independently. It is also common for coaching staff in professional clubs to keep an athletes training schedule, meaning even a single diet recall of the preceding 24-h may provide adequate energy/macronutrient intake data for assessment together with exercise activity, although this hypothesis has never been tested.

2.6.4 Food frequency questionnaire

Food frequency questionnaires (FFQs) provide a low burden method of dietary assessment due to self-administration. Briefly, FFQs consist of categories or lists of foods, with an option to select how often each is consumed within a specified period of time together with

a quantitative questionnaire to extract typical portion sizes. FFQs have been shown to provide reasonably accurate quantitative and qualitative estimations of selected nutrient intakes (Andersen et al., 2002, Kristjansdottir et al., 2006), and have been utilised within athletic populations (Fogelholm et al., 1992, Braakhuis et al., 2011), collegiate athletes (Sunami et al., 2016 Validity), and also validated for assessing antioxidant intakes of athletes when compared to a 7-day food-diary (Braakhuis et al., 2011). Sunami et al. (2016) found that a FFQ designed and validated for use with middle-aged persons had similar validity when used with young collegiate athletes (soccer, volleyball, track and field, judo, basketball, tennis), however, due to the logistic and technical requirements of appropriate questionnaire design and validation (Cade et al., 2002), the development of athletic group specific FFQs is lacking. Moreover, given the unique physical and training demands faced by rugby players, alongside a prerequisite for very high lean body mass (Duthie et al., 2006, Morehen et al., 2015), it would be difficult to utilize a non rugby specific FFQ within a rugby population who possess very particular dietary requirements. Furthermore, assessment of diurnal variation in energy intake is not possible with this method, meaning days with fluctuating energy intakes, such as carbohydrate loading in preparation for competition, would be incorporated into the mean total and overlooked.

2.6.5 Weighed food inventory method:

The weighed food inventory method is possibly more accurate than estimated diet records (Magkos and Yannakoulia, 2003). Precise measurements of foods are recorded before consumption alongside any food left after consumption to calculate total food consumed, allowing the practitioner to reasonably accurately analyse daily food intakes. Generally this method of dietary record is kept for 7-days and if the participant follows the

guidelines, can provide a appropriate quantitative log of food intake (Hackett, 2009). However, given the irregular training and eating schedules of athletes alongside the frequency of eating, availability of appropriate equipment to weigh food whilst at training, and lack of time around training, this method may prove difficult. It would require a highly motivated participant to keep an accurate account, especially for athletes who eat while on the move or those who eat at appropriate opportunities around their training schedule, which makes weighing food a large burden. It also makes eating out a near impossibility for athletes, altering their normal eating behaviours and reducing validity of the data.

2.6.6 Dietary analysis

Once information on dietary intake has been collected, the next step is to analyse the specific components of the diet. Methods of dietary assessment were traditionally developed as arduous hand written calculations, but with the advent of computerized technology, subject and practitioner burden was substantially reduced. Still, even for the most experienced practitioner, variable or erroneous coding is commonplace when analyzing dietary intakes due to the numerous methodological steps involved including; i) Interpreting the diary inputs or survey instrument ii), selecting the best fit item from the available choices and iii), quantifying the amount of each food or drink item (Braakhuis et al., 2003). Moreover, it is estimated that food diaries take a minimum of 45-min/day for the practitioner to analyse. Each additional day analysed reduces variability (~27%, 15% and 9% variability for energy intakes during 1-, 3- and 7-day food diaries respectively) although increases the likelihood of coding error (Braakhuis et al., 2003). Despite a large inventory of different foods and drinks detailed in modern food databases, a large number of foods consumed by the population are omitted. For example, meals containing numerous ingredients (e.g. curries, stir fries, stews), packaged meals or sport

supplements/foods, are often not listed within these databases (Braakhuis et al., 2003). Some modern software such as 'Nutritics' (Nutritics LTD, Dublin, Ireland) which has been utilised recently in elite sport (Robinson et al., 2014, Robinson et al., 2015), allow manual addition of new food, meals, and sport supplements/foods, including their nutrient composition. In some cases where manual entries are not possible, a practitioner may substitute the recorded food with another judged to have similar nutritional characteristics, or enter a group of ingredients that are judged to contribute to the total nutritional profile of the meal, although this practice further augments variability between practitioners. Professional interpretation, especially when quantifying food intakes to match foods from a computerized database, may also cause a difference to arise (Braakhuis et al., 2003). It is therefore imperative that the practitioner is trained and experienced in using computerized software in order to make accurate recommendations based on dietary analysis.

2.6.7 Total Energy expenditure (TEE)

Total energy expenditure (TEE) is comprised of three key components;

1. Exercise - contributing to ~50 % TEE in humans which will vary from person to person and depend on intensity and duration of exercise (Binns et al., 2015).
2. Thermic effect of food (TEF) – energy expended through increased metabolism whilst breaking down food in to utilizable components (Halton and Hu, 2004) accounting for ~10 % of TEE in humans (Halton and Hu, 2004, Stob et al., 2007).
3. Resting metabolic rate (RMR), comprised of three further sub-components - basal metabolic rate (BMR) + Non-exercise activity thermogenesis (NEAT) + Non-exercise physical activity (NEPA) contributing to ~60-75 % of TEE in humans. (Ferro-Luzzi, 2005, Shetty, 2005, Genton et al., 2010).

- BMR – energy expended while laying stationary on an empty stomach at a

comfortable room temperature (Ferro-Luzzi, 2005, Shetty, 2005, Genton et al., 2010).

- NEAT – energy expended through subconscious movement such as fidgeting (Levine et al., 2000).
- NEPA – energy expended through non-formal, yet intentional movement, such as carrying a bag (Levine et al., 2000).

2.6.8 Methods to assess TEE

RMR is often estimated using prediction equations (Cunningham, 1980), some which have been validated in athletic populations (Cunningham, 1991, ten Haaf and Weijs, 2014, Thompson and Manore, 1996) and are dependent on the procurement of accurate lean body mass data (LBM) i.e. LBM x 22 + 500 (Cunningham, 1991). Given the LBM of athletes in the original validation studies was ~46-63 kg (Cunningham, 1991), the appropriateness of these prediction equations for larger athletes such as rugby players is questionable. RMR has since been measured in elite RL players using indirect calorimetry (Moxus modular metabolic system; AEI Technologies Inc, Pittsburgh, USA), which reported an overestimation in the prediction equation of 16.5% (7.9 ± 0.4 *cf.* 9.2 ± 0.4 MJ; indirect calorimetry and Cunningham equation respectively; Cunningham, 1991). Given this discrepancy alongside the impracticality of sports teams performing RMR with indirect calorimetry, future studies might wish to develop prediction equations more suitable for athletes with large muscle mass. The measurement of TEE in contact sports is somewhat difficult given the suitability of the tools available. The most precise method of analysis is direct calorimetry (< 2% accuracy; Blaza and Garrow, 1983), however given the difficulty of application outside of a laboratory setting, this is rarely used in athletics studies. The most common methods to assess TEE in athletics studies therefore involve indirect calorimetry including; doubly labelled water (DLW) stable isotope method, commercial

heart rate monitors (CHRM), accelerometers, combined HR and activity monitors, and wearable micro-technology (summary of each can be seen in table 2.4).

Table 2.4 Methods to assess energy expenditure: Advantages and limitations of each. Adapted from Pinheiro Volp et al. (2011).

Method	Advantages	Limitations
Direct calorimetry	Highly sophisticated method, considered a gold standard for measuring total energy expenditure (TEE).	High complexity method, high cost and requires the confinement of the subject for 24-hr or more.
Indirect calorimetry	This method is considered a 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 measurements of EE	High cost, relatively complex. Requires trained personnel for its correct use and would be difficult to administer in the field
Doubly labeled water	This is a gold standard method with accuracy between 97-99%. It measures TEE precisely in free living subjects and because it uses deuterium (H2) and oxygen-18 (O18), 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 give information about substrate oxidation. Cannot analyse day-to-day variations in EE.
Accelerometers and heart rate monitors.	Inexpensive and lightweight method to assess total distances covered, distance covered at varied speeds, and duration of exercise.	Accuracy depends largely of 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 a tough training session.	Potential damage to the device and/or subject whilst performing contact based exercise means it must be removed during these periods. Similarly, device must be removed when bathing or if 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 if 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.

2.6.8 Portable commercial devices:

Given the reasonable financial cost and practicality of use, a number of portable commercial devices are commonly employed by both athletic and non-athletic populations to assess daily EE. A commercial heart rate monitor (CHRM) may provide an inexpensive and lightweight means to assess EE, although accuracy of these monitors largely depends on individual calibration (Strath et al., 2005), alongside other variables that can elevate HR such as thermoregulation (Brage et al., 2005). Accelerometers that can monitor duration of physical activity and distances covered at a variety of speeds are also commonly used in athletic populations. These devices are however restrictive as they are unable to detect changes in direction or collisions, and importantly, isometric exercise and physical exertions that are inherent of rugby match-play and contribute to EE. Given these difficulties, a combination of both devices has been proposed by several researchers to improve estimations of EE (Brage et al., 2005, Strath et al., 2005).

Multi-sensor wearable body monitoring technology such as the Actiheart (AH; Camntech Ltd, UK) or SenseWear armband (SWA; BodyMedia Ltd, Pitssburgh, US) may provide an effective alternative means of assessing TEE in rugby players allowing day-to-day comparisons and individual sessions to be analysed. The AH is a HR sensor with recording range between 31-250 bpm combined with a triaxial accelerometer, which has been reported to be technically valid and reliable in accurately monitoring EE in walking and running when compared with gas analysis (Brage et al., 2005, Crouter et al., 2008). Similarly, studies have demonstrated that the SWA provides accurate results for EE during low-to-moderate intensity physical exercise with a threshold for accurate measurements at intensities of around 10 METs (Drenowatz and Eisenmann, 2011). Given that the compendium of physical activities indicates an intensity of 8.3 METs for rugby competition (Ainsworth et al., 2011), the use of SWA for rugby appears appropriate.

Further the SWA using software version 5.1 (Bodymedia v 5.1, UK) has been shown to reliably estimate TEE, whereas SWA using software version 6.1 (Bodymedia v 6.1, UK) and AH both reported slight underestimations in TEE when validated against DLW (Farooqi et al., 2013).

2.7 Current dietary recommendations for rugby competition

Despite significant importance placed on the pre-season for physical development, and in-season strategies for optimizing competition, there is currently a lack of research into the training demands and nutritional intakes of elite rugby players during these periods. Metabolic and match demands data have been used to devise training programmes and nutritional strategies to enhance performance and/or delay fatigue in soccer (Maughan and Shirreffs, 2007), and such studies have also formed the basis of nutritional position stands (Burke et al., 2011). However, given the distinct differences in the both training and game characteristics between soccer and rugby, translation of these data for use in rugby may not be appropriate. Greater distances covered by soccer players (Varley et al., 2014, Bangsbo, 1994), multiple physical collisions observed in rugby that are not seen in soccer (Gabbett et al., 2013), rugby player's larger body masses, and the large inter- and intra-positional physiological characteristics of rugby not seen in soccer (Duthie et al., 2003, Morehen et al., 2015) mean the suitability of using such studies to inform nutritional practices of rugby is questionable. Accordingly, to understand the energy/nutritional requirements of elite rugby training for physiological adaptation (pre-season), and optimizing competition (in-season), quantification of the training loads and energy balance of rugby players during these distinct periods warrant investigation.

While limited empirical evidence exists, traditional nutritional practice in rugby has been to load with CHO in the days leading up to a game (Burke et al., 2011), including doses between 6-10 $g \cdot kg^{-1}$ body mass. Many professional rugby players might not strictly adhere to this advice, possibly because their large body mass makes such large CHO volumes difficult to consume (potentially ~1.3 kg of CHO per day for some larger players). Additionally, immediate consumption of CHO after team sport has been advised (Burke et al., 2011, Williams and Rollo, 2015) with current recommendations advising consumption of ~1.2 $g.kg^{-1}.BM.h^{-1}$ of CHO in the first 3-hr post-exercise to maximise the rate of muscle glycogen resynthesis (Ivy, 1998, Burke et al., 2011). Further, co-ingestion of protein (~0.3 $g.kg^{-1}.BM$) post-exercise will not only aid in repair and remodelling of skeletal muscle, but may also accelerate the rate of glycogen resynthesis due to an additional increase in circulating insulin concentrations (Tipton and Wolfe, 2004, Beelen et al., 2010, Moore, 2015). However, although not documented in the literature, anecdotal evidence suggests that some rugby players struggle to consume food and liquids immediately after match-play. Moreover, delaying CHO intake by ~2-hr post exercise may attenuate glycogen resynthesis (Ivy, 1998) which may impact subsequent performance, especially when recovery periods are short between competition. Accordingly, the effects of an acute CHO load on muscle glycogen concentration and performance in professional rugby warrants investigation. Similarly, the effects of delaying CHO consumption on the magnitude of muscle glycogen resynthesis post rugby match-play must be explored. As such, there is a definitive need to better understand muscle glycogen utilisation and repletion around rugby match-play thereby having practical implications for optimal CHO feeding strategies.

CHAPTER 3

GENERAL METHODS

This chapter provides details of methods that were employed in every subsequent study. Methods that were unique to a particular study are presented in the methods section of that particular study chapter.

3.1 Ethical approval and location of testing

The local ethical committee of Liverpool John Moores university approved all of the studies in this thesis. All subjects were fully informed of the nature of the testing verbally and in writing and were free to withdraw at any time during the studies. Exercise testing for studies 1 and 2 took place at Munster Rugby training facilities in Cork, S.Ireland (Figure 3.1) the competitive rugby match and tissue sampling for study 3 took place at Widnes Vikings training facilities (Figure 3.2) and the Rugby League Match Simulation Protocol (RLMSP) and tissue sampling for study 4 took place at Warrington Wolves training facilities (Figure 3.3) and also in the laboratories at Liverpool John Moores University. Gatekeeper consent was attained and risk assessments were thoroughly conducted for all venues.

Figure 3.1 – Munster Rugby training facilities for studies 1 and 2, Cork, S.Ireland. Indoor facilities with professional lifting platforms and running track allowing for consistent exercise and weather conditions.

Figure 3.2 – Widnes Vikings training facilities used for study 3. 4G field allowed for a consistent playing surface.

Figure 3.3 – Warrington Wolves indoor training facilities used for study 4. Indoor facilities allowed for controlled weather conditions and 4G field similar to the one used in study 3 for consistent exercise conditions.

3.2 Players

All players were male, and at the time of testing were free from any known illness or injury with no player under any pharmacological treatment (either prescribed or self-prescribed). The total number of players participating in these studies was 76; 61 of whom were professional rugby players and 15 of whom were university players. The characteristics of the players for the 4 studies can be seen in Table 3.1.

Table 3.1 – Summary of participant characteristics from all four studies. * denotes professional players.

	N	Age (yrs)	Height (m)	Weight (kg)
Study 1 (Chapter 4)	45*	25.5 (3.4)	1.86 (0.05)	90.6 (6.6)
Study 2 (Chapter 5)	44*	26.0 (3.3)	1.86 (0.05)	90.6 (6.5)
Study 3 (Chapter 6)	16*	18.1 (1.2)	1.82 (0.06)	88.4 (12.4)
Study 4 (Chapter 7)	15	20.9 (2.9)	1.77 (0.06)	87.3 (14.1)

3.3 Quantification of player loads

GPS technology was used to calculate total distance covered and average weekly training loads for RU forwards and backs during four 'typical' pre-season weeks, and four 'typical' in-season weeks. Seventeen GPS units were rotated around the team with each of the eight

positions represented by two units every session. The last unit was allocated to a player of interest selected by coaching staff. GPS technology was also used to analyse the match loads of RL game-play during a competitive fixture. Sixteen GPS units were fitted to a RL squad representing all playing positions. The minimax S4 (chapters 4 and 5) and S5 units (Chapters 6 and 7; Optmieye S4/S5 Catapult Innovations, Melbourne, Australia) were worn in a custom designed neoprene vest positioned between the scapulae (Figure 3.4), and movements were recorded sampling at 10 Hz. GPS units were used to collect data on total distance (m) and relative distance covered for standing/walking (0-0.2 $m \cdot s^{-1}$), jogging/cruising (2-4.4 $m \cdot s^{-1}$), striding (4.4-5.6 $m \cdot s^{-1}$), high-speed running (5.6-7.5 $m \cdot s^{-1}$) and sprinting (7.5 + $m \cdot s^{-1}$) based on in-house classification of speed zones, similar to those reported by O'Hara (2012). The exclusion criteria included a minimum of 8 satellite locks although our data report 12 or more for triangulated for most sessions, and a HDOP of <1.5. Data was downloaded <60 minutes post training and analysed (Catapult Sprint Software, Catapult Sport, Melbourne, Australia).

Tri-axial accelerometers and gyroscopes sampling at 100 Hz, also provided data on the number of maximal accelerations (>2.79 $m \cdot s^{-2}$), physical collisions, and repeated high-intensity efforts (RHIE). A RHIE was defined as three or more high acceleration (>2.79 $m \cdot s^{2}$), high-speed (5 $m \cdot s^{-1}$) or contact efforts each separated by less than 21 s (Austin et al, 2011). Accumulated PlayerLoad™, derived from the micro-technology device's embedded tri-axial accelerometer was collected and presented as an arbitrary value based on the combined rate of change of acceleration in three planes of movement; forward, lateral and vertical. Heart rate was also monitored (in chapters 6 and 7) using a coded transmitter unit (Polar, Oy, Finland) strapped to the chest with data transmitted and recorded to GPS units for later download and analysis.

Quantification of gym and pitch session training loads in Chapters 4 and 5 were assessed using the session rating of perceived exertion (sRPE; Foster et al., 2001). sRPE has been found to significantly correlate to internal (HR, r = 0.89; plasma lactate concentration, r = 0.86; Gabbett and Domrow, 2007) and external (total distance and varying speeds using GPS, and accelerometers) measures, and has been validated as a mean of quantifying training loads in rugby (Sirotic et al., 2014) and soccer (Alexiou and Coutts, 2008, Impellizzeri et al., 2004). Using a modified 10-point Borg Scale (Borg et al., 1987) individual RPEs were provided by each player ~20 minutes after a training session from which sRPE (AU) was calculated by multiplying RPE by total training time for field and gym sessions accumulatively. sRPE was also reported after performing a Rugby League Simulated Match Protocol (RLMSP) in Chapter 7, calculated the same as previously by multiplying RPE by total simulated match-play time.

Figure 3.4 – Catapult minimax S5 unit fitted in neoprene vest attached to players back during study 4.

3.4 Anthropometry assessment

The athletes were weighed (Seca Robusta 510, Hammer Steindamm, Hamburg, Germany) weekly in the morning prior to the commencing of training wearing a tee shirt and a pair of shorts only. Body fat was estimated using International Society for the Advancement of Kinanthropometry (ISAK) guidelines. Skinfold measurements were taken by two trained ISAK members of staff. One member of staff marked all players using a hypoallergenic eyeliner pen, a measuring tape (Bodymorph, Portsmouth, England) and a segmometer (Bodymorph, Portsmouth, England). The other member of staff took the skinfold measurements using skinfold callipers (Harpenden, Baty Intl, England). This remained consistent for all testing. Measurements included seven different skinfold sites (Tricep, Subscapular, Bicep, Supra-spinale, Abdominal, Thigh and Calf) and the right side only was measured. The mean of two measurements was taken. If the two measurements differed by more than 10 %, a third measure was taken and the median value taken. Data were reported as the sum of 7 sites although the percentage body fat was calculated using the method of Jackson et al. (1978) to allow for estimation of lean body mass.

3.5 Muscle Biopsy collection

Using a surgical pack (Nu-Care Products, UK) and sterile gloves (Nu-Care Products, UK), the area over the outside of the *vastus lateralis* muscle was carefully cleaned using a surgical scrub (Hydrex, Nu-Care Products, UK). A small amount of anaesthetic (Marcain 0.5%, Kays Medical Supplies, Liverpool, UK) was injected into and under the skin. A small, 4 – 5 mm incision was made in the skin using a surgical scalpel (Swann-Morton, Nu-Care Products, UK) in order to create an opening for the biopsy needle (Monopty 12g, BARD, Brighton, UK). The biopsy needle was inserted through the incision into the *vastus*

lateralis muscle and a small piece of muscle (20 – 50 mg) was quickly removed (Figure 3.5). A maximum of 4 samples were taken from each incision. The wound was cleaned and closed using steri strips (Nu-Care Products, UK) and dressed with a tegaderm film dressing (Nu-Care Products, UK). All muscle samples were immediately transferred to eppendorph tubes (Fischer Scientific, UK), snap frozen in liquid nitrogen and stored at -80°C for later analysis.

Figure 3.5 – Muscle being scraped from Microbiopsy needle (Monopty 12g, BARD, Brighton, UK) into eppendorph (Fischer Scientific, UK) after extraction from player. Picture taken in Widnes Vikings Changing room during Study 3.

3.6 Blood collection

Blood samples (5ml) were drawn from a superficial vein in the anticubital crease of the forearm using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson). Samples were collected in three vacutainers; Serum separating, EDTA and Lithium Heparin tubes (Nu-Care Products, UK), and were stored on ice (apart from serum) until

centrifugation (Sigma 4- 16KS, SIGMA, Germany) at 1500 RCF for 15min at 4° C (Figure 3.6). Following centrifugation, aliquots of serum and plasma were stored at -80° C for later analysis. All samples were recorded and tracked using the ProCuro database.

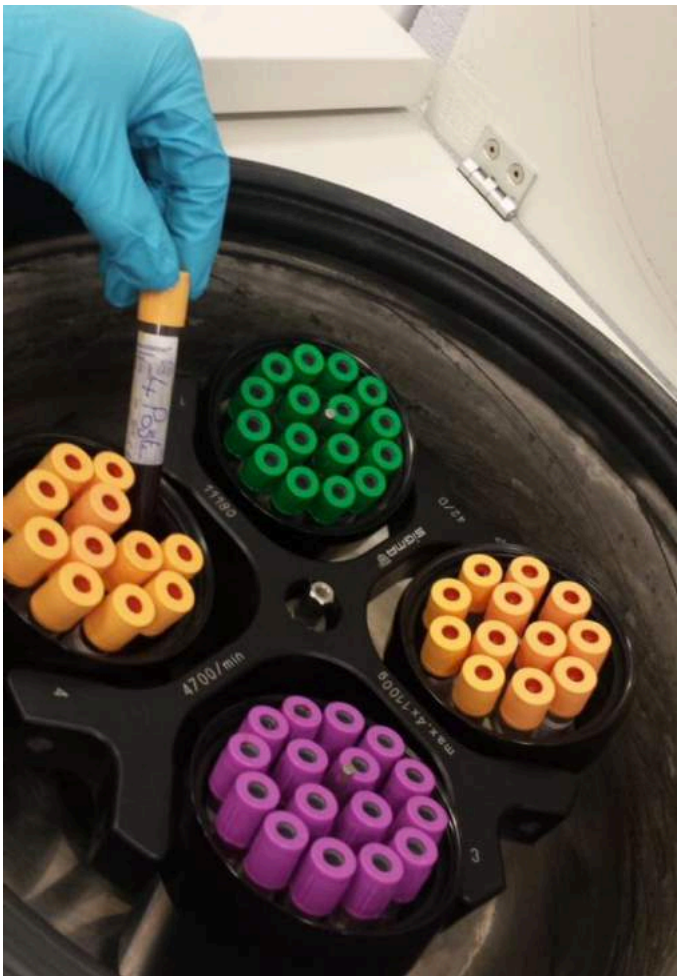


Figure 3.6 – Blood ready for centrifugation in LJMU physiology laboratory at 1500 RCF (Sigma 4- 16KS, SIGMA, Germany).

3.7 Muscle glycogen analysis

Muscle glycogen concentration was determined according to the method described by van Loon et al, (2000a). Eppendorphs containing muscle samples were carefully punctured at the top with a scalpel and placed into a freeze dryer (Mini Lyotrap, LTE Scientific, Oldham, UK) which subjected the samples to a temperature of -55 degrees under vacuum for a period of ~72-h. Approximately 2-3 mg of freeze dried samples was dissected free of

all visible non-muscle tissue, cut roughly in half with a scalpel, transferred to separate pre-weighed eppendorphs, and then reweighed for determination of sample weight (Figure 3.7). Samples were subsequently hydrolyzed by water bath incubation in 500 µl of 1 M HCI for 3-h at 100°C. Samples were removed and vortex mixed (Analog Vortex Mixer, Fischer Scientific™, Pittsburgh, USA) at approximately 2500 rpm at 30-min intervals before returning to incubation. After cooling to room temperature, samples were neutralized by the addition of 250 µl 0.12 $mol \cdot L^{-1}$ Tris/ 2.1 $mol \cdot L^{-1}$ KOH saturated with KC1. After centrifugation at 10,000 RCF for 10 min at 4° C, 200 µl of the supernatant was analysed by spectrophotometry in duplicate using Randox Daytona (Randox Laboratories, Antrim, UK) for glucose concentration according to the hexokinase method at 340 nM using commercially available kit (GLUC-HK, Randox Laboratories, Antrim, UK). Intra-assay coefficients of variation was <5%. Glucose concentrations were expressed as nM and converted to $mmol \cdot kg^{-1}\ d \cdot w^{-1}$.

$$\frac{0.75 \text{ x glucose concentration (nM) x } 1000}{\text{Muscle weight (mg)}}$$

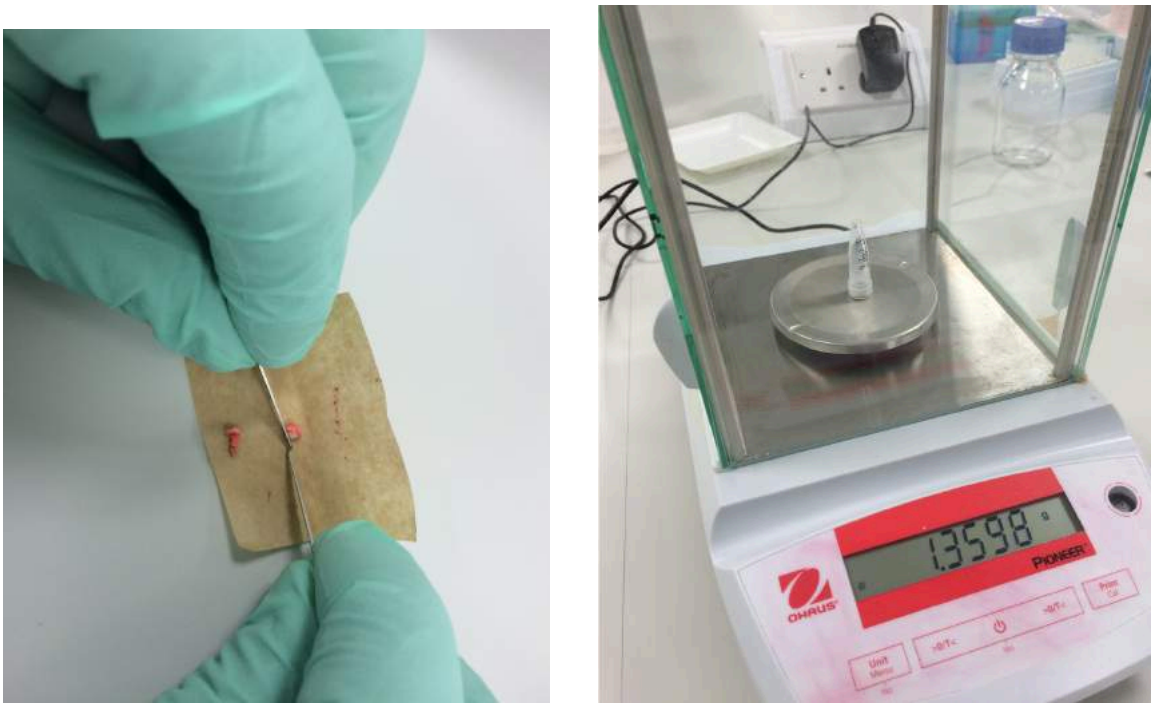


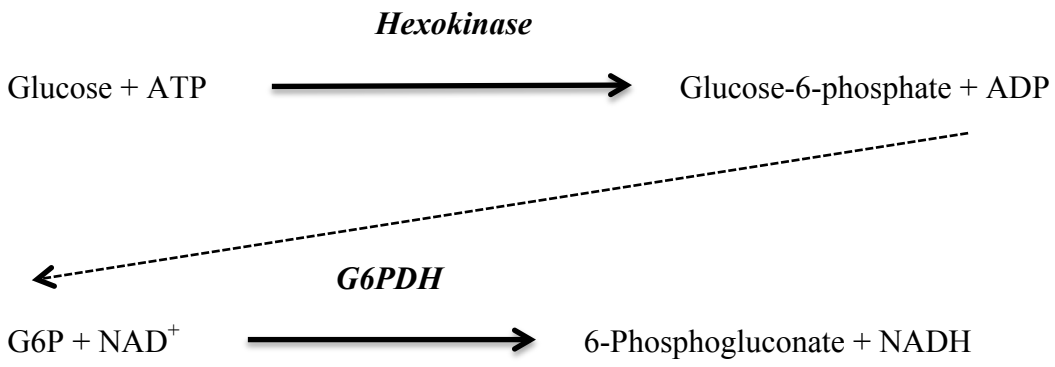
Figure 3.7 – Cutting freeze dried muscle sample in half and weighing eppendorph (Fischer Scientific, UK) with/without freeze dried muscle sample for determination of weight.

3.8 Blood metabolite analysis

Blood was analysed spectrophotometricaly for glucose, NEFA, and glycerol concentrations in chapter 6, and NEFA, glycerol and CK concentrations in chapter 7. Blood metabolites were analysed according to the hexokinase and colorimetric methods using commercially available kits (Randox, Laboratories, Antrim, UK) and expressed as $mmol \cdot L^{-1}$, $mmol \cdot L^{-1}$, $umol \cdot L^{-1}$and $U \cdot L^{-1}$ respectively.

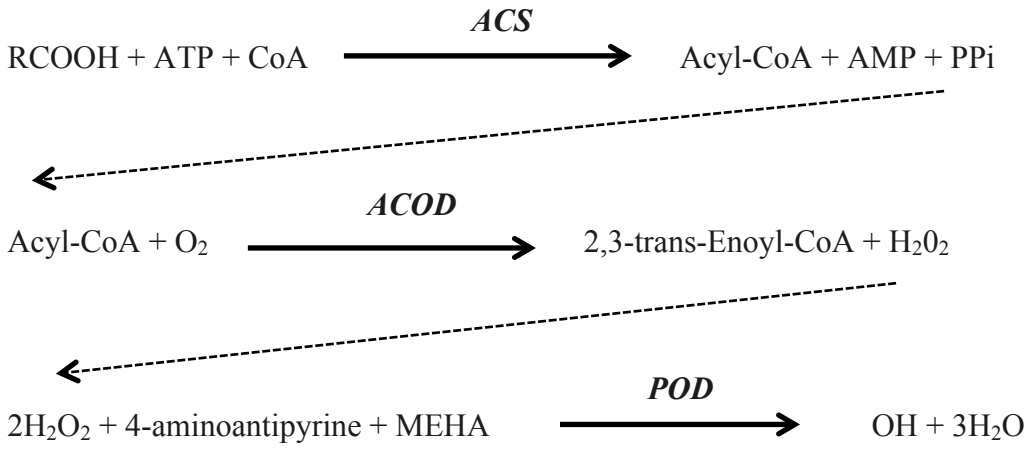
3.8.1 Glucose assay (Hexokinase method):

Glucose is first phosphorylated by hexokinase in a reaction with ATP. The product, glucose-6-phosphate (G6P), is then oxidised to 6-phosphogluconate in the presence of NAD+ in a reaction catalysed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD+ is reduced to NADH. Therefore the reaction can be monitored by measuring the increase in absorbance at 340 nM and this increase is directly proportional to the original glucose concentration.



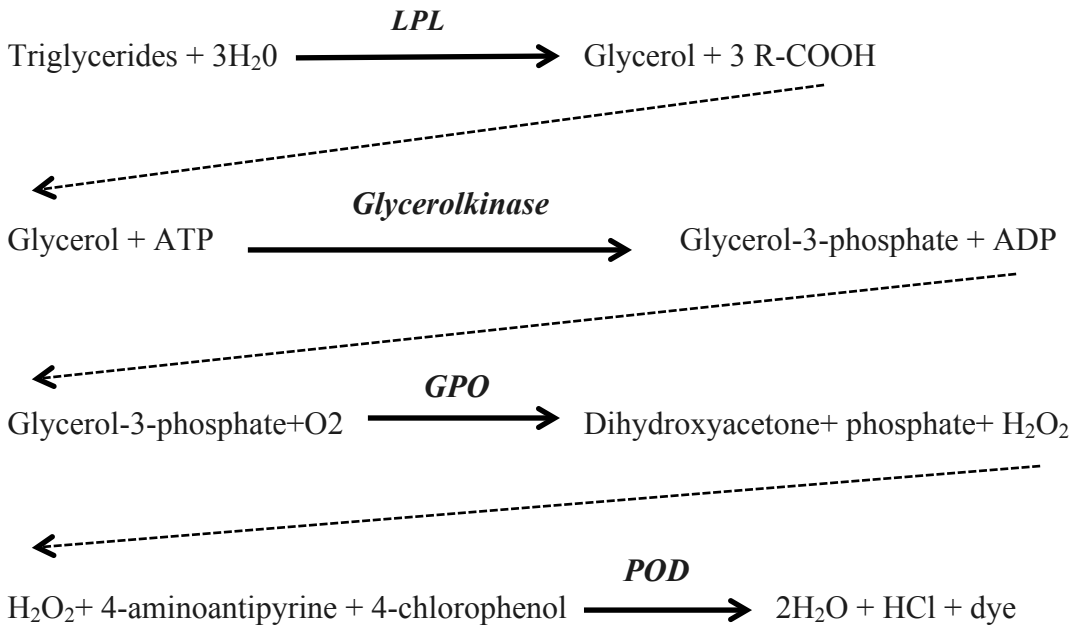
3.8.2 NEFA assay (Colorimetric method):

NEFA was first converted to Acyl-CoA, AMP and pyrophosphoric acid (PPi) by the action of Acyl-CoA synthetase (ACS) in the presence of coenzyme A (CoA) and adenosine 5-triphosphate disodium salt (ATP). The resulting Acy-CoA was then oxidised by the action of Acyl-CoA oxidase (ACOD) to yield 2,3-trans-Enoyl-CoA and hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide yields a blue-purple pigment by quantitative oxidation condensation with 3-Methyl-N-Ethyl-N-(beta-Hydroxyethyl)-Aniline (MEHA) and 4-aminoantipyrine (4-AA). The concentration of NEFA in the sample is determined by measuring the absorbance of the blue-purple pigment at 546 nM.



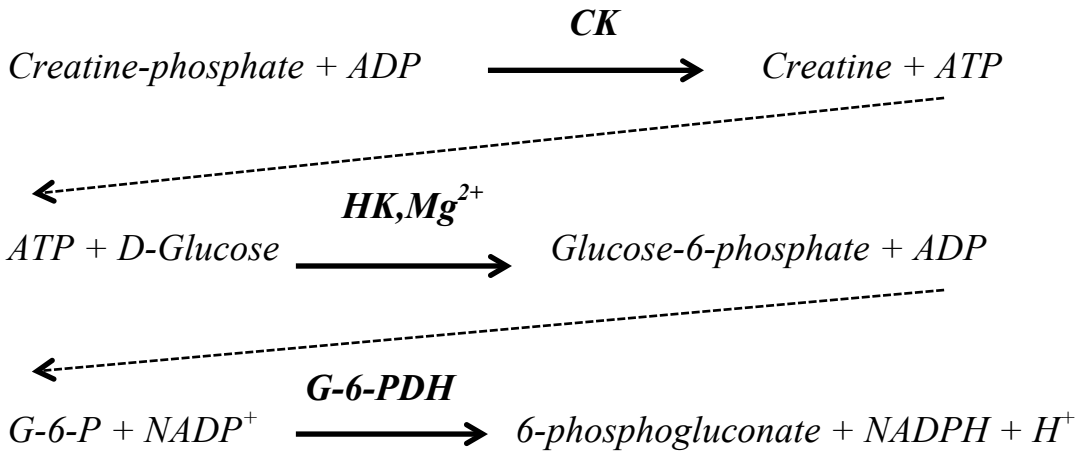
3.8.3 Glycerol assay (Colorimetric method):

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which then is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide (H_2O_2). In the presence of peroxidase (POD) the hydrogen peroxide yields a purple-blue pigment by quantitative oxidative condensation of 4-Chlorophenol and (4-AAP) 4-aminophenazone. The concentration of glycerol is subsequently determined by measuring the absorbance of the blue-purple pigment at 546 nM.



3.8.4 Creatine Kinase assay (modification of the IFCC method)

The CK procedure is a modification of the IFCC method (Horder et al., 1991). CK reversibly catalyzes the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalyzed by hexokinase (HK), which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidized by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/660 nM due to the formation of NADPH is directly proportional to the activity of CK in the sample.



3.9 Statistics

Given the applied nature of this research, magnitude-based inferential statistics (MBI) were employed to provide a standardized means of statistical analysis and information on the size of an effect. This allows for a more practical and meaningful explanation of the data that can be easily interpreted by practitioners and coaching staff (see section 8.3 for further detail). Differences in data (see specific study) were analysed using Cohen's effect size (ES) statistic ± 90% confidence limits (CL) and magnitude-based inferences, as suggested by Batterham and 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 and were considered 0.2, 0.6 and 1.2 for a small, moderate and large effect, respectively (Hopkins et al., 2009). The smallest worthwhile change was estimated as 0.2 x between-subject standard deviation (small ES). Effects with less certainty were classified as trivial and where ±90% CI of the ES crossed the boundaries of ES -0.2 and 0.2, the effect was reported as *unclear* (Hopkins et al., 2009). Threshold probabilities for a meaningful effect based on the 90% confidence limits (CL) were: <1%, *almost certainly not*; 1-5%, *very unlikely*; 5-25%, *unlikely*; 25-75%, possibly; 75-97.5%, *likely*; 97.5-99%, *very likely*; >99%, *almost certainly* (Hopkins et al., 2009). All calculations were completed using a predesigned spreadsheet (Hopkins, 2006).

CHAPTER 4

QUANTIFICATION OF TRAINING LOAD, ENERGY INTAKE AND PHYSIOLOGICAL ADAPTATIONS DURING A RUGBY PRE SEASON: A CASE STUDY FROM AN ELITE EUROPEAN RUGBY UNION SQUAD

This study was published in the Journal of Strength and Conditioning Research in 2014.

Bradley, W., Cavanagh, B., Douglas, W., Donovan, T. F., Morton, J. P., & Close, G. L. (2014). Quantification of training load, energy intake and physiological adaptations during a Rugby pre season: A case study from an Elite European Rugby Union Squad. *J Strength Cond Res*, *29*, 534-44.

4.1 Abstract

Objectives: Rugby Union is a high-speed collision sport consisting of an intermittent activity profile. Given the extreme physical demands of the sport, significant emphasis is placed on players possessing high lean body-mass whilst minimising body-fat. Anecdotally, the most significant changes in body composition are observed during the pre-season, however there are no objective data on the physiological demands and energy intake during this time. Forty-five (mean ± SD: age 26 ± 3.4 years, body-mass 101 ± 6.6 kg, height 185 ± 5 cm) elite professional rugby union players were monitored over a 10-week pre-season period. Training load was assessed using GPS and session RPE (sRPE), whilst also assessing changes in anthropometry and physical performance. Energy intake was assessed using 2 x 24-h diet recalls and analysed using Nutritics dietary analysis software. For forwards and backs respectively, mean weekly distance covered was 9774 m (1404) and 11585 m (1810) with a total mean weekly sRPE of 3398 (335) and 2944 (410) AU. Mean daily energy intake was 14.8 (1.9) and 13.3 MJ (1.9), carbohydrate intake was 3.3 (0.7) and 4.14 (0.4) $g.kg^{-1}$ body mass, protein intake was 2.52 (0.3) and 2.59 (0.6) $g.kg^{-1}$ body mass, and fat intake was 1.0 (0.3) and 0.95 (0.3) $g.kg^{-1}$ body mass for forwards and backs respectively. Markers of physical performance (1-RM strength, speed and repeated sprint tests) and anthropometry (body fat, and estimated lean mass) improved in all players. Interestingly, all players self-selected a 'low' carbohydrate 'high' protein diet. Based on physiological improvements the training load and energy intake seems appropriate, although further research is required to evaluate if such energy intakes would also be suitable for match day performance.

4.2 Introduction

Although there have been many studies on the energy intake of endurance sports such as cycling, running and swimming (Maughan, 1997), as well as a growing number of studies in soccer (Maughan, 1997, Russell and Pennock, 2011, Ono et al., 2012) there are few studies on the nutritional demands of non-soccer team sports (Mujika and Burke, 2010) and to the authors knowledge, no data evaluating the nutritional intake of elite RU players during training. Consequently, evidence based recommendations regarding the nutritional intake and nutrient composition required to fuel a rugby players training plan are currently lacking.

The daily carbohydrate (CHO) intake of athletes is perhaps one of the most controversial areas of modern sports nutrition. Whereas traditionally high CHO diets were unanimously supported for athletes engaged in team sports such as rugby, there is a modern trend towards lower CHO intakes in attempts to reduce body fat (Morton et al., 2010) as well as maximizing adaptations to exercise training (Morton et al., 2009, Hawley and Morton, 2014). The majority of studies that have suggested high CHO diets for team sports are based upon preventing exhaustion during simulated match play (Bangsbo et al., 1992, Akermark et al., 1996, Balsom et al., 1999b) rather than fuelling a training programme. To date there are no data on typical CHO intakes of elite RU players during training.

A review of literature surrounding protein ingestion in athletes reported that unlike carbohydrates, athletes usually meet (or exceed) the suggested daily intake of protein (Tipton and Wolfe, 2004, Tipton, 2011). Infact, it is common practice for athletes involved in strength and power sports such as rugby to consume amounts of protein well in excess of the amount required to maintain nitrogen balance (Alway et al., 1992) reflecting a desire to increase lean muscle mass and meet the physical requirements of the sport. Moreover,

the timing of protein intake in relation to exercise and other nutrient intakes may be equally important considerations for athletes wanting to increase their body mass (Tipton and Wolfe, 2004, Tipton, 2011). Despite this, there are currently no data available on the timing or total protein intakes of elite RU players.

Dietary supplements are becoming part of everyday life for the modern athlete. Supplements are generally used to aid an athlete to reach a macronutrient target, reverse a nutritional deficiency, or the belief that large quantities of a specific product can enhance performance or health. Although anecdotally supplements are routinely used in sports such as rugby, data on the specific supplements used, and more importantly if supplements are indeed required to fuel a training programme is currently lacking. Such data are essential to allow nutrition consultants to make informed decisions on the need for dietary supplements.

There is currently a lack of research into the training demands and nutritional intakes of elite RU players specifically during the pre-season, a crucial time in the year for physical development. These data would be of great importance to strength and conditioning research, allowing practitioners to make informed decisions with regards to players diets during this critical time of the year. Therefore the aim of this study was to 1) characterise the training demands of a RU pre-season using GPS technology and session RPE (sRPE), 2) report the changes in anthropometry and markers of physical performance over the (ten-week) pre-season and 3) evaluate the typical energy intakes and macronutrient intakes of elite RU players during the pre-season period, including the use of nutritional supplements. It was hypothesised that elite RU players consume less than the recommended dietary CHO intakes for team sports.

4.3 Methodology

4.3.1 Players

Forty-five elite RU players currently playing in the European Rabo Direct Pro 12 league volunteered for this study. The sample population was collected on the first team squad which included 12 current international players and 4 British & Irish Lions. Ethical approval was granted by the local ethics committee of Liverpool John Moores University. A summary of the participant characteristics can be seen in Table 4.1.

4.3.2 Experimental design

Players returned to training at the rugby club following a 4-week off-season. The first week back in training was in early July and this was classed as week-1. All baseline tests including strength, speed, and anthropometry were performed during week-1 (see Chapter 3.4 For methods of anthropometry assessment). Players then began a 10-week pre-season training programme prescribed by the club (a summary of the pre-season plan can be seen in Table 4.2.). During the 10-week pre-season, running activity was monitored at every session using GPS technology (see section 3.3) with players prescribed the same unit every session. At the end of the pre-season (week-10) all tests were retaken to monitor changes over the pre-season. All performance tests were performed as part of the club's normal pre-season training regime and were routinely performed by all of the players who were therefore familiar with each test. At the end of the pre-season players performed a 24-h diet recall of two typical training days. Of the 45 players that completed the pre-season, 20 players completed the diet recall analysis due to time pressures on the players and 17 completed the GPS analysis due to limited equipment.

Table 4.1. Mean (SD) Summary of the characteristics of the participants at the start of the study including the number that took part in each phase of the research. * Indicates *almost certainly* different from Backs. FWD = forwards.

	N		Age (y)		Height (m)		Weight (kg)	
	FWD	**Back**	**FWD**	**Back**	**FWD**	**Back**	**FWD**	**Back**
Anthropometry & Performance Tests	25	20	26 (3.6)	25 (3.1)	1.89* (0.06)	1.83 (0.05)	109.3* (6.9)	91.7 (6.6)
Diet Recall	10	10	24.4 (4)	24.2 (2)	1.92* (0.05)	1.82 (0.05)	108.1* (7.5)	89.6 (5)

Table 4.2. Typical Pre-season training week. Only the field based sessions were tracked using GPS.

	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Sat**	**Sun**
AM	Lower Body gym (60 mins)	Upper Body gym (60 mins)	**OFF**	Lower Body gym (60 mins)		**OFF**	**OFF**
Mid-AM			**OFF**	Gym Circuit (30 mins)	On Feet Conditioning (45 mins) and rugby (1hr)	**OFF**	**OFF**
PM	On Feet Conditioning (45 mins) and rugby (1hr)	On Feet Conditioning (45 mins) and rugby (1hr)	**OFF**		Upper body gym (60 mins)	**OFF**	**OFF**

4.3.3 Maximal strength and power

Upper and lower body maximum strength were determined using free weights (back squat, bench press, Romanian Dead Lift and weighted chins) during a normal training session in the team's gymnasium. Each player warmed up (stretching and mobility for 1-hr) prior to weight training, and once warmed up performed a 3-5 repetition max test on the selected exercises. The 1-repetition max was then calculated using the prediction equation of

Brzycki (1993). All strength-testing sessions were conducted by an experienced and qualified strength and conditioning coach and verbal encouragement was given to the athlete during all testing sessions.

4.3.4 Speed

Timing gates (Brower Timing System, Utah, USA) were set up in the club's gymnasium at 0 m and 10 m measured using fixed markings on the floor for consistency. Players in turn took up their desired starting stance behind the 0 m timing gate with instruction that they must not move or sway backwards before setting off. The player set off in his own time breaking the first set of gates starting the TC-Timer and sprinted at full pace through the 10 m gates. This was repeated three times and the best score from three attempts was recorded. A further gate was set up at 20 m and this process was repeated for 20 m sprints.

4.3.5 Conditioning

1 x 60 s and 3 x 60 s shuttle tests were performed on all players. Gridlines were marked on a full size rugby pitch (100 m) at 5 m intervals from 0 m to 30 m using a trundle wheel (Nedo, Sheffield, UK). The athletes lined up at the 0 m mark and set off at the sound of the whistle. Running to and crossing the furthest gridline, they turned 180 degrees and continued running, repeating this at both ends until 60 seconds had ensued. At 60 s the total distance covered was recorded by coaching staff. This was repeated a further two times at 5 minute intervals (1:4 work:rest). The total distance covered from the first run (1 x 60) and the cumulative score from all three efforts (3 x 60) were recorded.

4.3.6 24-h diet recall

A 24-h food recall was used to assess typical energy intake and macronutrient composition of the athletes. Two x 24-h diet recalls were performed on 20 professional players, and the mean of the 2 days was reported. Players were interviewed in a private room and asked to recall their nutrition intake over a typical training day. Specific details were teased out by the nutritionist, such as brands of foods, and portion size was estimated (Cheyette, 2012). The macronutrient intake was then calculated by either using the manufactures website to obtain energy and macronutrient composition or using MyDailyPlate diet analysis software (www.livestrong.com.myplate). Given that there is no gold standard tool to assess energy intake, the choice of method is dependent upon the population being measured (Magkos and Yannakoulia, 2003). A 24-h diet recall was selected since the aim of the tool was to assess the energy intake of a group of elite players rather than assess the energy intake of an individual player.

4.4 Statistics

Magnitude-based inferential statistics were employed to find differences in movement characteristics, anthropometry, performance testing, and 24-hour dietary recall between forwards and backs (see section 3.9).

4.5 Results

4.5.1. Player Load and GPS assessment

Table 4.3. Mean (SD) weekly GPS data reported in $m \cdot s^{-1}$ for the forwards (FWD) and Backs. Accelerations are defined by efforts performed >2.79$m \cdot s^{-2}$. RHIE is defined as a cluster of three user defined high intensity efforts performed <21s apart (contacts, accelerations or sprints).

- Indicates *almost certainly* or *likely* different from Backs.

	Total Distance (m)	Contacts	0 – 2 $m \cdot s^{-1}$	2 - 4.4 $m \cdot s^{-1}$	4.4 - 5.6 $m \cdot s^{-1}$	5.6 - 7.5 $m \cdot s^{-1}$	7.5 + $m \cdot s^{-1}$	RHIE	Accelerations	Mean weekly sRPE
FWD	9774* (1404)	105* (53)	3893 (994)	3738 (397)	1723 (606)	417* (84)	4* (4)	16 (6.8)	125* (34.1)	3398* (335)
Back	11585 (1810)	74 (19)	4522 (829)	4470 (984)	1655 (428)	894 (178)	22 (18)	13 (5.9)	157 (53.8)	2944 (410)

Session RPE and GPS data can be seen in Table 4.3. The mean weekly sRPE was calculated by combining all of the sRPE giving a cumulative value of 3398 (335) and 2944 (410) AU for forwards and backs respectively. Backs *almost certainly* covered greater total distances (ES; ±90% CL: 1.28 ±0.59) and higher running speeds between 5.6-7.5 $m \cdot s^{-1}$ (1.8; ±0.72). Furthermore, backs *very likely* covered greater distances at speeds of 7.5+ $m \cdot s^{-1}$ and *likely* performed more accelerations (0.88; ±0.59) than the forwards. Conversely, the forwards *likely* performed more contacts (0.7; ±0.71), and sRPE was *almost certainly* greater (1.4; ±0.66) than the backs. Sub analysis of the GPS data revealed that differences between groups at the lower running speeds, (i.e. 0-2, 2-4.4 and 4.4-5.6 $m \cdot s^{-1}$) and RHIE's were *unclear* (0.06; ±0.36, 0.11; ±0.72, 0.17; ±0.32, and 0.02; ±0.61 respectively).

4.5.2. Changes in anthropometry, strength, speed and conditioning

Table 4.4. Mean (SD) anthropometric measures, estimated 1 repetition max (1-RM) for the squat, romanian deadlift (RDL), bench press, and weighted chin, speed over 10m and 20m and conditioning results for 3 x 60 second shuttle and 1 x 60 second shuttle for FWD (forwards) and backs. SO7 = Sum of 7 skinfolds, BF% = Body fat %, FFM = Fat free mass.

* Indicates *almost certainly*, *very likely* or *likely* different from Backs.
# Indicates an *almost certainly*, *very likely, likely, possibly* or *unlikely* difference from Pre.

	Forwards		Backs	
	Pre	Post	Pre	Post
Weight (kg)	109.3 ± 6.9*	109.4 ± 6.4*	91.7 ± 6.6	91.5 ± 6.6
SO7 (mm)	93.2 ± 25.9*	87.6 ± 25.8*	68.6 ± 13.1	63.5 ± 15.7
BF%	13 ± 3*	12.2 ± 3.4*	9.3 ± 2	8.5 ± 2.4
FFM (kg)	94.9 ± 4.5*	95.8 ± 4.5*	83.1 ± 5.4	83.8 ± 5.4
Squat (kg)	201 ± 27*	215 ± 32* #	175 ± 12	196 ± 17 #
RDL (kg)	170 ± 14*	190 ± 14* #	147 ± 19	161 ± 22 #
Bench (kg)	135 ± 12*	141 ± 13* #	122 ± 10	128 ± 11 #
Weighted Chin (kg)	143 ± 7*	148 ± 8* #	132 ± 11	137 ± 8 #
10m (s)	1.8 ± 0.05*	1.73 ± 0.04* #	1.63 ± 0.06	1.63 ± 0.05
20m (s)	3.13 ± 0.08*	3.0 ± 0.08* #	2.89 ± 0.12	2.86 ± 0.06 #
3 x 60 s shuttle (m)	820.5 ± 29.7*	832.4 ± 20.9* #	842.1 ± 19.5	859.6 ± 16.7 #
1 x 60 s shuttle (m)	284.7 ± 9.8*	295 ± 8.5* #	296.7 ± 8.9	305 ± 4.3 #

Changes in anthropometry can be seen in Table 4.4. Forwards weight, SO7, BF% and FFM were *almost certainly higher* than the backs at week-1 (2.27; ±0.45, 1.36; ±0.51, 1.29; ±0.46 and 1.98; ±0.46) and week-10 (2.29; ±0.44, 1.2; ±0.5, 1.19; ±0.46 and 1.97; ±0.46)

respectively. Forwards *likely reduced* in SO7 (-0.35; ±0.17) and BF% (-0.38; ±0.19), while changes in weight and FFM were *very unlikely* (-0.01; ±0.1) and *unlikely* (-0.16; ±0.08) respectively over the course of the pre-season. Backs *very likely* and *almost certainly reduced* in SO7 (-0.46; ±0.16) and BF% (-0.45; ±0.15) respectively, while changes in weight and FFM were *almost certainly not* (0.03; ±0.07) and *very unlikely* different (0.13; ±0.05) respectively over the course of the pre-season.

Changes in strength over the pre-season can be seen in Table 4.4. Both groups *almost certainly* and *very likely improved* across all lifts over the course of the pre-season; Squat (0.46; ±0.27 and 1.53; ±0.7), RDL (1.28; ±0.34 and 0.64; ±0.35), bench press (0.58; ±0.13 and 0.53; ±0.12) and weighted chins (0.71; ±0.27 and 0.4; ±0.18) for forwards and backs respectively. The forwards were *almost certainly* and *very likely stronger* than the backs on all lifts; Squat (1.79; ±1.02 and 0.93; ±0.86), RDL (1.78; ±1.08 and 2.18; ±1.22), bench press (1.18; ±0.62 and 1.16; ±0.62) and weighted chins (1.58; ±0.84 and 1.46; ±0.61) at week-1 and week-10 respectively.

Changes in speed and conditioning measures can be seen in Table 3.4. The backs were *almost certainly* quicker than the forwards over 10 m (3.42; ±0.7 and 2.2; ±0.65) and 20 m (2.73; ±0.92 and 1.85; ±0.65) sprint tests at week-1 and week-10 respectively. Forwards *almost certainly improved* over 10 m but improvements over 20 m were *very unlikely* over the course of the pre-season. Backs improvements over 10 m were *unlikely* but *possibly improved* over 20 m over the course of the pre-season. Backs *almost certainly* covered greater distances than the forwards during the 1 x 60 m (1.14; ±0.56 and 1.11; ±0.44) and 3 x 60 m (1.06; ±0.73 and 1.23; ±0.52) tests at week-1 and week-10 respectively. Both groups *almost certainly* improved their 1 x 60 m distance (0.99; ±0.3 and 0.87; ±0.34) with the forwards *likely* and the backs *very likely improving* their 3 x 60 m distance (0.39; ±0.26 and 0.83; ±0.4) over the course of the pre-season (forwards and backs respectively).

4.5.3. Dietary analysis

Table 4.5. Mean (SD) energy intake and macronutrient profile obtained from the 24-h dietary recall for the forwards (FWD) and backs including contribution from dietary supplements.

* Indicates *almost certainly*, *very likely* or *likely* different from Backs.

	Protein			Carbohydrate			Fat			Energy	
	Total (g)	**g·kg**	**Range**	**Total (g)**	**g·kg**	**Range**	**Total (g)**	**g·kg**	**Range**	**MJ**	**Range**
FWD	273.6 (44.7)	2.52 (0.3)	2.1 to 2.8	356.2 (63)	3.3 * (0.7)	2.5 to 4.8	108.27 (31.9)	1.0 (0.3)	0.4 to 1.5	14.8 * (1.9)	12.2 to 17.1
of which supplements	50.2 (28.6)	0.46 (0.3)	0.4 to 0.9	26.6 (25.5)	0.24 * (0.2)	0.1 to 0.5	0.05 (0.03)	0.005 (0.0)	0.0 to 0.1	1.5 (0.7)	1.4 to 2.8
Back	228.8 (50.5)	2.59 (0.6)	1.6 to 4.0	367.7 (42.4)	4.14 (0.4)	3.6 to 5.2	83.9 (24.4)	0.95 (0.3)	0.7 to 1.4	13.3 (1.9)	11.3 to 16.7
of which supplements	49.4 (13)	0.56 (0.2)	0.5 to 1.0	50.7 (15.5)	0.57 (0.2)	0.3 to 0.7	0.05 (0.02)	0.03 (0.0)	0.0 to 0.1	1.8 (0.4)	0 to 2.5

Data from the 2 x 24-hr diet recall (presented in $g \cdot kg^{-1}$ body mass) can be seen in Table 4.5. Mean daily energy intake was *likely higher* for the forwards (ES; ±90% CL: 0.75; ±0.69) with supplemented energy intake *unclear* between groups (0.58; ±0.99). Mean energy intake was 14.8 ± 1.9 and 13.3 ± 1.9 MJ for forwards and backs respectively of which 1.5 ± 0.7 and 1.8 ± 0.4 MJ (14 and 10 % of total calories) was from dietary supplements. Backs CHO consumption was *almost certainly higher* (0.17; ±0.65) and supplemented CHO *very likely higher* than the forwards (3.14; ±1.87). Mean CHO intake was 3.3 ± 0.7 and 4.14 ± 0.4 $g \cdot kg^{-1}$ (46 and 40 % of total calories) for forwards and backs respectively of which 0.24 ± 0.2 and 0.57 ± 0.2 $g \cdot kg^{-1}$ was from dietary supplements. Difference in total and supplemented protein intakes were *unclear* between groups (0.04; ±0.59 and 0.35; ±1.06 respectively). Mean protein intake was 2.52 ± 0.3 and 2.59 ± 0.6 $g \cdot kg^{-1}$ (29 and 31% of total calories) for forwards and backs respectively of which 0.46 ± 0.3 and 0.56 ± 0.2 $g \cdot kg^{-1}$ was from dietary supplements. Finally, differences in total and supplemented fat intake were *unclear* between groups (0.17; ±0.65 and 0.36; ±0.95 respectively). Mean fat intake was 1.0 ± 0.3 and 0.95 ± 0.3 $g \cdot kg^{-1}$ (24 and 28 % of total calories) for forwards and backs respectively of which 0.05 ± 0.03 and 0.03 ± 0.02 $g \cdot kg^{-1}$ was from dietary supplements. A summary of the supplement use can be seen in Table 4.6.

Table 4.6. Supplements used by the players including serving size.

Supplement	Serving
Whey Protein Concentrate	30 g
Maltodextrin	As directed by dietary group
Beta-alanine	3 g
Creatine	5 g
HMB	3 g
L-Glutamine	5 g
Omega 3 fish oils	1100mg (765 mg EPA & 240 mg DHA)
Multi vitamin	1 tablet (100 % RDA)
Probiotic (acidophilus)	1 tablet (2 billion microorganisms)
CLA	3 g
Electrolyte tablets	1 tablet in 500 ml water

4.6 Discussion

The aim of the present study was threefold 1), to quantify the training loads and running distances covered during a typical training week 2), report the changes in anthropometry and physical performance and 3), evaluate the dietary habits of elite rugby players over a pre-season. It is reported for the first time that distances of ~10-12 km are covered during a typical week, the total weekly load was ~3400-3700 AU and the daily energy intake of elite RU players was 11.3– 17.1 MJ. Interestingly, the players in the present study all self-selected what could be classed as a low CHO / high protein diet with all players failing to meet current recommendations for CHO intake (Burke et al., 2011). It is important to note that these guidelines do not reflect the outcome of an experimental study, and are based rather on the formulated opinions of one experienced practitioner. The guidelines do however clearly state that "These general recommendations should be fine-tuned with individual consideration of total energy needs, specific training needs, and feedback from training performance" allowing for personal interpretation in any given sporting context (Burke et al 2011). Given the improvements in physical performance and body composition, along with the data suggesting relatively low running distance and low sRPE covered in a pre-season week, it could be argued that for RU players, CHO intakes of 2.5-5 $g.kg^{-1}$ body mass are not '*low*' and are in fact '*appropriate'* for this group of athletes.

GPS analysis of the training sessions revealed that the running distances performed were not high, with drills tailored towards repeated high intensity efforts to simulate game intensities. During the weeks studied, players only covered a total distance of 9.8 ± 1.4 km and 11.6 ± 1.8 km for the forwards and backs respectively, and were given three full days of rest. During a typical week, significant differences were found in total distance, conditioning distance, contacts and accelerations between the forwards and the backs. These differences are due to the game specific training drills performed by each group,

with the forwards performing significantly more contacts than the backs such as tackling, rucking and mauling and the backs performing significantly more accelerations and engaging in more ball-in-hand running play than the forwards. It should be noted however that given the relatively static isometric activities performed by forwards such as scrummaging and rucking, it is possible that the GPS analysis fails to capture some of their high intensity efforts which would add to their sRPE. Furthermore, differences in distances covered at a 'sprint' threshold are likely as a consequence of using absolute rather than relative speed thresholds, and data must be interpreted with caution.

A great importance is placed on monitoring player load for injury prevention due to the relationship between training loads and training injury rates (Gabbett, 2004). Session RPE was therefore recorded following all training sessions to quantify training load. Weekly sRPE of ~3398 and ~2944 were observed for forwards and backs respectively, which are somewhat surprisingly lower than those seen in professional soccer players during pre-season where values of ~4343 AU have been reported (Jeong et al., 2011). This lower sRPE in rugby compared with soccer may be a reflection of the different training methods between the sports with rugby players performing more gym based sessions and fewer fields based running sessions. Moreover, it may also represent more rest days being utilised in rugby for recovery as a consequence of the high amount of physical collisions performed in training. For example, Jeong et al. (2011) report one rest day per week compared with the three rest days reported in this study. Given that there was a significantly greater weekly sRPE reported in forwards compared with backs despite lower total running distances covered by forwards than backs, these data suggest that GPS is not able to assess the relatively static but highly demanding efforts performed during forward specific training such as scrummaging and supports the use of sRPE alongside GPS to monitor training loads.

One of the main goals of the pre-season training programme in rugby is to optimise body composition. Although the mean changes in the sum of seven skinfold sites were relatively modest (decrease of 5.6 ± 6.0 and 5.12 ± 4.8 mm for forwards and backs respectively), peak losses of body fat were substantial (decrease of 19.3 and 18.8 mm for forwards and backs respectively). Based on prediction equations (which it is acknowledged have substantial limitations), this would account to ~3 % drop in body fat. Importantly, peak increases in lean body mass of 2.9 kg and 2.4 kg for forwards and backs respectively were reported. Similar observations were made in a RU pre-season which found an ~11 mm reduction in sum of eight skinfolds (Argus et al., 2010), and also in amateur rugby league players during the pre-season with an average decrease of ~6 mm in sum of seven skinfolds (Gabbett, 2005a). Although the mean increase in lean mass could be described as modest, it is important to note that in highly trained professional athletes, increases in lean mass become increasingly difficult given that the players will have been engaged in a resistance training programme for several years. Similarly, the somewhat modest mean losses of body fat may also represent the fact that not all players were attempting to lose body fat and therefore interpretation of mean data in elite sporting environments should be taken with caution. Alongside the improvements in body composition, improvements in physical performance were also reported. Strength significantly improved across all tests for both groups with an average improvement of 8% which is similar to previous findings of 11% strength improvements in a RU pre-season (Argus et al., 2010). Speed also significantly improved over both 10 m and 20 m for forwards, and markers of conditioning improved for both groups. It must be noted that the conditioning test utilised in this study was designed in-house to best fit the clubs facilities and requirements (previous data existed from this cohort using this test), rather than utilising a validated protocol. As such, no reliability data exisits nor validation performed, making comparisons between other teams or team sports difficult, and data must be interpreted with extreme caution. Despite

significant improvements illustrated in this study, the relationship between training volume and the magnitude of adaptation remains unknown, and such data may provide crucial information for the programming of appropriate training loads to elicit optimal physiological adaptation. Nevertheless, the data from this study provide a realistic benchmark for other professionals to aim for during a pre-season.

Analysis of the 24-h recall revealed that the energy intakes on a typical training day were only 14.8 MJ (range 12.2 – 17.1) and 13.3 MJ (range 11.3 to 16.5) for forwards and backs respectively. These data are similar to that reported in professional RL (~14 MJ; Morehen et al., 2015) and surprisingly not too dissimilar to soccer players where values of 12.8 MJ were reported (Maughan, 1997) despite rugby players having significantly more lean mass than soccer players. This similar energy intake despite much larger lean mass in rugby players may be accounted for by the lower weekly sRPE reported in rugby players, although this suggestion requires further investigation. It should also be stressed that the study by Maughan (1997) used a 7-day weighed food intake compared with a 24-h dietary recall in this study, which may compromise the comparison between the two studies. Moreover, it cannot be excluded that the relatively low energy intake observed in the present study could be related to the fact that energy intake was assessed in the form of a 24-h recall and these particular days were not typical of the players general eating patterns. However, all players reported that the day described was 'typical' and the author therefore has no reason to believe that this is the reason for the low energy intakes. It is also possible that the players in the present study may have intentionally (Bingham, 1987b, Burke et al., 1991, Deakin, 2000) or unintentionally (Bingham, 1987b) underreported the total energy intake. However, since approximately half of the daily nutrition consumed was observed by the author including a meal provided on arrival at the club, whey protein pre- and post-training, and a lunch provided post-training, this is deemed unlikely. Rather, the present

data may reflect the fact that during a pre-season, despite the need to fuel training sessions, a major goal of many players is to maximise body composition and reduce body fat. It could be speculated that players' diets were influenced heavily by advice given by the clubs nutritionist and foods provided by the club, however, anecdotally this practice is a common feature within modern elite sports clubs and reflects accurately a 'typical day' within an elite rugby players life. Moreover, nutrition support at this particular club advised carbohydrate periodisiation around training, and therefore low carbohydrate intakes were as a consequence of players' own nutritional choices in and away from the club. Given the fact that all players improved their physical markers of performance, combined with no player appearing to lose lean muscle mass, it is hard to argue that the players were under consuming on a daily basis. To fully answer this question though, it would be necessary to measure energy expenditure alongside energy intake during a typical training week, which are key aims of Chapter 5 of this thesis.

Carbohydrate intake in the present study fell below the recommended values for elite athletes and actually could be classified as values for athletes engaged in light exercise or skill based sports (Burke et al., 2011). Interestingly, the mean CHO intake reported of 3.3 (0.7) and 4.1 (0.4) $g.kg^{-1}$ for forwards and backs respectively were similar to that reported by Maughan (1997) in professional soccer players (3.4 $g.kg^{-1}$), and more recently (Milsom et al., 2014) in a case study of a professional premier league soccer player (~4 $g.kg^{-1}$). In recent years the guidelines for CHO intake have changed significantly dropping from 8-12 $g.kg^{-1}$ in the 2004 guidelines (Burke et al., 2004a) to 6-10 $g.kg^{-1}$ in the 2011 guidelines (Burke et al., 2011) for athletes engaged in moderate to high intensity exercise lasting 1-3 hours. The most recent guidelines do however clearly state that CHO intake must match the athletes training goals (Burke et al., 2011). It is possible that given the mass of rugby players, the guidelines given for most athletes in g.kg body mass, even in the updated

recommendations, remain too high for rugby players. For example, in the present study, if one of the 110 kg athletes were to consume 8 $g.kg^{-1}$ of CHO instead of the reported 3.7 $g.kg^{-1}$ this would involve an additional ~8 MJ per day which may have resulted in the players gaining unwanted body mass. Whilst there is unquestionable support that a high CHO diet leading up to team sport based games improves playing performance (Jardine et al., 1988, Hawley et al., 1997), the present data supports the notion that the CHO intake of the athletes for training purposes should reflect the specific training demands (Burke et al., 2011). Given the current chapter describes only 'typical' energy intakes of rugby players, the day-to-day variation in energy balance is currently unknown. Furthermore, energy expenditure (EE) was not assessed in the current study and consequently this key variable is still unknown in professional rugby players.

It could be agued that the players in the present study over-consumed protein with values of 2.5 and 2.6 $g.kg^{-1}$ being reported for forwards and backs respectively, which is considerably higher than the guidelines of 1.8 $g.kg^{-1}$ body mass for strength based athletes (Tipton and Wolfe, 2004) and much higher than the 1.4 $g.kg^{-1}$ body mass reported in soccer players (Maughan, 1997). Consequently a reduced protein intake may have allowed for more CHO to be consumed. However, given that 2.5 $g.kg^{-1}$ has been recommended for athletes attempting to maintain muscle mass whilst decreasing body fat (Mettler et al., 2010), it is possible that the protein intakes were in fact appropriate. Moreover, studies have clearly shown that protein consumed post-exercise is beneficial in promoting muscle protein synthesis (Rasmussen et al., 2000), with some research suggesting 0.3 $g.kg^{-1}$ body mass as an optimum level post-exercise (Borsheim et al., 2002, Miller et al., 2003, Mori et al., 2010). In the present study, it was noted that approximately 0.3-0.6 $g.kg^{-1}$ body mass of the total 2.6 $g.kg^{-1}$ body mass was consumed as protein supplements and this was predominantly in the form of whey protein being taken before and after daily training

sessions. Given that there are no reported dangers of consuming these intakes of protein in a non-diseased state (Tipton, 2011), combined with the desire to maintain, if not increase lean mass by all of the rugby players in this study, it would appear that the reported protein intakes of the players were appropriate. Dietary fat intakes in the present study were approximately 1.0 and 0.95 $g.kg^{-1}$ body mass for forwards and backs respectively, which is in line with the current recommendations (Bishop et al., 1999). The majority of the fat came from foods rather than supplements including regular consumption of oily fish, meat products and cooking oils. Given the important roles of these fats in health and performance, it would be unwise to suggest a reduction in dietary fat intake and again it is likely that the rugby players are consuming the correct levels of this macronutrient.

The use of supplements in sports is one of the most controversial areas of sports nutrition from both a risk management and efficacy perspective. All players consumed whey protein on a daily basis and this tended to be consumed around training. Players consumed approximately 0.3-0.6 $g.kg^{-1}$ of whey protein supplements per day that equated to approximately 2 x 30 g protein supplements. Some players also consumed supplementary CHO post-training with the backs consuming more than the forwards. This difference in supplementary CHO likely reflects the greater desire of the forwards to reduce body fat during the pre-season given that 'mid-training' sports drinks and post-training CHO supplementation was completely self-selected at the club. Additional supplements taken by the rugby players included beta-alanine, creatine, Beta-hydroxy beta-methylbutyrate (HMB), omega 3 fish oils, multi vitamins, probiotics, Conjugated linoleic acid (CLA), glutamine and electrolyte tablets (Table 4.6). Importantly all supplements were provided by batch-tested companies to reduce the risk of contamination. It must be stressed however that the supplements used in this study may reflect advice given by the club as opposed to

being a reflection of the general practices of all rugby teams. A comprehensive appraisal of supplement use within rugby is now clearly warranted.

4.7 Summary

The present study has for the first time attempted to quantify the training demands of elite rugby players during a pre-season, monitor changes in performance, as well as evaluate dietary intakes. Significant improvements were reported in the physical performance and anthropometry of rugby players observed over a 10-week pre-season, despite CHO consumption falling significantly short of suggested levels and total energy intake being less than may have been expected. These data may be accounted for by the fact that the overall training load, in terms of both sRPE and running distances covered, was not especially high. It could therefore be suggested that the 'relatively low' CHO intake observed in the present study was sufficient to fuel a rugby pre-season, although whether this CHO intake would be suitable for in-season is currently unknown. It may therefore be pertinent to quantify the training loads of the in-season whilst assessing energy intakes and expenditures of elite RU players over a full training week. Furthermore, day-to-day variations in energy balance should also be assessed which may be achieved using a 6-day food diary (to avoid interference with game day practices) and wearable micro-technology.

CHAPTER 5

ENERGY INTAKE AND EXPENDITURE ASSESSED 'IN SEASON' IN AN ELITE RUGBY UNION SQUAD

This study has been published in the European Journal of Sports Science in 2015 and features as a case study in the book "The Science of Rugby".

Bradley, W. J., Cavanagh, B., Douglas, W., Donovan, T. F., Twist, C., Morton, J. P., & Close, G. L. (2015b). Energy intake and expenditure assessed 'in-season' in an elite European rugby union squad. *Eur J Sport Sci.* 2015; 15(6):469-479.

5.1 Abstract

Rugby Union is a complex, high-intensity intermittent collision sport with emphasis placed on players possessing high lean body-mass and low body-fat. After an 8-12 week pre-season focused on physiological adaptations, emphasis shifts towards competitive performance. However, there are no objective data on the physiological demands or energy intake (EI) and expenditure (EE) for elite players during this period. Forty-four (mean ± SD: age 26 ± 3.4 years, body-mass 101 ± 6.6 kg, height 185 ± 5 cm) elite professional rugby union players completed a 36-week in-season. In-season training load was assessed using GPS and session RPE (sRPE), alongside six-day assessments of EE and EI measured using wearable SenseWear technology and a 6-day food diary respectively. Mean weekly distance covered was 7827 ± 954 m and 9572 ± 1233 m with a total mean weekly sRPE of 1776 ± 355 and 1523 ± 434 AU for forwards and backs, respectively. Mean weekly EI was 16.6 ± 1.5 and 14.2 ± 1.2 MJ, and EE was 15.9 ± 0.5 and 14 ± 0.5 MJ for forwards and backs respectively. Mean carbohydrate intake was 3.5 ± 0.8 and 3.4 ± 0.7 $g.kg^{-1}$ body mass, protein intake was 2.7 ± 0.3 and 2.7 ± 0.5 $g.kg^{-1}$ body mass, and fat intake was 1.4 ± 0.2 and 1.4 ± 0.3 $g.kg^{-1}$ body mass for forwards and backs respectively. All players who completed the food diary self-selected a 'low' carbohydrate 'high' protein diet during the early part of the week, with carbohydrate intake increasing in the days leading up to a match, resulting in the mean EI matching EE. Based on EE and training load data, the EI and composition seems appropriate, although further research is required to evaluate if this diet is optimal for match day performance.

5.2 Introduction

In chapter 4 carbohydrate intakes of 3.3 ± 0.7 and 4.1 ± 0.4 $g \cdot kg^{-1}$ for forwards and backs respectively, were reported during a RU pre-season. These data are similar to those reported in professional soccer players (3.4 $g \cdot kg^{-1}$; Maughan, 1997), but lower than intakes generally suggested for team-sports engaged in high intensity exercise programmes where values of 6-10 $g \cdot kg^{-1}$ have been recommended (Burke et al., 2011). To date there are no data on typical macronutrient intakes of elite rugby players during in-season training.

To implement a valid nutritional plan it is important to understand the day-to-day energy requirements of an athlete. Due to the physicality of rugby, the measurement of EE is somewhat difficult given that many of the tools available would not be suitable either through danger to the athlete or to the equipment. Currently the doubly labelled water (DLW) stable isotope method is considered the gold standard for measuring EE (Ekelund et al., 2002), however this technique does not allow day-to-day comparisons to be made. Multi-sensor, wearable body monitoring technology might therefore provide an effective means of assessing daily EE in free-living rugby players.

Although in chapter 3 the training demands and nutritional intakes of an elite RU pre-season were reported, to date there are no studies showing the training demands and energy intakes and expenditures during the competitive season. Due to the importance of competitive performance, these data would be of great significance to strength and conditioning research literature allowing informed decisions to be made with regards to players' diets during this competitive period.

Therefore the aim of this study was to i) characterize the weekly external and internal training demands of a RU in-season using GPS technology and session RPE (sRPE) ii), evaluate the typical energy intakes and macro- and micronutrient intakes, and iii) analyse

the energy expenditures of elite RU players during the in-season period. It was hypothesised that elite rugby players would consume less than the recommended 6-10g.kg CHO per day (Burke et al., 2011) whilst meeting or exceeding recommended protein intakes (Tipton, 2011).

5.3 Methodology

5.3.1 Players

Forty-four elite RU players currently playing in the European Rabo Direct Pro 12 league volunteered for this study. The sample population was collected on the first team squad, which included 12 current international players and 4 British & Irish Lions. Ethical approval was granted by the local ethics committee of Liverpool John Moores University. A summary of the participant characteristics can be seen in Table 5.1.

Table 5.1. Mean (SD) Summary of the characteristics of the participants at the start of the study including the number that took part in each phase of the research. * Indicates *almost certainly* different from backs. FWD = forwards.

	N		Age (yrs)		Height (m)		Weight (kg)	
	FWD	**Back**	**FWD**	**Back**	**FWD**	**Back**	**FWD**	**Back**
GPS	24	20	26 (3.6)	26 (3.1)	1.89* (0.06)	1.83 (0.05)	110.1* (6.1)	92.1 (7)
6-day food diary & energy expenditure	7	7	28 (2.8)	25.1 (3.8)	1.92* (0.07)	1.84 (0.06)	110.1* (6.2)	93.6 (5.9)

5.3.2 Experimental design

Players began in-season training at the rugby club after a 12-week pre-season period. The first week of in-season training started in early October and this was classed as Week 13. Players then began 3 x 12-week in-season training macrocycles as prescribed by the club. During the 'in season', running activity was monitored at every training session using GPS technology, and session RPE (sRPE) was used to quantify the overall training load (see chapter 3.3). GPS equipment was rotated equally around all players with all positions represented equally. Food diaries were completed as part of the club's normal in-season

training regime and were routinely performed by all of the players who were therefore familiar with this method. During weeks 32 (n = 5), 33 (n = 5) and 34 (n = 4) of the season 14 players (7 forwards and 7 backs) wore SenseWear armbands and completed a detailed six-day food diary to assess energy expenditure and nutrient intake. A typical in-season training week is depicted in Table 5.2.

Table 5.2. A typical In-season training week. Training days are shown in relation to game day rather than days of the week. Only the field based sessions were tracked using GPS.

	Game Day -5	**Game Day -4**	**Game Day -3**	**Game Day -2**	**Game Day -1**	**Game Day**	**Game Day +1**
AM	Mobility	Gym extras & Meetings	Mobility	Gym extras & Meetings	**REST**	**GAME**	**RECOVERY**
Mid-AM	Strength (1hr)	Field (45 mins)	Strength (1hr)	Rugby (1hr)	**REST**	**GAME**	**RECOVERY**
PM		Rugby (1hr)			**REST**	**GAME**	**RECOVERY**

5.3.3 Energy Intake (6-day food diary)

A six-day food diary was used to analyse player macronutrient and micronutrient intakes and reported as days away from a game (Game day -5, -4, -3, -2, -1 and game day +1) in megajoules (MJ). This time period is believed to provide reasonably accurate and precise estimations of habitual energy and macronutrient consumption (Braakhuis et al., 2003). Players were instructed to document a complete account of all foods and fluids ingested over a six-day period. Specific training on completing the food diary was given by the teams nutritionist who was a Sport and Exercise Nutrition Register (SENr) accredited practitioner with over 10 years experience working in professional sport. All players were instructed to give careful attention to detail such as timing of nutrient intake, estimation of

volumes and quantities, and to provide specific brand names where possible. To enhance reliability, food diaries were reviewed and cross checked using a 24-h recall by the team's nutritionist after one day of entries to ensure accurate input as previously suggested (Thompson and Subar, 2008). The nutrient intakes were calculated using Nutritics professional diet analysis software (Nutritics Ltd, Ireland) to obtain energy and macro- and micronutrient composition.

Figure 5.1 – Player taking pictures of prepared food to aid with food diary entries.

5.3.4 Energy Expenditure

SenseWear Pro2 wearable armband (SWA; BodyMedia, USA) was used to assess energy expenditure. Five armbands were rotated between the athletes over a three-week period during the same macrocycle. Athletes wore the armband 24-h a day for six days, except during water or heavy contact based activities. The SWA were removed on match day to avoid disruption during match preparations and also due to contacts sustained during

competition. The armband was worn on the back of the upper right arm and utilised a two-axis accelerometer, heat flux sensor, galvanic skin response sensor, skin temperature sensor, and a near-body ambient temperature sensor to capture data leading to the calculation of energy expenditure using a proprietary algorithm. SenseWear computer software (BodyMedia 5.1, USA) was used to analyse player energy expenditure and reported as days away from competition (Game day -5, -4, -3, -2, -1 and game day +1) in MJ. 07:00 was chosen as the 24-h start point determined by average player wake-up time according to the clubs daily monitoring.

Figure 5.2 – Player wearing one of five SenseWear armband during training.

5.4 Statistics

Magnitude-based inferential statistics were employed to find differences in movement characteristics and energy intake and energy expenditure between forwards and backs (see section 3.9).

5.5 Results

5.5.1 Weekly external and internal training load

Table 5.3. Mean (SD) weekly GPS data reported in $m \cdot s^{-1}$ for the Forwards (FWD) and Backs. Accelerations are defined by efforts performed >2.79$m \cdot s^{-2}$. RHIE is defined as a cluster of three user defined high intensity efforts performed <21s apart (contacts, accelerations or sprints). Accels = Accelerations.

* Indicates different from backs.

	Total Distance	Contacts	0 – 2 $m \cdot s^{-1}$	2 - 4.4 $m \cdot s^{-1}$	4.4 - 5.6 $m \cdot s^{-1}$	5.6 - 7.5 $m \cdot s^{-1}$	7.5 + $m \cdot s^{-1}$	RHIE	Accels	sRPE
FWD	7827* (954)	80* (25)	3940* (487)	3020* (438)	665* (175)	194* (141)	4* (17)	19.2 (7.9)	15.3* (9.6)	1776* (355)
Back	9572 (1233)	50 (22)	4462 (679)	3460 (603)	993 (196)	617 (232)	40 (61)	15.4 (10.3)	46 (14.6)	1523 (434)

Session RPE and GPS data can be seen in Table 5.3. The mean weekly sRPE was calculated by combining all of the sRPE giving a cumulative value of 1776 (335) and 1523 (410) AU for forwards and backs respectively. Backs *almost certainly* covered greater total distances (ES; ±90% CL: 1.32; ±0.67), higher running speeds between 5.6-7.5 $m \cdot s^{-1}$ (1.8; ±0.72) and speeds of 7.5+ $m \cdot s^{-1}$ (4.57; ±2.13) than forwards. Further, backs *very likely* performed more accelerations (2.34; ±1.29) than the forwards. Conversely, the forwards *almost certainly* performed more contacts (2.38; ±1.31) and sRPE was *very likely* greater (1.78; ±0.76) than the backs. Sub analysis of the GPS data revealed that the backs likely covered more distance at the lower running speeds, (i.e. 0-2, 2-4.4 and 4.4-5.6 $m \cdot s^{-1}$; 0.36; ±0.16, 1.1; ±0.52, and 0.79; ±0.51 respectively). Difference in RHIE's between the forwards and backs was unclear (0.27; ±0.39).

5.5.2 Energy intake and expenditure

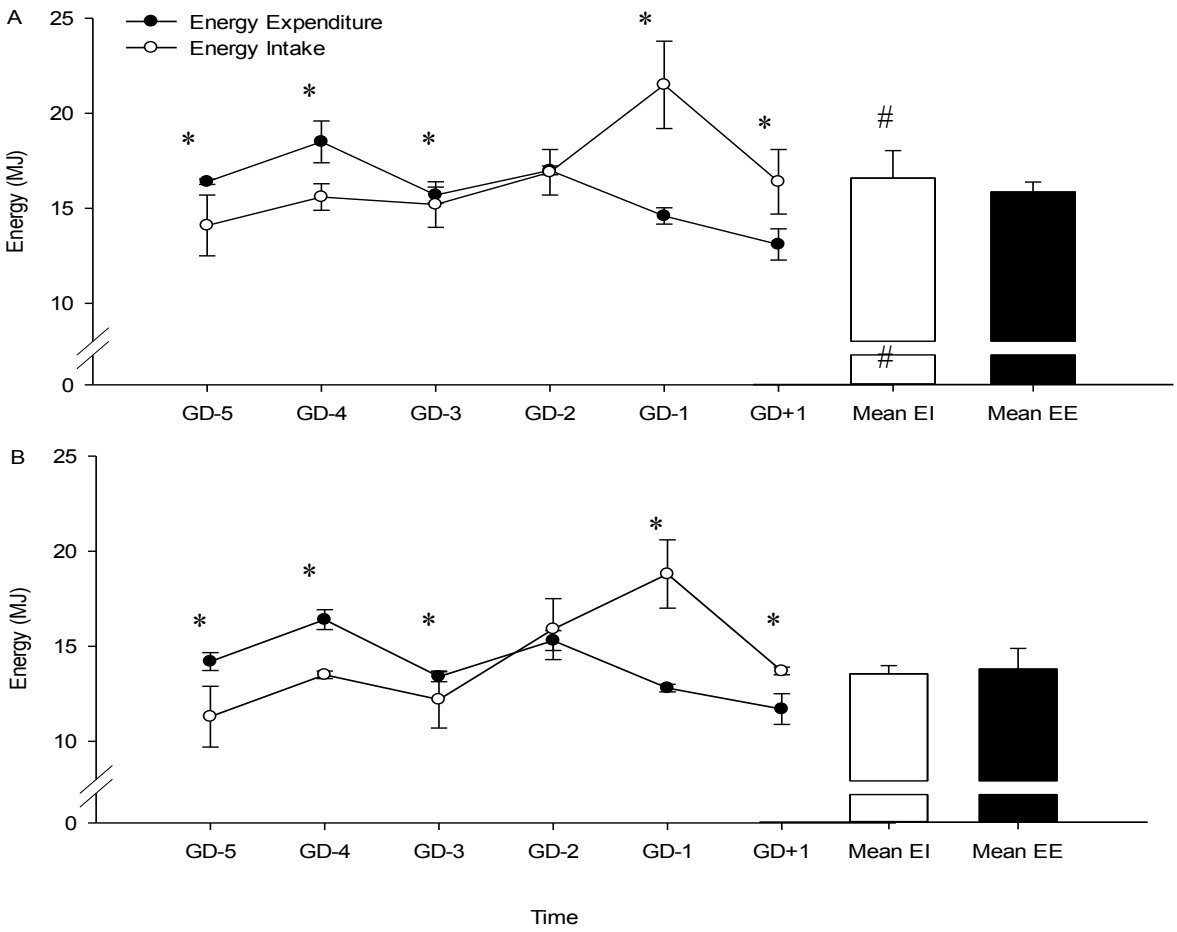


Figure 5.3. Energy intake (EI) and energy expenditure (EE) of 14 elite rugby union players over a 6-day period (Game day -5, -4, -3, -2, -1 and +1) taken from SenseWear armband data and 6-day food diary analysis. Figure A = Forwards, figure B = Backs.

* Indicates difference between EI and EE (*almost certainly, very likely and likely*).
# Indicates different from backs (*very likely*).

Energy intake (EI) and expenditure (EE) over the six assessment days, presented in megajoules (MJ), are shown in Figure 5.3. Mean EI and EE was 16.6 (1.25) MJ and 15.9 (0.53) MJ, and 14.2 (1.2) MJ and 14 (0.47) MJ for forwards and backs respectively, with both EI and EE *very likely* higher in the forwards than the backs (0.76; ±0.49 and 1.0; ±0.6 respectively). EI was *very likely* lower than EE on GD-5 (15.83; ±11.68), GD-4 (2.25;

±0.86) and GD-3 (1.99; ±1.28), *almost certainly* higher than EE on GD-1 (3.23; ±0.88) and GD+1 (1.89; ±0.77), and differences were *unclear* on GD-2 (0.27; ±0.54) for the forwards. EI was *very likely* lower than EE on GD-5 (5.66; ±4.32) and GD-3 (5.76; ±4.96), *almost certainly* lower than EE on GD-4 (4.45; ±0.85), *almost certainly* higher than EE on GD-1 (16.34; ±4.74), and differences were *unclear* on GD-2 (0.03; ±1.71) for the backs.

EI *likely* increased from GD-5 on GD-4 (0.75; ±0.9) and GD+1 (0.87; ±0.9), and *very likely* increased on GD-2 (1.49; ±1.21) and GD-1 (12.56; ±1.27) with changes on GD-3 *unclear* (0.44; ±1.11) for the forwards. This coincided with a *very likely* reduction in EE on GD-3 (3.43; ±1.91) and an *almost certain* reduction in EE on GD-1 (10.02; ±2.34) and GD+1 (17.56; ±5.79) with a *very likely* and *almost certainly* increased EE on GD-4 (10.63; ±5.41) and GD-2 (3.37; ±0.96) respectively for forwards. EI *likely* increased from GD-5 on GD-4 (0.92; ±0.93) and GD+1 (1.01; ±0.87) and *very likely* increased on GD-2 (1.47; ±1.15) and GD-1 (2.23; ±1.11) with changes on GD-3 *unclear* (0.19; ±0.93) for the backs. This coincided with a *very likely* reduction in EE on GD-3 (1.31; ±0.48) and GD+1 (454; ±1.53), and an *almost certain* reduction in EE on GD-1 (2.29; ±0.46) with an *almost certain* increase in EE on both GD-4 (3.07; ±0.29) and GD-2 (1.62; ±0.66) for backs.

5.5.3 Macronutrient profile

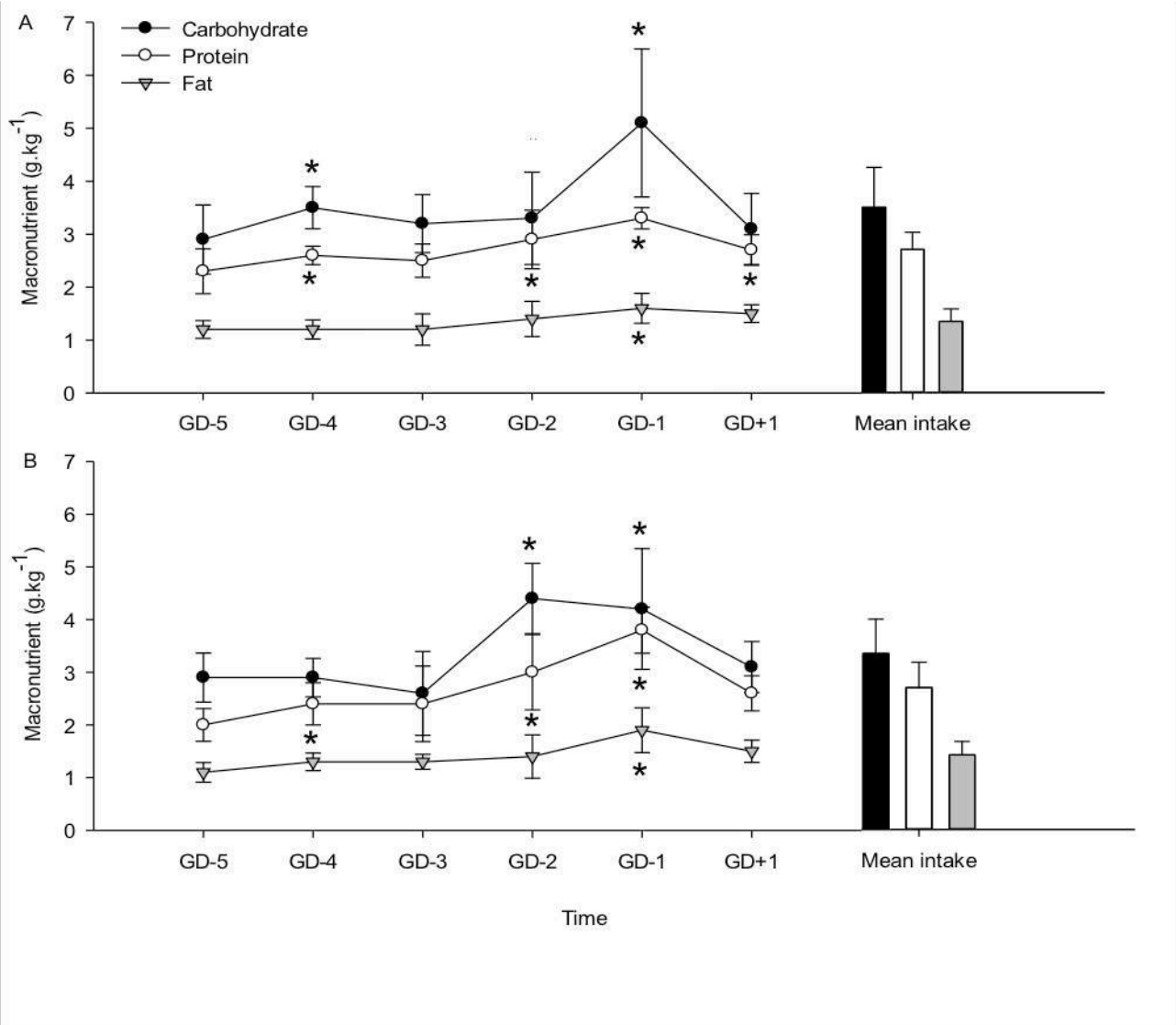


Figure 5.4. Macronutrient intakes (Carbohydrate, Protein and Fats) of 14 elite rugby union players over a 6-day period (Game day -5, -4, -3, -2, -1 and +1) taken from 6-day food diary analysis. Figure 5.4A = Forwards, figure 5.4B = Backs.

* Indicates different from GD-5 (*almost certainly, very likely and likely*).

Macronutrient intakes from six-day food diaries (presented in $g \cdot kg^{-1}$ body mass) can be seen in Figure 5.4. Difference in CHO between forwards and backs was *unclear* (0.16; ±0.51), with values of 3.5 (0.8) $g \cdot kg^{-1}$ (38 % total calories) and 3.4 (0.7) $g \cdot kg^{-1}$ (37 % total calories), respectively. Similarly, differences in mean weekly protein intake between the forwards and backs was *unclear* (0.24; ±0.62) with values of 2.7 (0.5) $g \cdot kg^{-1}$ (30 % total

calories) and 2.7 (0.3) $g \cdot kg^{-1}$ (30 % of total calories), respectively. Mean fat intake was also *unclear* between positions (0.21; ±0.56), with values of 1.4 (0.2) and 1.4 (0.3) $g \cdot kg^{-1}$ (32 and 33 % of total calories) for forwards and backs, respectively.

Forwards *likely* and *almost certainly* consumed a higher CHO intake on GD-4 (0.72; ±0.75) and GD-1 (2.01; ±1.28) respectively when compared with GD-5 (the first day of the week). Differences in CHO intake from GD-5 were *unclear* on GD-3 (0.45; ±0.84), GD-2 (0.41; ±1.42) and GD+1 (0.27; ±0.88) for the forwards. This coincided with a *likely* increase in protein intake on GD-4 (0.67; ±0.71) GD-2 (0.98; ±0.96) and GD+1 (0.73; ±0.75) respectively, and an *almost certain* increase in protein intake on GD-1 (1.72; ±0.71). Differences in protein intake from GD-5 were *unclear* on GD-3 (0.34; ±0.8). Differences in fat intake from GD-5 were *unclear* for GD-4 (0.32; ±0.76), GD-3 (0.43; ±0.65), GD-2 (0.23; ±0.75), and GD+1 (0.29; ±0.56) but *very likely* increased on GD-1 (1.56; ±1.52).

Backs *almost certainly* and *very likely* consumed a higher CHO intake on GD-2 (1.98; ±0.93) and GD-1 (1.74; ±1.34) respectively when compared with GD-5. Differences in CHO intake from GD-5 were *unclear* on GD-4 (0.5; ±1.08), GD-3 (0.07; ±1.18) and GD+1 (0.29; ±0.68) for the backs. This coincided with a *very likely* and an *almost certain* increase in protein intake on GD-2 (1.93; ±1.27) and GD-1 (3.26; ±0.83) respectively, with differences from GD-5 *unclear* on GD-4 (0.44; ±0.77), GD-3 (0.12; ±0.73) and GD+1 (0.23; ±0.89) for the backs. Differences in fat intake from GD-5 were *unclear* for GD-4 (0.37; ±0.55), GD-3 (0.29; ±0.97), GD-2 (0.31; ±0.32), and GD+1 (0.59; ±0.83) but *almost certainly* increased on GD-1 (2.58; ±1.14) for the backs.

5.5.4 Micronutrient profile

Table 5.4. Mean (SD) 6-day micronutrient profile (minerals and vitamins) compared with *RDA's* for each micronutrient obtained from 6-day food diaries. Reported as a weekly average for all 14 players who completed the 6-day food diary. Minerals: K = Potassium, Ca = Calcium, Mg = Magnesium, Fe = Iron, Zn = Zinc, Cu = Copper. Vitamins: A, D, E, K, B6, B12, C. RDA's are taken from: Whiting and Barabash (2006). ↑ Indicates greater than RDA, ↓ indicates lower than RDA.

Minerals		Average (mg)	*RDA*	Vitamins		Average (µg)	*RDA*
K	↑	6258 ± 1691	*4700*	**A**	↑	2287 ± 1488	*900*
Ca	↑	1733 ± 694	*1000*	**D**	↑	8 ± 5	*5*
Mg	↑	681 ± 171	*400*	**E (mg)**	↑	17 ± 8	*15*
Fe	↑	24 ± 9	*8*	**K**	↓	97 ± 60	*120*
Zn	↑	23 ± 8	*11*	**B6 (mg)**	↑	5 ± 2	*1.3*
Cu	↑	3 ± 1	*0.9*	**B12**	↑	12 ± 6	*2.4*
				C (mg)	↑	168 ± 132	*90*

Daily average micronutrient intakes for the squad taken from six-day food diaries can be seen in Table 5.4. Mean micronutrient intakes met and exceeded RDA's for physical activity for all minerals and vitamins apart from vitamin K which fell 24µg under this RDA but met and exceeded the RDA for general health.

5.6 Discussion

The aims of the present study were 1) to quantify the external and internal training loads during a typical in-season training week for elite RU players and 2), evaluate the EI and EE of elite rugby players during the competitive season. It is reported for the first time that distances of ~8-10 km are covered by elite RU forwards and backs during a typical in-season week, which equates to a total weekly internal load of ~1500 - 1800 AU. Daily EE and EI of elite RU players during this same training period were 14-16 and 14-17 MJ, respectively. Considerable variation in the day-to-day EE was also observed, with peak EE occurring early in the week and tapering down in preparation for competition. Interestingly, although EI also varied on a day-to-day basis, the temporal pattern did not match EE with EI being the lowest when EE was the highest and EI increasing in preparation for game-day. This inverse pattern may be essential to allow players to load with CHO in preparation for game day without excessive total energy intake during the week which over the course of the season may lead to unwanted gains in body fat.

GPS analysis of the training sessions revealed that weekly total distances of 7.8 ± 1 km and 9.6 ± 1.2 km were covered by forwards and backs, respectively. These distances were achieved over a five-day period as players were given rest days before and after game day. Backs covered more distance in all speed zones, along with a greater number of maximal accelerations but less collisions than forwards. These differences probably reflect the contrasting training regimes between RU forwards and backs. For example, forwards engage in more activities that involve tackling, rucking, mauling and line-outs, while the backs perform more acceleration and ball-in-hand running play. Interestingly, the frequency of RHIE was similar for positional groups despite clear differences in the movement characteristics of forwards and backs. This is probably explained by how the GPS software detects RHIE, which is defined as three consecutive efforts (sprint, contact

or acceleration) each separated by <21 s (Gabbett et al., 2013). So, while both positional groups perform a similar number of high intensity bouts, the movement actions that determine the RHIE are likely to be different between forwards and backs.

A weekly sRPE of ~1778 and ~1522 AU was observed for forwards and backs. These values are lower than those seen in elite RU players during pre-season (~2900-3400 AU; Chapter 4) and reflects the periodization of a rugby training programme. Indeed, lower training loads during the competitive season are deliberately administered to allow optimal recovery and for players to peak around games, whereas higher training loads are used in the pre-season when physiological adaptation is key and competition not a priority. Whilst not reported in this thesis, variations in weekly sRPE or indeed variation in sRPE during different periods of the season are likely evident due to this training periodisation. This was observed in a case study of futsal players conducted by Rabelo et al, (2015) who found that the pre-season elicited higher sRPE values than the first competitive period (COMP1), inter competition period (INTER-COMP), and second competitive period (COMP2). Interestingly, the COMP1 period revealed lowest sRPE and training intensities which is probably explained by a greater number of games (38 in total) in this period. Furthermore, higher training intensities were observed later in the season due to lower game volumes resulting in higher sRPE. This information is currently unavailable for rugby and may help to provide a clearer insight of periodisation and training demands of the rugby season.

Despite backs experiencing a higher external load during a training week, forwards did exhibit a higher internal load (sRPE) than backs. While sRPE is an appropriate measure of training load in rugby players (Lovell et al., 2013), variances in perceptual responses will be influenced by several internal and external factors. Here, differences in perceived weekly load between positions is probably explained by higher numbers of collisions experienced in training by forwards. Indeed, collisions have been purported to contribute

significantly to the variance in sRPE between players during rugby training (Lovell et al., 2013). These findings reaffirm the complexity of factors influencing perceptual measures of training load and the necessity to adopt both internal and external measures to monitor training in rugby.

Mean energy expenditure was lower than those values reported in-season using DLW for RL forwards (21.5 MJ) and backs (20 MJ; Morehen et al, 2016) which may be due to differences in match-play and training demands between rugby codes. However, weekly sRPE were similar between studies meaning that the lower EE reported in this study may reflect i), a difficulty in wearable technology to quantify anaerobic contributions to training and ii), missing data for short time periods that the technology was removed during contact and water submersion. Measures of HR which have been used to predict EE in football (Bangsbo, 1994, Esposito et al., 2004), RL (Coutts et al., 2003) and RU (Cuniffe et al., 2009) using methods outlined by Spurr et al, (1988), could have been utilised during contact periods while the SWA was removed. However, given that both technologies measure completely different metrics, alongside a lack of validation between the devices, combining this data may be wholly inappropriate. One further possibility involves the use of one of many regressional equations applied to the acceletometer data in order to estimate EE, however, prediction equations developed using moderate-intensity lifestyle activities tend to overestimate the energy cost of walking, sedentary, and light activities, whilst underestimating the energy cost of most other activities (Crouter et al., 2006).

Energy expenditure changed during the training week for both forwards and backs, with higher EE elicited during the first four days of the training week and significantly reducing around competition (Figure 5.1). The six-day food diary revealed changes in EI during the

training week for both forwards and backs, following an inverse trend to EE (Figure 5.1). Fluctuations in EI represent lower intakes during the first 4-days of the training week concurrent with higher EE. This is likely attributed to rugby players attempting to reduce or maintain body fat before significantly increasing EI by increasing carbohydrate intake leading up to competition in an attempt to increase muscle glycogen concentration. It is possible, however, that players might have intentionally (Bingham, 1987a, Deakin, 2000) underreported their total energy intake, although since approximately half of the daily nutrition consumed was observed by the authors, this seems unlikely.

Although present data indicate lower training loads and total distances compared to those of RU players in pre-season (Chapter 4), mean EI was slightly higher in-season for both forwards (15.8 *cf.* 14.8 MJ) and backs (14.1 *cf.* 13.3 MJ). This may be attributed to players increasing total EI in the days leading up to competition. It must be stressed, however, that the pre-season study used a 24-h dietary recall, which might compromise the comparison between the two studies. Interestingly, while EE and EI differ on a day-to-day basis, mean EE and EI were surprisingly similar for forwards (16.6 and 15.8 MJ) and backs (14.2 and 14.1 MJ). This suggests that although athletes might fail to meet energy requirements on some training days, light training days or rest days before a game correspond with players increasing EI (mainly through CHO increases) to maximise muscle glycogen stores. The lower EI early in the week may be necessary to prevent a positive energy balance that over the course of a season could result in unwanted gain in body-fat.

Interestingly, all the players again self-selected what could be classed as a low CHO / high protein diet (similar to that observed in Chapter 4) for the first four days of the training week, and increased CHO intake the day before game day. This practice contravenes earlier recommendations for CHO intakes of 8-12 $g \cdot kg^{-1}$ (Burke et al., 2004a), as well as more recent guidelines that state values of 6-10 $g.kg^{-1}$ (Burke et al., 2011) for athletes

engaged in moderate to high intensity exercise lasting 1-3 hours. Therefore it could still be argued that players in the current study failed to meet the daily recommended CHO requirements. However, current guidelines also clearly state that CHO intakes should be designed to meet the fuel requirements of the training programme (Burke et al., 2011). It is therefore proposed that players are attempting to match CHO intakes with training demands such that CHO intakes of 4-6 $g \cdot kg^{-1}$ body mass are not '*low*' and are in fact '*appropriate'* for this group of athletes providing CHO intake is increased in the day before and after a game. Given that CHO intake altered significantly over the week and was the main macronutrient contributor in the daily EI fluctuations, it is suggested that the players are indeed following the recent guidelines and matching their CHO intakes to the fuel requirements of the training programme. Players could be using some periods of lower CHO intake to enhance training adaptions (Morton et al., 2009, Hawley and Morton, 2014) and for the maintenance of low body fat (Morton et al., 2010), yet still increasing muscle glycogen concentrations in preparation for competition (Hawley et al., 1997). Interestingly, backs utilised a two-day load compared with a single day by the forwards. This might reflect 1), a lower CHO intake in the first three days to reduce body fat or 2), a purposeful attempt to increase glycogen more aggressively than the forwards due to the varying physiological challenges of the positions.

The cycling of CHO intake reported in the present study might be a suitable way of maintaining weekly energy balance yet still allowing sufficient CHO intake to increase muscle glycogen and thus enhance match day performance. Playing performance is unquestionably improved with a high CHO diet leading up to team sport based games (Jardine et al., 1988, Hawley et al., 1997), and although a significant increase in CHO intake was reported in the days leading up to competition, the intakes reported in this study are still below recommended CHO intake for elite athletes. It is still possible that such

intakes are not optimal for match day performance and future studies should now attempt to measure pre- and post-game muscle glycogen demands in elite rugby.

Protein intakes of 2.7 $g \cdot kg^{-1}$ reported in the present study were similar to values reported in an elite RU pre-season (2.5 and 2.6 g $g \cdot kg^{-1}$; Chapter 4). These intakes are much higher than the 1.4 $g \cdot kg^{-1}$ reported in soccer (Maughan, 1997) and 1.8 $g \cdot kg^{-1}$ described for strength based athletes (Tipton and Wolfe, 2004). However, to maintain muscle mass whilst decreasing body fat, protein intakes of 2.5 $g \cdot kg^{-1}$ have been recommended (Mettler et al., 2010) suggesting that the protein intakes in this study might in fact have been appropriate. Moreover, the athletes in the present study would have deliberately timed protein intakes around training in an attempt to maximise muscle protein synthesis, which might explain these higher protein intakes. The backs significantly increased protein intake from four days before the match. However, this higher protein intake early in the week could simply have been used as a CHO substitute given that CHO intake simultaneously reduced at this time. Dietary fat intakes in the present study were approximately 1.4 $g \cdot kg^{-1}$ body mass, slightly higher than the current recommendations (Bishop et al., 1999) but similar to those seen in elite Australian athletes (Burke et al., 2003). Consumption of oily fish, meats, and the use of cooking oils accounts for most of the fat intake, and although intakes were high, given the importance of healthy fats for performance it would be unwise to suggest a reduction in dietary fat intake.

Micronutrient intakes met and exceeded the RDAs for physical activity (Whiting and Barabash, 2006) for all minerals and vitamins apart from vitamin K, which fell slightly below the guidelines for physical activity (-24 µg, less than 1 small stem of broccoli, see Table 5.4). These values did, however, meet and exceed the RDA for general health. Although supplement use is common practice in sport with 40 to 100% of athletes using supplements (Baume et al., 2007), it seems inappropriate to supplement the athletes in this

study with a multi-vitamin or a mega dose single vitamin supplement given the lack of any micronutrient deficiencies (Whiting and Barabash, 2006). The exception to this could be vitamin D with recent data suggesting the current RDA for general health is too low (Holick and Chen, 2008) and deficiencies are commonplace in many athletes including rugby players (Close et al., 2013).

5.7 Summary

To conclude, for the first time this study has attempted to quantify the training demands and assess energy expenditure, intake and micronutrient intakes of elite rugby players during the in-season. It is reported that mean energy intake and expenditure followed an inverse trend, with expenditure exceeding intake during the first four-days of the training week and then reversed in the day leading up to competition with intake exceeding expenditure. This is likely due to a heavier training load and players desire to maintain body fat during the beginning of the training week, followed by a decrease in training load and increase in CHO intake leading up to competition in order to maximise glycogen concentration. Interestingly, mean energy intake exceeded expenditure for both forwards and backs despite CHO consumption falling short of recommended guidelines. This is likely attributable to relatively low training loads and running distances that attempt to provide sufficient stimulus to maintain player strength and fitness during the in-season, while reducing residual fatigue and promoting competition preparation. Alongside no micronutrient deficiencies, the current dietary practices of these elite rugby players are sufficient to fuel training during the in-season, providing energy intake and CHO are increased leading up to a match. Playing performance is however unquestionably improved with a high CHO diet leading up to team sport based games (Jardine et al., 1988, Hawley et al., 1997), and although a significant increase in CHO intake was reported in the days

leading up to competition, the intakes reported in this study are still below recommended CHO intake for elite athletes (Burke et al., 2011). It is therefore possible that such intakes are not optimal for match day performance. Future studies should now attempt to measure the metabolic demands and glycogen utilisation of elite rugby match-play after consuming low CHO (~3g.kg; habitual intake of rugby players reported in Chapters 4 and 5), or higher CHO (~6g.kg; intake reported for GD-1 in this chapter).

Given the unique opportunity to collect muscle biopsies from professional rugby players, combined with similar demands outlined in Chapter 2.1 and Table 2.1, the remaining 2 chapters will now focus on RL players.

CHAPTER 6

MUSCLE GLYCOGEN UTILISATION DURING RUGBY MATCH PLAY: EFFECTS OF PRE-GAME CARBOHYDRATE

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Bradley WJ, Morehen JC, Haigh J, et al. Muscle glycogen utilisation during Rugby match play: Effects of pre-game carbohydrate. *J Sci Med Sport.* 2016.

6.1 Abstract

Although the physical demands of Rugby League (RL) match-play are well-known, the fuel sources supporting energy-production are poorly understood. Muscle glycogen utilisation and plasma metabolite responses to RL match-play were therefore assessed after a relatively high (HCHO) or relatively low CHO (LCHO) diet. Sixteen (mean ± SD age; 18 ± 1 years, body-mass; 88 ± 12 kg, height 180 ± 8 cm) professional players completed a RL match after 36-h consuming a non-isocaloric HCHO (n=8; 6 $g \cdot kg \cdot day^{-1}$) or LCHO (n=8; 3 $g \cdot kg \cdot day^{-1}$) diet. Muscle biopsies and blood samples were obtained pre- and post-match, alongside external and internal loads quantified using Global Positioning System technology and heart rate, respectively. Data were analysed using effects sizes ± 90% CI and magnitude-based inferences. Differences in pre-match muscle glycogen between HCHO and LCHO (449 ± 51 and 444 ± 81 $mmol \cdot kg^{-1}$d.w.) were *unclear*. HCHO (243 ± 43 $mmol \cdot kg^{-1}$d.w.) and LCHO (298 ± 130 $mmol \cdot kg^{-1}$d.w.) were *most* and *very likely* reduced post-match, respectively. For both HCHO and LCHO, differences in pre-match NEFA and glycerol were *unclear*, with a *almost certain* increase in NEFA and glycerol post-match. NEFA was *likely* greater in LCHO compared with HCHO post-match (1.45 ± 0.51 $mmol \cdot l^{-1}$ and 0.95 ± 0.39 respectively), whereas differences between the 2 groups for glycerol were *unclear* (123.1 ± 39.6 and 98.1 ± 33.6 $mmol \cdot l^{-1}$), LCHO and HCHO respectively. Professional RL players can utilise ~40 % of their muscle glycogen during a competitive match regardless of their CHO consumption in the preceding 36-h, although less variability in starting muscle glycogen concentration was observed with consumption of ~600g CHO.

6.2 Introduction

In chapters 4 and 5 CHO intakes of 3.3 ± 0.7 and 4.1 ± 0.4 $g \cdot kg^{-1}$ were reported during a RU pre-season, and 3.5 ± 0.8 $g \cdot kg^{-1}$ and 3.4 ± 0.7 $g \cdot kg^{-1}$ were reported in-season for forwards and backs respectively. Although these dietary intakes appear to be appropriate for rugby training, key information is missing for us to make accurate dietary recommendations for rugby competition. Metabolic and match demands data have helped to devise physiological training programmes and nutritional strategies to enhance performance and/or delay fatigue in soccer (Maughan and Shirreffs, 2007). Such studies have also formed the basis of nutritional position stands (Burke et al., 2011), which have then been translated for use in rugby. However, there are distinct differences in the game characteristics between rugby and soccer, most notably the greater distances covered by soccer players (Bangsbo, 1994, Varley et al., 2014) and the multiple physical collisions observed in rugby that are not seen in soccer (Varley et al., 2014). Therefore, the suitability of using such studies to inform nutritional practices of rugby is questionable. Despite limited empirical evidence, traditional nutritional advice in rugby has been to load with CHO in the days leading up to a game (Burke et al., 2011), including doses between 6-10 $g \cdot kg^{-1}$ body mass. Many professional rugby players might not strictly adhere to this advice (Chapters 4 and 5), possibly because their large body mass makes such large CHO volumes difficult to consume (potentially ~1.3 kg of CHO per day for some larger players). Accordingly, the effects of an acute CHO load on muscle glycogen concentration and performance in professional rugby players warrants investigation.

Although the game demands of professional rugby have been well described (Gabbett et al., 2012, Twist et al., 2014, Evans et al., 2015b) the sources of energy fuelling these workloads are not well understood (Krustrup et al., 2006). As such, there is a need to better understand muscle glycogen utilisation during rugby match play thereby having practical

implications for optimal CHO loading strategies. Given that both codes of rugby poses similar physiological demands (Duthie et al., 2003, Gabbett et al., 2012, Twist et al., 2014) and nutritional behaviours (Morehen et al., 2016, Chapters 4 and 5 of this thesis), and with a unique opportunity arising consenting the extraction of muscle fibres from professional RL players around a competitive match, a decision was made to complete the final two studies in RL. The aim of the present study was to therefore quantify the physiological and metabolic demands of RL match-play in conditions of 'high' and 'low' CHO availability. To this end, professional male academy players were studied during a competitive RL match having followed 36-h of either a relatively high (6 $g \cdot kg^{-1}$; HCHO) or low (3 $g \cdot kg^{-1}$; LCHO) CHO diet. It was hypothesised that i), a competitive RL game would result in muscle glycogen depletion in players similar to that seen after competitive soccer games and ii), players consuming 6 $g \cdot kg^{-1}$ CHO would perform better in the second half compared with those players that followed the 3 $g \cdot kg^{-1}$ CHO diet.

6.3 Methodology

6.3.1 Players

Sixteen professional male rugby players aged 18 (1.2) years, weighing 88.4 (12.4) kg and height 1.8 (0.06) m, from a Super League rugby club academy volunteered to take part in this study. The sample population was collected on the academy squad which included 6 current senior players. Players were medically screened for the suitability of the use of prescription anaesthetic Marcain by a trained practitioner. Measurements included; mean arterial pressure using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, FL), height and body mass (SECA, Hamburg, Germany) and a pre-biopsy questionnaire. Ethical approval was granted by the local ethics committee of Liverpool John Moores University.

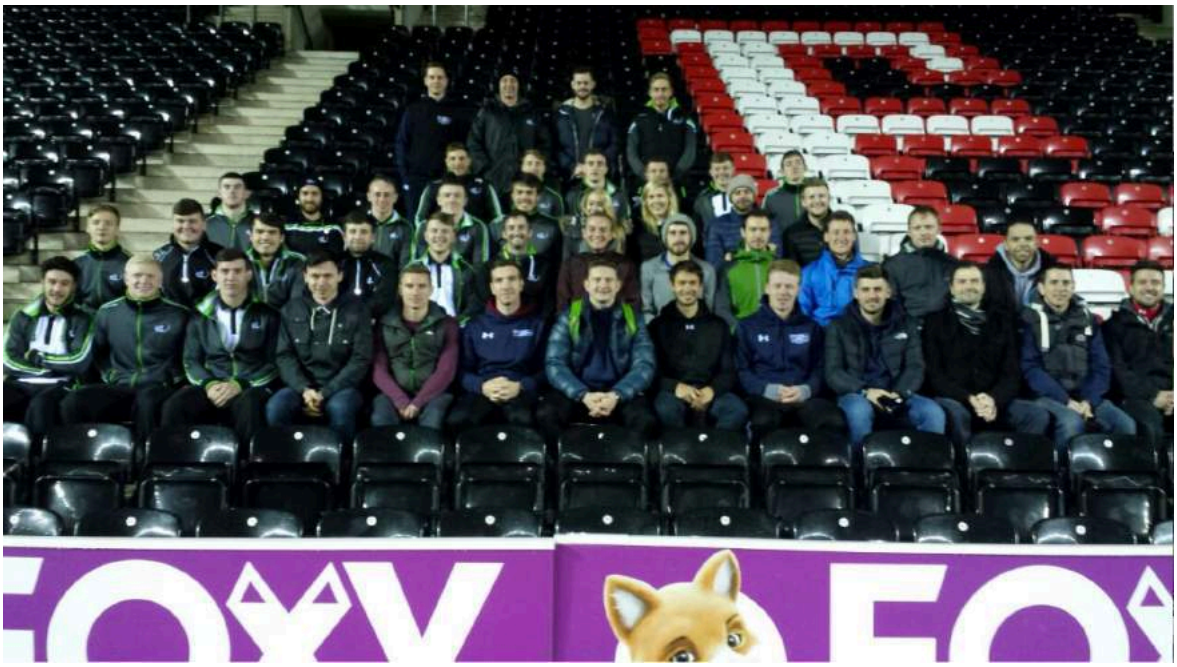

Figure 6.1 – All players and research staff who took part in the first muscle biopsy study to be conducted in elite rugby at Widnes Viking training facilities.

6.3.2 Experimental design

Participants played in a scheduled 80-minute 13-a-side RL game with a 10-minute half time period. Muscle biopsies and blood were collected and analysed (see sections 3.5 - 3.8) alongside assessments of urine osmolality and countermovement jumps pre- and post-match for all participants. Distances covered, player load, and heart rate were recorded throughout the match (see Chapter 3.3). A typical five-day lead into the match and the match day timings are depicted in Figure 6.2. Individualised diets were bought (Figure 6.3) designed and provided to all participants (Tables 6.1a and 6.1b). Player body mass was recorded and grouped to the closest 10 kg (70, 80, 90, 100 or 110 kg) which was used to prescribe the CHO content of the individual's diet. In a randomised design, players were initially divided into forwards and backs, ranked according to body mass, and then block randomised to one of two diet groups comprising either relatively high CHO (~6 $g \cdot kg^{-1}$ CHO, ~1.8 $g \cdot kg^{-1}$ protein and 0.7 $g \cdot kg^{-1}$ fat) or low CHO (~3 $g \cdot kg^{-1}$ CHO, ~1.8 $g \cdot kg^{-1}$ protein and 0.7 $g \cdot kg^{-1}$ fat). On game day, players consumed the same prescribed breakfast, at ~10:00AM, and then consumed a standardised pre-match meal ~4hr prior to kick off comprising 250g Tilda basmati rice and 125g chicken breast (~60g CHO, 30g PRO). Groups were fluid matched consuming ~3.5 L of fluid with only CHO intake differing between the two dietary groups. Foods were selected, purchased, and distributed by the clubs nutritionist and delivered to the players accompanied by a meal plan. All players were given strict instructions to follow the diet beginning the day before the match. Players were explicitly instructed not to consume any foods or liquids other than what was provided and to finish all meals provided. All players self-reported that they had strictly adhered to the diets prescribed to them.

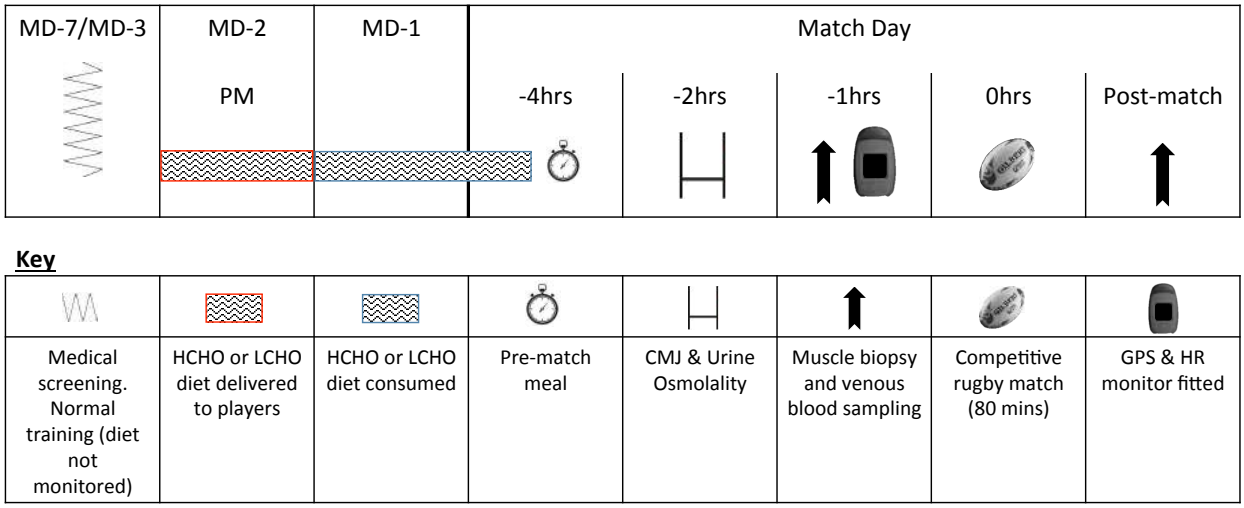

Figure 6.2. Schematic representation of the lead in to the trial and the game day timings.

Figure 6.3 – Food preparation for study 3.

Table 6.1a Example diet for 100kg player following ~3g·kg^{-1} CHO, ~1.8 g·kg^{-1} protein and ~0.7 g·kg^{-1} fat. GD = Game day.

Time		Description	Nutrition				
GD-1	GD		CHO (g)	Pro (g)	Fat (g)	kCAL	Fluid (ml)
08:00	10:00	Coco pops (100g)	85	5	2.5	387	
		Semi skimmed milk (200ml)	10	6.5	3.2	48	200
		Orange juice (200ml)	21	1	0	112	200
		Protein shake	0	20	0	118	250
		Total	**116**	**31.5**	**5.7**	**665**	**650**
10:30		Water	0	0	0	0	500
		Banana	23	1	0	105	
		Muller rice	40	6.4	4.4	218	
		Protein shake	0	20	0	118	250
		Total	**63**	**27.4**	**4.4**	**441**	**750**
13:00		Tilda flavoured brown rice (250g)	58.2	7	8.2	316	
		Chicken breast (125g)	0	29	8	130	
		Broccoli (40g)	0.5	1.5	0	44	
		Avocado (1/2 medium)	6	1.5	13.5	138	
		Water	0	0	0	0	500
		Total	**64.7**	**39**	**29.7**	**628**	**500**
15:00		One banana	23	1	0	105	
		Protein shake	0	20	0	118	250
		Water	0	0	0	0	450
		Total	**23**	**21**	**0**	**223**	**700**
17:30		Tilda flavoured brown rice (250g)	58.2	7	8.2	316	
		Chicken breast (125g)	0	29	8	130	
		Broccoli (40g)	0.5	1.5	0	44	
		Avocado (1/2 medium)	6	1.5	13.5	138	
		Water	0	0	0	0	500
		Total	**64.7**	**39**	**29.7**	**628**	**500**
21:00		Protein shake	0	20	0	118	250
		Total	**10**	**20**	**3.2**	**318**	**250**
		TOTAL	**325.4**	**177.9**	**65.1**	**2643**	**3350**

Table 6.1b Example diet for 100kg player following ~6 g·kg^{-1} CHO, ~1.8 g·kg^{-1} protein and ~0.7 g·kg^{-1} fat. GD = Game Day.

Time		Description	Nutrition				
GD-1	GD		CHO (g)	Pro (g)	Fat (g)	kCAL	Fluid (ml)
08:00	10:00	Coco pops (100g)	85	5	2.5	387	
		Semi skimmed milk (200ml)	10	6.5	3.2	48	200
		Orange juice (200ml)	21	1	0	112	200
		Bread Roll x 1	30	5	3	180	
		Strawberry Jam (15g)	9	0	0	30	
		Total	**155**	**17.5**	**8.7**	**757**	**400**
10:30		Water	0	0	0	0	250
		Banana	23	1	0	105	
		Muller rice	40	6.4	4.4	218	
		Protein shake	0	20	0	118	250
		Total	**63**	**27.4**	**4.4**	**441**	**500**
13:00		Tilda flavoured brown rice (250g)	58.2	7	8.2	316	
		Chicken breast (125g)	0	29	8	130	
		Broccoli (40g)	0.5	1.5	0	44	
		Bread Roll x 1	30	5	3	180	
		Avocado (1/2 medium)	6	1.5	13.5	138	
		Energy drink	47	0	0	187	500
		Total	**141.7**	**44**	**32.7**	**995**	**500**
15:00		Muller Rice (200g)	40	6.4	4.4	218	
		Protein shake	0	20	0	118	250
		Energy drink	47	0	0	187	500
		Total	**87**	**26.4**	**4.4**	**523**	**750**
17:30		Tilda flavoured brown rice (250g)	58.2	7	8.2	316	
		Chicken breast (125g)	0	29	8	130	
		Broccoli (40g)	0.5	1.5	0	44	
		Bread Roll x 1	30	5	3	180	
		Energy drink	47	0	0	187	500
		Total	**135.7**	**42.5**	**19.2**	**857**	**500**
		Protein shake	0	20	0	118	250
21:00		Energy drink	47	0	0	187	500
		Total	**47**	**20**	**0**	**305**	**700**
		TOTAL	**629.4**	**177.8**	**69.4**	**3878**	**3350**

6.3.3 Muscle Biopsy

A muscle biopsy was taken pre- and post-match within a 30-min period (Figure 6.4), as described in section 3.5. Following the pre-match biopsy, the incision was closed with steri-strips (Nu-Care Products, UK), and wrapped with a tegaderm dressing (Nu-Care Products, UK). This was then strapped with rugby lifting tape to prevent coming off during the game. Although the increase in inflammatory markers after a muscle biopsy have been well documented (Van Thienen et al, 2014) given our key measure was glycogen, it was decided that post-match biopsies would be taken from a new incision close to the original site (~2 cm proximal) after the same procedures.

Figure 6.4 – Widnes Vikings Players room set up for Muscle Biopsy and Blood letting. Four biopsy beds each prepared with materials necessary for tissue collection, three dewers with liquid nitrogen ready for muscle samples to be snap frozen, and a small selection of the practitioners who assisted with this study.

6.3.4 Countermovement Jump

Countermovement jump (CMJ) height was estimated from an individual's flight time using a contact timing mat (Just Jump, Probiotics Inc, Alabama) to assess lower body power. Players stood on the mat in socks or bare feet as still as possible and upright with body mass evenly distributed over both feet. Players were instructed to place hands on hips and keep them there throughout the test. The player squatted down quickly until the knees were flexed at ~90° at which point they immediately jumped vertically as high as possible before landing back on the mat. Take off and landing position was assumed to be the same, with any jumps deviating from this technique repeated (Figure 6.5). The maximum flight time (s) of the three trials was recorded and used to calculate jump height (cm) using the formula: $4.9 \times (0.5 \times \text{flight time})^2$ (Aragon, 2000). The Just jump system (JJS) is reported to provide a reliable (CV = 3.7%) and valid measurement of jump height, when compared with jump height values derived from a three-camera motion capture system ($r = 0.967$, $p < 0.01$; Thomasson and Comfort, 2012)

Figure 6.5 – CMJ being performed by Widnes Vikings player. Data collected pre- post- and 24-h-post match-play to assess lower body power.

6.3.5 Urine Osmolality

Players were asked to provide a urine sample in a 30 ml container (Sterilin universal, Sterilin, UK) upon arriving at the club and as soon as possible after the match. Samples were analysed for osmolality measured in mOsm·kg using a handheld osmometer (Osmocheck, PerformBetter, UK) which has previously been validated (Sparks and Close, 2013).

6.4 Statistics

Magnitude-based inferential statistics were employed to find differences in i) movement characteristics from the first and second halves of a competitive RL match between LCHO and HCHO groups, and ii), urine osmolality, CMJ height, muscle glycogen concentration and blood metabolites before and after a competitive RL match between LCHO and HCHO groups (see section 3.9).

6.5 Results

6.5.1 Muscle glycogen

Mean and individual muscle glycogen data are presented in Figure 6.6. Differences in muscle glycogen between HCHO and LCHO were *unclear* during the first (ES; ±90 % CL: 0.05; ±1.31) and second halves (0.65; ±1.93). Muscle glycogen *almost certainly* reduced from pre- to post-match by 45 ± 9.5 % (4.96; ±1.43) and *very likely* reduced by 38.2 ± 17.5 % (-2.08; ±1.21) for HCHO and LCHO respectively.

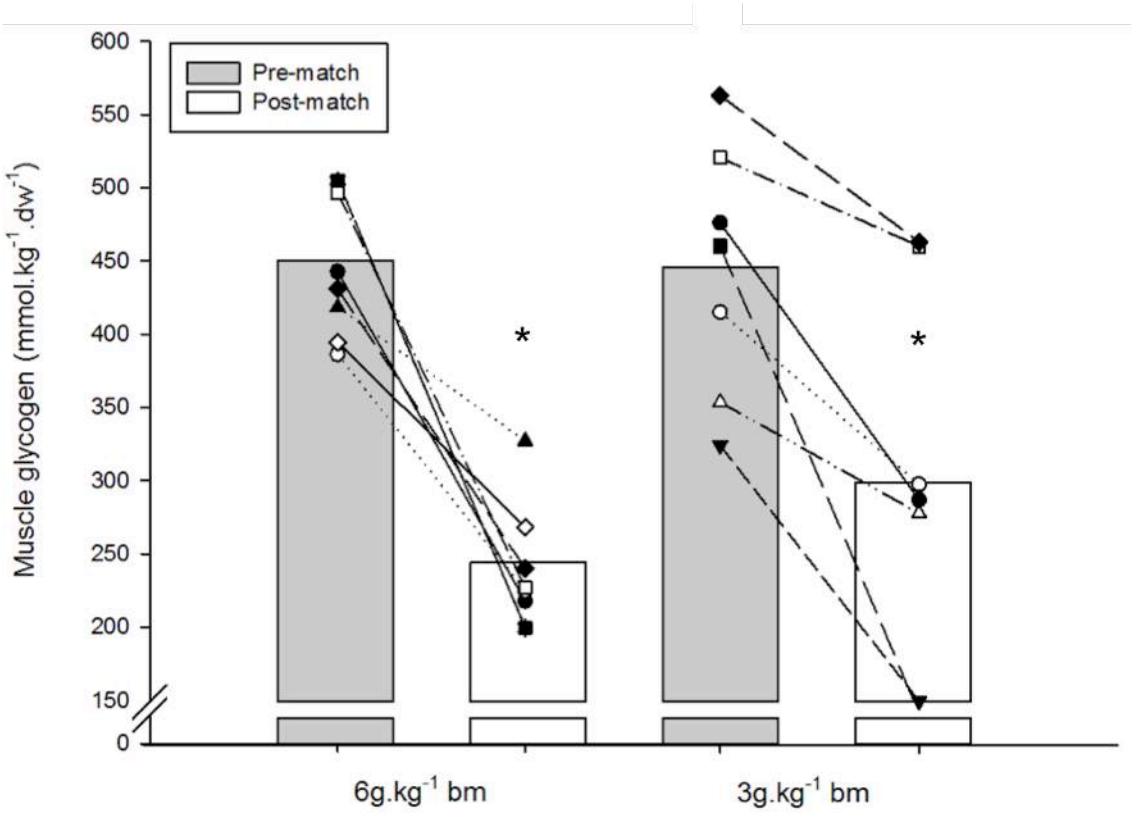

Figure 6.6. Muscle glycogen values pre- and post-competitive rugby game presented in mmol·kg^{-1} dry weight^{-1} taken from 16 professional rugby league players. Mean data (bars) and individual data (lines). Muscle samples were taken using the micro-biopsy technique.

* Indicates *almost certainly* and *very likely* different from pre-match.

6.5.2 Plasma glucose, glycerol and NEFA

Blood glucose, glycerol and NEFA data are presented in Figures 6.7a, b and c respectively. Differences in plasma glucose between HCHO and LCHO were *unclear* during the first (-1.86; ±0.96) and second halves (-0.06; ±0.77). Changes in plasma glucose from pre- to post-match were *unclear* for both HCHO and LCHO groups with a -1 ± 18.8 % reduction (-0.15; ±3.02) and 9.3 ± 18.2 % increase (1.11; ±2.07) respectively.

Differences in plasma glycerol between HCHO and LCHO were *unclear* during the first (0.4; ±0.79) and second halves (0.66; ±0.91). Plasma glycerol *almost certainly* increased from pre- to post-match by 52 ± 24.4 % (2.94; ±1.38) and by 122 ± 68 % (1.7; ±0.64) for HCHO and LCHO respectively.

Differences in NEFA between HCHO and LCHO were *unclear* during the first (0.27; ±0.74) and *likely* higher for LCHO during the second half (0.95; ±0.93). NEFA concentration *almost certainly* increased from pre- to post-match by 172.6 ± 97.3 % (1.21; ±0.42) and 226.7 ± 175.9 % (1.89; ±0.82) for HCHO and LCHO respectively.

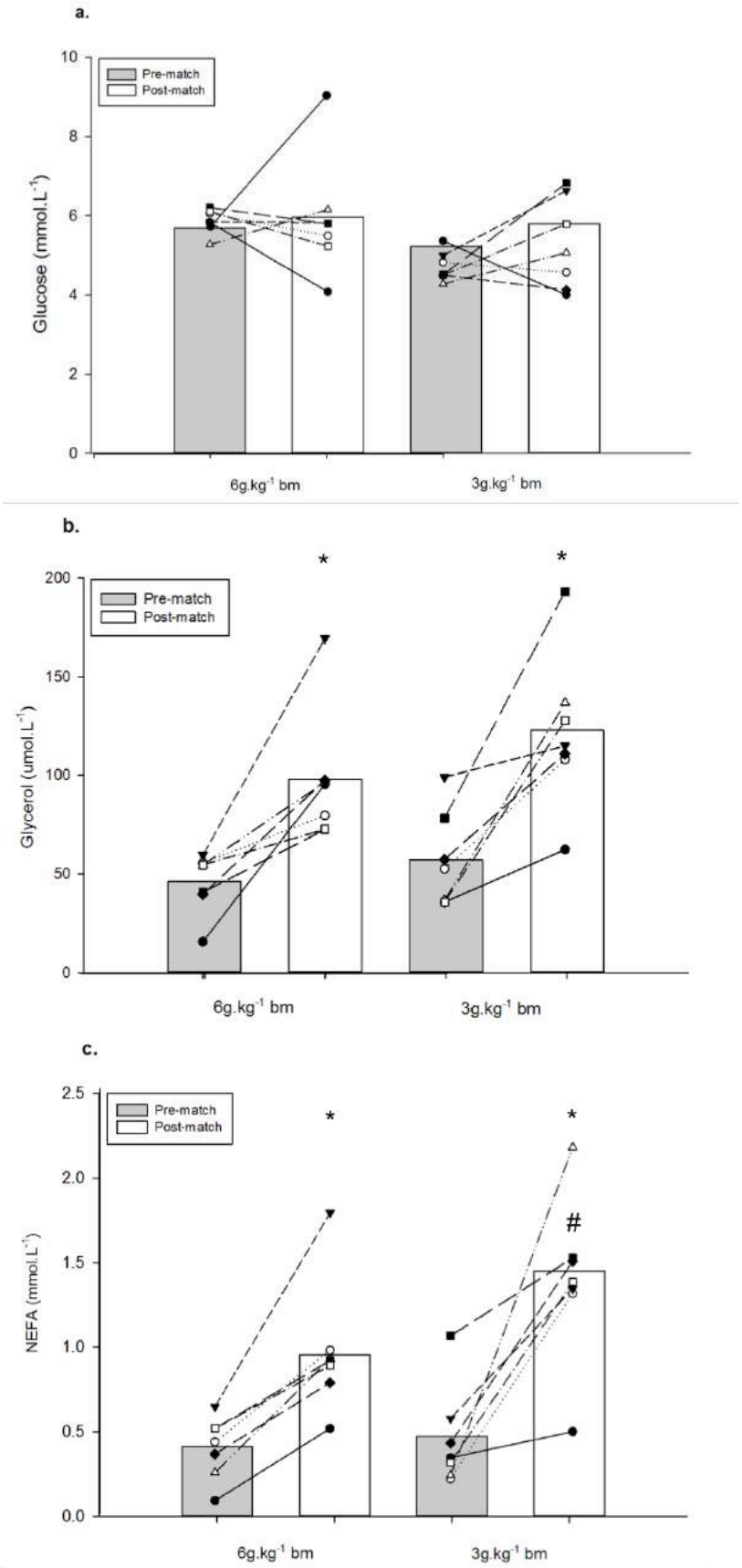

Figure 6.7a, b, c. Plasma glucose, glycerol and non-esterified fatty acid (NEFA) concentrations pre- and post-competitive rugby game presented in mmol·L^{-1}, umol·L^{-1} and mmol·L^{-1} respectively. Mean data (bars) and individual data (lines). * Indicates *almost certainly* different from pre-match. # Indicates *likely* different from 6g·kg.

6.5.3 GPS and heart rate

Differences in overall total distance relative to playing time (0.22; ±0.63), low intensity activity (-0.13; ±0.80) and high intensity running (-0.31; ±0.58) were *unclear* between LCHO and HCHO. Total distance relative to playing time ($m \cdot min^{-1}$) from the first to the second half was *unclear* in LCHO (-0.23; ±0.68) but *possibly* decreased in HCHO (-0.30; ±0.45). A change in low intensity activity from the first and second half was *unclear* in LCHO (0.04; ±0.60) and HCHO (-0.08; ±0.65). Conversely, high intensity running in the second half was *very likely* lower for LCHO (-1.42; ±0.69) and *likely lower* in HCHO (-0.55; ±0.36). Differences in percentage of heart rate peak ($\%HR_{peak}$) between HCHO and LCHO were *unclear* during the first (0.74; ±0.67) and second halves (0.01 ± 0.82). $\%HR_{peak}$ *possibly* reduced from first to second half (-0.23; ±0.15) and *likely* reduced (-0.48; ±0.63) for HCHO and LCHO respectively. First and second half movement characteristics for LCHO and HCHO are shown in Table 6.2.

Table 6.2 Mean (SD) game GPS data reported in $m \cdot min^{-1}$ for 6g·kg and 3g·kg dietary conditions. RHIE is defined as a cluster of three user defined high-intensity efforts performed <21 seconds apart (contacts, accelerations or sprints).

* Indicates *very likely*, *likely*, or *possibly* decreased from 1st half.

	$m \cdot min^{-1}$		Low Intensity $m \cdot min^{-1}$		High Intensity $m \cdot min^{-1}$		Player load$\cdot min^{-1}$		RHIE		$\%HR_{peak}$	
Half	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
6 g·kg	93.7 (12.4)	85.3 (13.1)	78.9 (8.8)	74.9 (9.2)	14.8 (5.4)	10.3* (4.3)	7.3 (3.2)	7.9 (1.8)	0.4 (0.5)	0.6 (1.2)	82.9 (6.1)	82.5* (7.5)
3 g·kg	89.4 (9.8)	86.9 (9.7)	76.7 (10.3)	77.2 (9.5)	12.7 (1.9)	9.7* (2.1)	9 (2.3)	8.2 (1.4)	1.5 (1.7)	1.6 (1.9)	81.9 (7.2)	78.4* (10.5)

6.5.4 Countermovement jump

Average height jumped by players in the HCHO group was 50.4 ± 5.7 cm pre-match, 49.4 ± 6.4 cm post-match, and 42.6 ± 5.12 cm 24-h post-match. Average height jumped by players in the LCHO group was 53.3 ± 8.9 cm pre-match, 53.3 ± 8 cm post-match, and 46.6 ± 6.7 cm 24-h post-match. Differences in CMJ between HCHO and LCHO were *unclear* pre- (0.39; ±1.05) post- (0.5; ±0.86) and 24-h post-match (0.62; ±0.86). CMJ *possibly* reduced from pre- to post-match (1.01; ±0.54) and any change was *unlikely* (0.37; ±0.57) for HCHO and LCHO respectively. CMJ *almost certainly* reduced 24-hours post-match (-1; ±0.26) and (-0.75; ±0.16) for HCHO and LCHO respectively.

6.5.5 Urine osmolality

Average urine osmolality was 354.4 ± 226.3 *cf.* 593.8 ± 299 $mOsmol \cdot l^{-1}$ pre-match, and 677.8 ± 170.2 *cf.* 685 ± 197.8 $mOsmol \cdot l^{-1}$ post-match for HCHO and LCHO respectively. HCHO were *likely more* hydrated than LCHO pre-match (0.74; ±0.67) but this was *unclear* post-match (0.01; ±0.82). Hydration *very likely* reduced from pre- to post-match by (1.01; ±0.54) and *possibly* reduced (0.37; ±0.57) for HCHO and LCHO respectively.

6.6 Discussion

The aim of the present study was to quantify the physiological and metabolic demands of RL match play in conditions of high and low CHO availability. It is reported for the first time that RL match-play can induce an approximate 40 % glycogen depletion and moreover, manipulation of energy in the form of CHO did not appear to affect resting glycogen availability, glycogen utilisation, or markers of match-play work load. Analysis of players' internal and external loads revealed these data were consistent with competitive Super League match-play intensities (Waldron et al., 2011), with mean total distance covered relative to match time of ~85-94 $m \cdot min^{-1}$ and heart rates 78-83 $\%HR_{peak}$. Interestingly, no differences were found in total, high and low intensity running distance, RHIEs, or $\%HR_{peak}$ between the HCHO and LCHO conditions during the first or second half. In the second half, there were *likely* and *very likely* reductions in high intensity running distance, for the HCHO and LCHO groups, respectively. The GPS data therefore suggest no major advantage of consuming more energy in form of CHO to enhance the high intensity running capabilities of professional RL players. This being said, a study by Sykes et al, (2011) assessed the running demands of 8 x 10-min segments of RL match-play and reported that whilst there were little differences in overall relative running speeds covered between quarters, large reductions in high and very high intensity running locomotive rates were observed in the final quarter indicating fatigue. Therefore given that information relating to more precise periods of play are missing from the current thesis chapter, it is unclear to what extent temporal match fatigue was elicited due to the dietary intervention. Furthermore, tactical changes and the ability of players to control locomotive rate means future studies using simulated match-play should be employed to confirm these findings.

Despite major differences in the match day demands between soccer and rugby, similar pre- and post-match muscle glycogen concentrations were reported (Krustrup et al., 2006). The relatively high muscle glycogen concentrations observed in the present study were achieved after CHO intakes typically ingested by players during the training week (Chapter 4; ~3 $g \cdot kg^{-1}$; 240 – 330 g CHO) and leading up to competition (Chapter 5; ~6 $g \cdot kg^{-1}$; 480 – 660 g CHO) which could be described as low or relatively high CHO diets, respectively. Alongside findings of energy balance despite 'relatively low' CHO intakes inrugby players (Chapter 5), given that previous research by Krustrup et al, (2006, 2011) showed only modest ~40% muscle glycogen utilisation during competitive soccer match-play and severe muscle glycogen did not occur after RU match-play (Jardine et al., 1988), intakes of ~3 and ~6$g \cdot kg^{-1}$ were deemed appropriate for this study. Although no dietary analysis was performed before the study, we speculate that the training and nutrition of the players earlier in the week resulted in a reasonable muscle glycogen concentration before the 36-hours CHO load leading into the game. This is pertinent given that the magnitude of muscle glycogen resynthesis is heavily influenced by the starting muscle glycogen concentration (Zachwieja et al., 1991, Price et al., 2000, Jentjens and Jeukendrup, 2003a). Indeed, it is typical in rugby for training to taper towards match day whilst CHO intake gradually increases (Chapter 5) and therefore it is feasible that some of the players started the one-day load with adequate muscle glycogen concentrations. Moreover, 3-6 $g \cdot kg^{-1}$ CHO in athletes with a high muscle mass, i.e. rugby players (Morehen et al., 2015) results in a total CHO intake of ~300-600 g and might not be considered a low CHO intake for team-sport athletes. Given that the studies used to formulate CHO intake guidelines have recruited athletes with a much lower body mass (van Hall et al., 2000, van Loon et al., 2000b), it is suggested that absolute rather than relative amounts might be more appropriate when prescribing CHO recommendations for rugby players. However, this suggestion requires further investigation. Interestingly, analysis of individual players

revealed that the group receiving ~600 g of CHO for 36-h pre-match demonstrated a more homogenous pre-match glycogen concentration. Moreover, no player in this group finished the match with glycogen concentrations <200 $mmol \cdot kg^{-1}$d.w. which has previously been reported as the threshold for impaired sarcoplasmic reticulum Ca^{2+} release rate and a potential mechanism for muscular fatigue (Ortenblad et al., 2011). In contrast, 2 players from the group receiving ~300g CHO presented with concentrations <150 $mmol \cdot kg^{-1}$d.w. post-match-play. Despite a potential decline in muscle force production impacting match-play for these 2 players, no differences were found in distance covered, high speed running, or RHIE's occurrences when compared with the 6 $g \cdot kg^{-1}$ group. This is thought to be in relation to the playing ability of these two players, both of whom train with the senior RL squad, and may therefore posess a greater ability to work under fatigue at this standard of match-play. It is therefore speculated that ~600 g of CHO may be recommended for 36-h pre-match for competitive rugby players, although future studies would be required to assess the efficacy of this suggestion as well as assessing glycogen utilisation in single fibres (Krustrup et al., 2006).

Analysis of urine osmolality revealed that those on the HCHO diet were significantly more hydrated prior to the game than those on the LCHO diet with osmolality scores of 354 and 594 $mOsmol \cdot l^{-1}$ respectively. These pre-match values are likely due to sports drinks being utilised for the HCHO dietary intervention to aid in CHO consumption despite the two groups being fluid matched. This may suggest that the sports drinks were better at maintaining euhydration than plain water, or that the players did not consume as much liquid in the LCHO group, possibly due to taste reasons. It is possible however to conclude that adding CHO drinks into a players pre-match loading strategy not only makes achieving the required CHO intake more manageable but may also help to hydrate the player prior to the game.

Analysis of the CMJ data suggested that there was no significant difference between the 2 groups in terms of recovery from the game. All players presented with significantly reduced CMJ the day after the game, which is likely due to muscle damage causing a reduction in maximal force-generating capacity (Clarkson et al., 1992, Clarkson and Sayers, 1999). This decrease in CMJ was not affected by manipulating energy in the form of CHO in the days leading into the game, confirming previous suggestions that pre-exercise CHO manipulation does not affect recovery from damaging exercise (Close et al., 2005).

Increased lipid mobilization was evidenced by large increases in NEFA and glycerol concentrations after the match for both the HCHO and LCHO group. Although there was no statistical difference between the two CHO groups, analysis of individual responses suggested that the increase in both NEFA and Glycerol were greater in the LCHO group despite similar pre-match muscle glycogen concentrations. Considering the intermittent nature of a rugby match, elevated lipid oxidation, despite what would appear sufficient muscle glycogen concentrations even at the end of the match, might reflect periods of low intensity activity (walking or jogging) and those unique to rugby such as the rest period after a try is scored, penalty kicks, setting up a scrum, and when the ball was out of play. Interestingly, pre- and post-match NEFA concentrations were similar to those observed in football (Krustrup et al., 2006) despite differences in match-play activity. Combined with the similar reductions in post-match muscle glycogen, these data suggest that RL and football might possess similar metabolic demands. The reduced lipid mobilisation in the HCHO group may also suggest CHO oxidation was enhanced in this group which could add further weight to suggestion that ~600 g is the preferred CHO dose leading into a game.

6.7 Summary

This study has for the first time attempted to quantify the metabolic demands of professional RL match-play whilst manipulating energy in the form of CHO. It is reported that competitive RL match can result in ~40 % muscle glycogen depletion and that match-day performance variables did not differ between the 6 $g \cdot kg^{-1}$ or 3 $g \cdot kg^{-1}$ CHO conditions. However, further analysis suggested that the higher CHO intake results in a more homogenous pre-match glycogen concentration between the players, and no player in this group presented with concentrations <200 $mmol \cdot kg^{-1}$d.w post-match-play. Therefore, despite no differences in movement characteristics between the low and the high CHO groups, it is postulated that an absolute amount of ~600 g CHO 36-h pre-match is recommended strategy for rugby league players. Future studies might wish to further titrate these CHO recommendations as well as assessing the effects of the timing of CHO intake on the magnitude of glycogen repletion after rugby match-play. To reliably assess the magnitude of muscle glycogen repletion, it would be necessary for participants to finish exercise with similar muscle glycogen concentrations in order for any dietary intervention to be meaningful. It may be necessary therefore to perform rugby exercise under controlled conditions, utilizing a simulated rugby match.

CHAPTER 7

METABOLIC DEMANDS AND REPLENISHMENT OF MUSCLE GLYCOGEN AFTER SIMULATED RUGBY MATCH PLAY

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7.1 Abstract

Although the physical and metabolic demands of Rugby league (RL) match-play are well-known, current nutritional guidelines for recovery may not be appropriate given the damaging nature of RL. The metabolic requirements of a rugby league match simulation protocol were examined alongside the timing of carbohydrate provision on glycogen resynthesis in damaged muscle. In a randomized pairs design, fifteen (mean ± SD: age 20.9 ± 2.9 yrs, body-mass 87.3 ± 14.1 kg, height 177.4 ± 6.0 cm) male university RL players consumed a 6g·kg·day^{-1} CHO diet for 7-days, completed a time to exhaustion test (TTE) and a glycogen depletion protocol on day-3, followed by a Rugby League simulated-match protocol (RLMSP) on day-5 and a TTE on day-7. Players were randomly prescribed either an immediate or delayed (2-h-post) re-feed post-simulation. Muscle biopsies and blood samples were obtained post-depletion, before and after simulated match-play, and 48-h after match-play with PlayerLoad and heart-rate collected throughout the simulation. Data were analysed using effects sizes ± 90% CI and magnitude-based inferences. PlayerLoad (8.0 ± 0.7 AU·min-1) and %HRpeak (83 ± 4.9%) during the simulation were similar to values reported for RL match-play. Muscle glycogen *very likely* increased from immediately after to 48-h post-simulation (272 ± 97 *cf.* 416 ± 162 mmol·kg^{-1}d.w.; 0.88 ± 0.66) after immediate re-feed, but changes were *unclear* (283 ± 68 *cf.* 361 ± 144 mmol·kg-1d.w.; 0.7 ± 1.23) after delayed re-feed. CK *almost certainly* increased by 77.9 ± 25.4% (0.75 ± 0.19) post-simulation for all players. TTE performance revealed no difference between conditions. The RLMSP displayed player loads comparable to professional RL match-play, although difficulties in replicating physicality reduced glycogen utilisation. Further, it is possible to replete muscle glycogen in damaged muscle employing an immediate re-feed strategy.

7.2 Introduction

In chapter 6 it was reported that a competitive RL match can result in ~40 % muscle glycogen depletion and that a higher CHO intake (~600 g) results in a more homogenous pre-match glycogen concentration when ingested 36-h prior to match-play. Although these dietary intakes appear to be appropriate for rugby competition, key information is missing for us to make accurate dietary recommendations for recovery after rugby competition. Furthermore, studies to the examine the metabolic requirements imposed on players are challenged by the large inter-match variations in movement characteristics observed in RL match play (Kempton et al., 2014). Match score, posession, and tactical decisions, can heavily influence match-play activities such as high speed running or contacts (Twist et al., 2014), and therefore muscle glycogen utilisation. Moreover, dietary intakes in the days leading up to match-play have been shown to cause variability in pre-match concentrations (Chapter 6), further augmenting the lack of control and difficulty in assessing any meaningful effect from an intervention. Accordingly, a case for utilising a standardised approach such as a simulated RL match-play protocol replicating the physiological demands and movement patterns of real match-play, might be appropriate to permit greater control whilst conducting dietary intervention studies. Observations from a RL simulation (Sykes et al., 2013) reported similar total distance covered and $\%HR_{peak}$ to elite RL match-play (Waldron et al., 2011), suggesting an accurate reflection of real match movement demands. However, the metabolic demands and glycogen utilisation of this RL simulation remain unknown and warrant investigation.

Despite high volumes of eccentric muscular contraction present throughout real or simulated RL match-play through repeated bouts of acceleration, deceleration, rapid changes in direction, and impacts (McLellan et al., 2011a), it is currently unknown how the resulting muscle membrane damage (Proske and Morgan, 2001) affects muscle glycogen

repletion in the following days. Furthermore, inflammatory cells present within damaged muscle have an affinity for glucose oxidation, competing with glycogen depleted muscle cells for blood glucose (Costill et al., 1990), resulting in a reduction in glycogen synthesis.

Therefore the aims of the present study were to 1) examine the metabolic demands of a simulated RL match and compare with previously published data from professional RL match play and 2), assess the efficacy of an immediate or delayed CHO re-feed on muscle glycogen resynthesis in damaged muscle after a simulated match. Accordingly, this study assessed university RL players who performed a Rugby League match simulation protocol (RLMSP; Sykes et al., 2013), after which consuming either an immediate or delayed re-feed. It is hypothesised that 1), the simulated match would result in similar muscle glycogen utilisation to that previously reported in a professional RL match (Chapter 6), 2), the immediate re-feed would result in greater muscle glycogen resynthesis compared with the delayed re-feed and 3), with a correct re-feeding strategy it will be possible to replenish a damaged muscle after a simulated rugby match.

7.3 Methodology

7.3.1 Players

Fifteen university RL players (mean ± SD: age 20.9 ± 2.9 years, body-mass 87.3 ± 14.1 kg, height 177.4 ± 6.0 cm) playing in British Universities and College Sports (BUCS) Northern 1A league volunteered to take part in this study (Figure 7.1). Four of the players had previously played for Super League academies and all players were experienced players. Players were medically screened for the suitability of the use of prescription anaesthetic Marcain by a trained medical practitioner. Measurements included; mean arterial pressure using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, FL), height and body mass (SECA, Hamburg, Germany) and a pre-biopsy questionnaire. Ethical approval was granted by the local ethics committee of Liverpool John Moores University.

Figure 7.1 – Group 1 with Soulmate food delivery, comprised of 6g·kg·day^{-1} CHO, 2 g·kg·day^{-1} protein and 1g·kg·day^{-1} fat according to player body weight.

7.3.2 Experimental design

In a matched pairs design and using a random number generator (body mass used to match pairs), players were randomly allocated into one of two groups, those being immediate re-feed or delayed re-feed. Testing took place over two separate weeks in an indoor training facility to ensure an identical playing surface and weather conditions. Over a 7-day period, players consumed a standardised diet and completed; a time to exhaustion test (TTE) followed by a glycogen depleting protocol, a Rugby League match simulation protocol (RLMSP) to deplete muscle glycogen (Sykes et al., 2013), and a final TTE (Figure 7.2). Muscle biopsies and blood were collected post-depletion, pre- and post-simulation, and 48-h post-simulation and analysed (see sections 3.5 - 3.8). Muscle biopsies were taken in a randomised order from alternate legs at each time point, with the second incisions on each leg made close to the original site (~2 cm proximal). Due to technical issues, blood samples were not collected on 5 players therefore all performance, HR and muscle biopsy data are reported as *n*=15, whereas bloods data are presented on *n*=10.

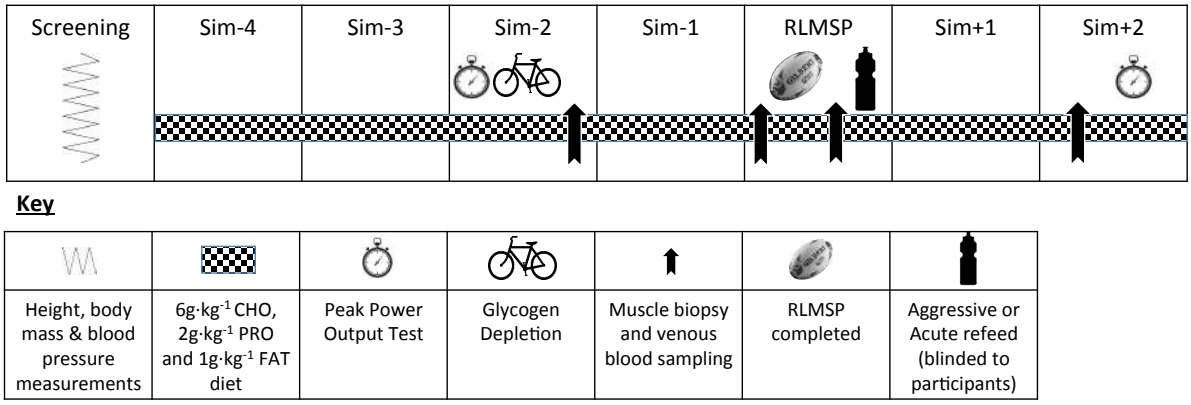

Figure 7.2. Schematic representation of screening process, testing protocols, muscle biopsy and venous blood sampling and dietary intervention, all expressed as days away from simulation (sim-4, -3, -2, RLMSP, -1, +1, +2).

7.3.3 Dietary Intervention

Individualised 7-day diets were designed and provided to all participants comprised of $6g \cdot kg \cdot day^{-1}$ CHO, $2\ g \cdot kg \cdot day^{-1}$ protein and $1g \cdot kg \cdot day^{-1}$ fat according to body weight, with habitual diet ingested prior to intervention (Table 7.1). Although some may not consider $6g \cdot kg \cdot day^{-1}$ CHO to be a 'high' intake, this has previously been shown to adequately elevate muscle glycogen for rugby competition (Chapter 6). Moreover, these intakes are typically ingested by rugby players during the training week and leading up to competition (Chapter 5). All meals were designed and distributed to the players by a SENr accredited practitioner and prepared by catering food and drink supplier 'Soulmate Food' complete with meal plans. All players were given strict instructions to follow the diet explicitly, not to consume any foods or liquids (apart from water) other than what was provided, and to finish all meals provided. Players self-reported that they had strictly adhered to the diets prescribed to them. All supplements prescribed were informed sport.

Table 7.1. Sample diet designed by Soulmate food for one of the players (66kg). ~6g·kg·day^{-1} CHO, 2 g·kg·day^{-1} protein and 1g·kg·day^{-1} fat.

		Meal	Macronutrient Values CHO (g)	PRO (g)	Fat (g)
Saturday	Lunch	Pork Gyoza with Thai Wild Rice Salad	120.9	31.8	15.8
	P.M. Snack	Gingerbread Fudge with Chocolate Protein Shake	24.2	25.9	18.5
	Dinner	White Fish & Wild Mushroom Miso Broth with Rice Noodles	105.2	31.2	8.6
		Daily Intake	**250.3**	**88.9**	**42.9**
Sunday	Breakfast	Cinnamon & Dried Fruit Muesli	95.8	27.9	12.3
	A.M. Snack	Honey Chicken, Pitta & Yuzu Dip	39.4	31.5	19.3
	Lunch	Chicken, Roast Squash & Millet Salad	103.7	34	12.2
	P.M. Snack	Cinnamon, Orange & Ginger Flapjack with Vanilla Protein Milk	64.5	25.1	16.3
	Dinner	Hungarian Pulled Beef Goulash with Bulgur Wheat	95.8	37.2	12.1
		Daily Intake	**399.2**	**155.7**	**72.2**
Monday	Breakfast	Orange and Cranberry Overnight Oats	123	30.1	16.7
	A.M. Snack	Rosemary Beef Bites with Artichoke Dip	11.4	32.6	14.9
	Lunch	Thai Chilli and Kale Soup with Chipotle Chicken Wrap with Bagel	126.7	36	14.9
	P.M. Snack	Banana & Blueberry Protein Muffin	38.1	25.6	14.8
	Dinner	Lamb Stew with Baby Roast Potatoes	90	28.6	10.7
		Daily Intake	**389.2**	**152.9**	**72**
Tuesday	Breakfast	Cranberry & Coconut Muesli	92.3	31.2	17.2
	A.M. Snack	Blueberry & Oat Protein Bar	60	22.8	6.1
	Lunch	Roast Salmon & Mango Quinoa Salad with Coconut Dressing	96	39	20.7
	P.M. Snack	Cinnamon, Orange & Ginger Flapjack with Milk	62	25.1	20.6
	Dinner	Prawn & Purple Potato Laksa with Extras	93.7	30.1	6.4
		Daily Intake	**404**	**148.2**	**71**
Wednesday	Breakfast	Super Berry Muesli	102.1	31.3	11.9
	A.M. Snack	Za'atar Chickpea Dip with Oatcakes & Chicken Bites	43.2	28.9	10.1
	Lunch	Deli Couscous Salad with Chicken	106.4	41.4	31.9
	P.M. Snack	Mint Chocolate Bar with Protein Shake	56.8	25.6	18.6
	Dinner	Gammon & Pineapple with Turmeric Noodles	84.6	29.6	5.2
		Daily Intake	**393.1**	**156.8**	**77.7**
Thursday	Breakfast	Almond, Goji & Chocolate Muesli with Milk	110	31.1	9.9
	A.M. Snack	Beetroot, Apple & Ginger Flapjack with Strawberries & Cream Protein Shake	39.8	34.9	13.7
	Lunch	Deli Couscous Salad with Chicken	106.4	45.4	31.9
	P.M. Snack	Pine Nut & Sunblush Tomato Hummus with Pitta & Chicken Bites	50.7	17.8	13.6
	Dinner	Steak & Chips with Chimichurri & Rice	96.6	32.8	8.1
		Daily Intake	**403.5**	**162**	**77.2**
Friday	Breakfast	Berry Coconut Porridge	86.5	21	21.7
	A.M. Snack	Red Velvet Protein Cupcake with Smoothie	40.8	29.7	15.9
	Lunch	Spiced Squash Soup with Ras el Hanout Chicken Wrap & Bagel	105.6	43.5	7.4
		Daily Intake	**232.9**	**94.2**	**45**

7.3.4 Post exercise re-feed

Players were randomly allocated to one of two dietary re-feed conditions; immediate (IM) or delayed (DE) ingestion of CHO post-RLMSP. IM group consumed a beverage containing 90 g CHO (My Protein Maltodextrin) and 30 g PRO (My Protein True Whey), followed by a further 90 g CHO (My Protein Maltodextrin) beverage 1-hour later. DE group consumed visually identical beverages containing 30 g PRO (My Protein True Whey), followed by a zero calorie hypotonic sports drink (ASDA zero sport) 1-hour later, and all participants continued with prescribed diets 2-h post-simulation. Post-exercise drinks were delivered as an absolute, rather than relative dose of CHO and protein given that there is currently no consensus as to the optimal amount of CHO and protein post rugby match-play.

Table 7.2 Post-RLMSP re-feed intervention. Note – only difference in re-feed was the 2 x 90g CHO (square box).

	Immediate	**Delayed**
Days 1-7	6 g·kg CHO^{-1} 2 g·kg PRO^{-1} 1 g·kg FAT^{-1}	6 g·kg CHO^{-1} 2 g·kg PRO^{-1} 1 g·kg FAT^{-1}
Post-simulation	90 g CHO + 30 g PRO	0 g CHO + 30 g PRO
+1-h	90 g CHO	0 g CHO
+2-h	Continued prescribed diet	Continue prescribed diet

7.3.5 Time to exhaustion test

Players performed a maximal incremental cycling test to volitional fatigue on a Lode ergometer (Daum Electronic Premium 8i, Furth, Germany) for determination of peak power output (PPO) and time to exhaustion (TTE). Tests were started every 5-min with players in different areas of the laboratory with partitioning screens to ensure that there was no competition bias. The maximal incremental protocol commenced at 150 W for 2-min, with work rate increased by 30 W every minute thereafter until exhaustion (Hawley, Noakes, 1992). TTE was recorded as a measure of performance, and the highest power output (W) attained used to inform exercise intensity during the glycogen depletion protocol. After the TTE test, players were given 15-min to rest before beginning an intermittent glycogen-depleting cycling protocol (Pedersen et al., 2008).

7.3.6 Glycogen depleting protocol

The purpose of the glycogen depletion was to reduce muscle glycogen, followed by 48-hr of controlled CHO intake to ensure that all players commenced the RLMSP with comparable muscle glycogen. Pre-exercise muscle glycogen concentrations are shown to be extremely variable between participants without this pre-trial depletion (Chapter 6). An adapted version of the protocol used by Pedersen et al, (2008) was used for the glycogen depleting exercise protocol (Figure 7.3). After a 5-min warm-up at a self-selected intensity, participants commenced cycling at 90 % of PPO for 2-min followed immediately by 1-min of an active recovery at a self-selected intensity. This work-recovery protocol was maintained until the participants were unable to complete 2-min at 90 % PPO, determined as an inability to maintain a cadence of 60 rpm for 15 s. When players could not maintain 2-min at 90 %, the work bouts were reduced to 1.5 min, while maintaining the 1-min

recovery bouts at a self-selected intensity. When players were unable to maintain the 1.5-min at 90 %, the work period was reduced to 1-min. Once the participants could not maintain 90 % of PPO for 1-min, the intensity was lowered to 80 %, 70 % and finally 60 % of PPO following the same work to rest pattern. Exercise was terminated when players could not complete 1-min of cycling at 60 % of PPO at a cadence of 60 rpm. This protocol was chosen so as to maximally deplete muscle glycogen in both Type I and Type II muscle fibres and in an attempt to standardize muscle glycogen concentration between participants (Kuipers, Keizer, Brouns, Saris 1987). Verbal encouragement was given and water was consumed *ad libitum* throughout exercise.

Figure 7.3 – Glycogen depleting protocol using an adapted version of the protocol used by Pedersen et al, (2008) on Lode ergometer (Daum Electronic Premium 8i, Furth, Germany). Blue partitioning screens used during time to exhaustion test (TTE) to avoid competition bias. LJMU Physiology labs.

7.3.7 Rugby League Match Simulation Protocol

An adapted version of the protocol used by Sykes et al, (2013) was used for the RLMSP (Figure 7.4). The movements for the protocol are based on the mean locomotive speeds and activities of whole-match players established during competitive RL matches (Sykes et al., 2011). The protocol lasted for 92 min (2 x 46 min separated by 10-min to simulate half-time), replicating the mean time that a player spends on a pitch including stoppages. The protocol comprises 40 identical cycles lasting 2-min 18 s. Each cycle comprised Part A (a 44.4 s cycle performed twice), designed to replicate typical ball in play movement patterns and Part B (a 49.3 s cycle performed once), designed to replicate typical ball out of play movements. The specific movements are as follows:

Part A:

- 10.5 m jog (2.9 m s^{-1}) from yellow to red cones followed by 180° turn;
- 10.5 m walk (1.1 m s^{-1}) from red to yellow cones followed by 180° turn;
- 20.5 m maximal effort sprint from yellow to blue cones;
- 8 m deceleration to white cone followed by alternate: Contact with tackle bag, 4s wrestle bag to the left and right, stand bag back up OR simulated contact (down and up off the ground). *These alternated movements comprise the only adaptation to the protocol*.
- 13 m jog (2.9 m s^{-1}) from white to green cones;
- 15.5 m walk (1.1 m s^{-1}) from green to yellow cones.

Part B

- 10.5 m walk (1.1 m s^{-1}) from yellow to red cones followed by 180° turn;
- 10.5 m walk (1.1 m s^{-1}) from red to yellow cones followed by 180° turn;
- 6.00 s passive rest at yellow cone;
- 15.5 m jog (2.9 m s^{-1}) from yellow to green cone followed by 180° turn;
- 15.5 m walk (1.1 m s^{-1}) from green to yellow cones;
- 4.75 s passive rest at yellow cone.

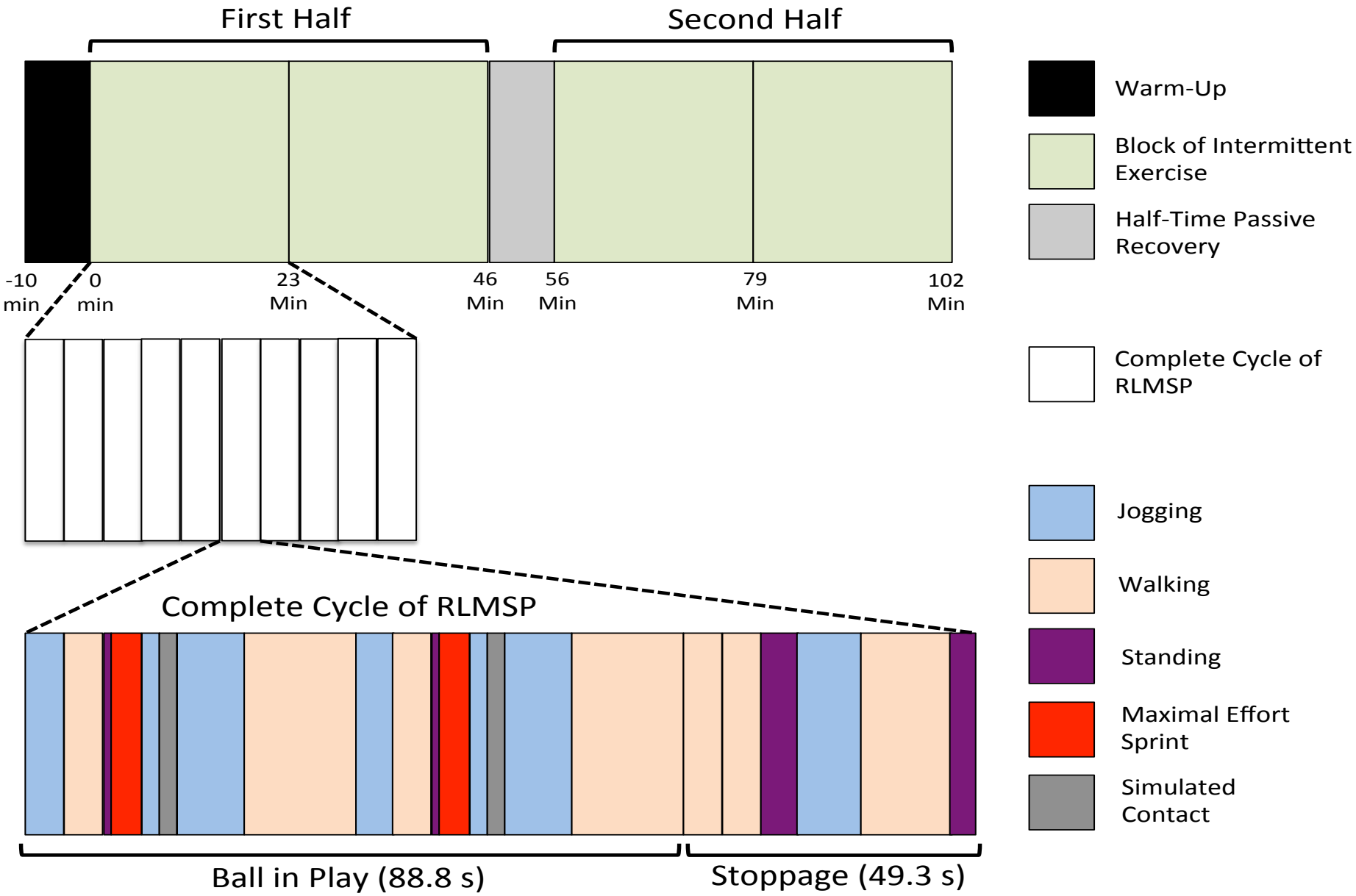

Figure 7.4. Schematic representation of the exercise pattern of the rugby league match simulation protocol adapted from Sykes et al, (2013).

Participants move between a series of cones positioned on a 28.5 m linear course (Figure 7.5) at locomotive speeds determined from the analysis of activity patterns in senior elite rugby league matches (Sykes et al., 2011). The locomotive speeds are dictated by an audio CD, with changes being signaled by a ‘‘beep’’ and an instructive voice command, such as, ‘‘Jog to white’’ (cone).

To replicate collisions during match-play, participants were required to alternate between tackling a tackle bag and wrestling the bag from left to right, or lying prone on the floor with their waist level with the white cones and chest on the floor, to hold for 3 s and then regain their feet as rapidly as possible at the next beep (Figure 7.6). These movements were included in an attempt to replicate the physical exertion of contacts yet still control the reproducibility of the protocol. Although participants performed this movement more frequently than contacts in the competitive match, the percentage of total time spent performing this movement was similar to that observed in contact during a match (4.2 ± 1.9%; Sykes et al., 2009).

Players performed the protocol in 2 groups (n=8 and n=7; using lanes laid out side-by-side) and verbal encouragement was provided throughout each trial. Accumulated PlayerLoad™ (AU), heart rate, perceived exertion (RPE) and sRPE was collected as described in chapter 3.3.

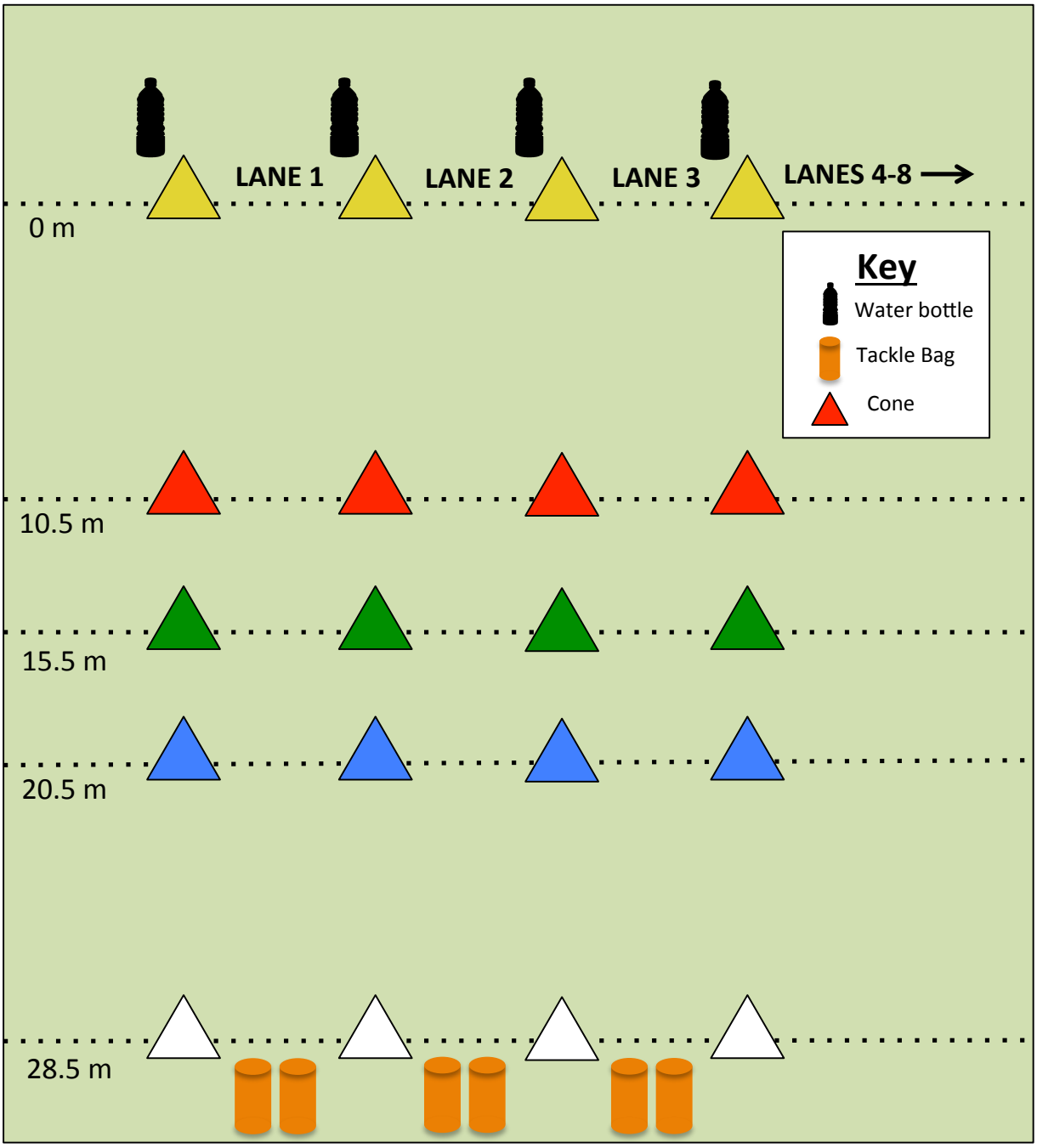

Figure 7.5. Schematic representation of the layout of the testing area used for the rugby league match simulation protocol (Sykes et al., 2013).

Figure 7.6 – Rugby League Match Simulation Protocol in motion, players performing a tackle during Part A of the simulation. Warrington Wolves indoor training facilities.

7.4 Statistics

Magnitude-based inferential statistics were employed to find differences in i) muscle glycogen concentrations and blood metabolites before and after rugby match-play and 48-hours after rugby match-play and ii), TTE scores between the pre- and re-test between immediate and delayed dietary groups (see section 3.9).

7.5 Results

7.5.1 Player load, sRPE and heart rate

Differences in PlayerLoad™ during the RLMSP were *unclear* (2 ± 9%, ES; ±90% CI; 0.02; ±0.01) between the immediate (7.3 ± 0.43 AU·min^{-1}) and delayed re-feed (8.0 ± 0.33 AU·min^{-1}) groups. Similarly, differences in %HR_{peak} (84 ± 4 *cf.* 82 ± 6%; 2 ± 6 %, 0.03; ±0.08) and sRPE (407 ± 105 *cf.* 458 ± 124; 7 ± 11 %, 0.54; ±0.85) were *unclea*r between immediate and delayed re-feed groups, respectively.

7.5.2 Muscle glycogen

Muscle glycogen data are reported in Figure 7.7. All players sufficiently depleted muscle glycogen after the depletion protocol (<50 mmol·kg^{-1}d.w.) and mean muscle glycogen concentrations were relatively high before commencing the simulation after following the standardized diet (337 *cf.* 376 mmol·kg^{-1}d.w. in immediate and delayed re-feed groups, respectively). Muscle glycogen concentrations were decreased immediately after the simulation by 25 ± 14% (-1.38; ±0.90, *very likely*) and 24 ± 12% (-1.6; ±0.92, *very likely*) in the immediate and delayed re-feed groups, respectively. Muscle glycogen concentrations were increased 48-h after the simulation by 51 ± 47% (0.88; ±0.66, *very likely*) in the immediate re-feed but only 24 ± 49% (0.7; ±1.23, *unclear*) in the delayed re-feed group.

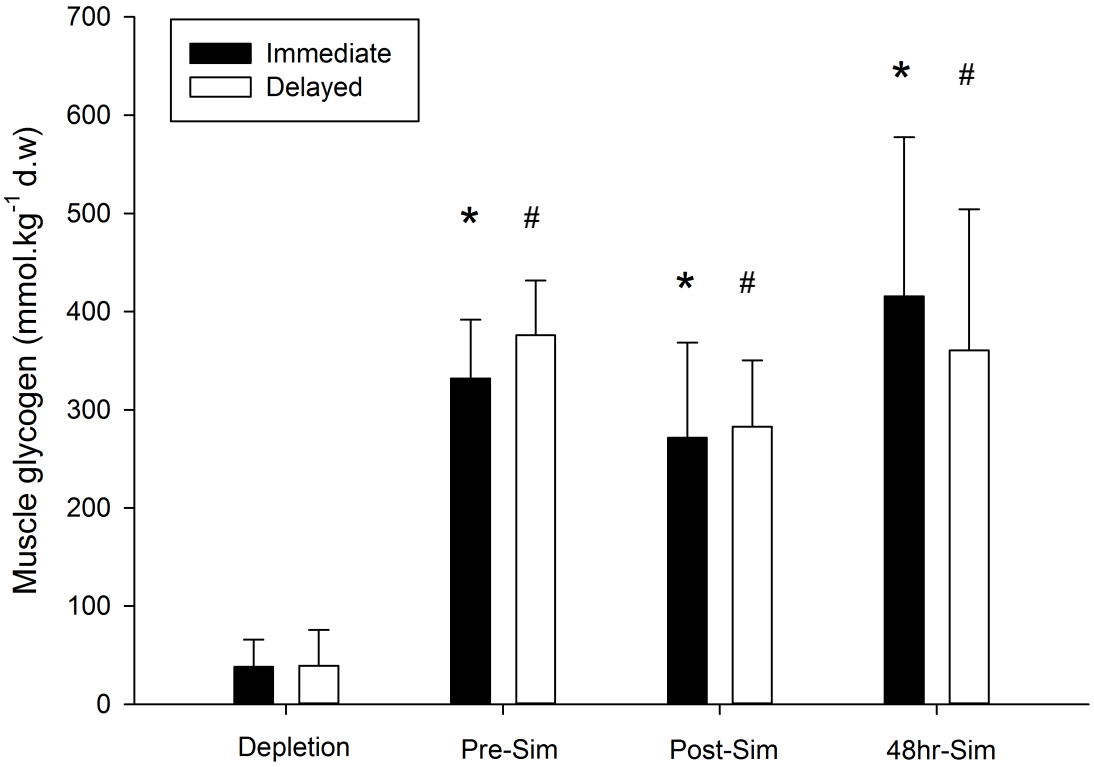

Figure 7.7 - Muscle glycogen concentrations at four time points: Depletion, Pre- and Post-simulation, and 48-h Post-simulation presented in mmol·kg^{-1} d.w. taken from 15 university rugby league players. * = difference in immediate group from previous time point. # = difference in delayed group from previous time point.

7.5.3 Serum glycerol, NEFA and CK

Changes in blood metabolites (plasma glycerol, NEFA and CK) are reported in Figure 7.8. Glycerol concentrations were increased after the simulation by 60 ± 60% (0.78; ±0.52, *likely*) and 103 ± 23% (4.13; ±0.76, *almost certainly*) in the immediate and delayed re-feed group, respectively. Glycerol concentrations were *unclear* between groups before (0.42; ±1.26) and after the simulation (-0.32; ±1.47), although the delayed group presented higher concentrations than the immediate re-feed group 48-h after (1.36; ±0.87, *very likely*). NEFA concentrations were increased after the simulation by 1049 ± 581% (2.01; ±0.40, *almost certainly*) and 998 ± 1060% (2.4 ± 0.86, *almost certainly*) in the immediate and delayed re-feed group, respectively. Differences between groups in NEFA concentrations were *unclear* before (0.29; ±0.76), immediately (0.10; ±0.87), and 48-hr after the

simulation (0.58; ±0.92). CK concentrations were increased after the simulation by 128 ± 43% (0.60; ±0.18, *very likely*) and 84 ± 41% (0.63; ±0.23, *very likely*) in the immediate and delayed re-feed group, respectively. CK concentrations remained higher but decreased at 48-h after by 29 ± 38% (-0.42; ±0.61, *likely*) and 30 ± 20% (-0.55; ±0.43, *likely*) in the immediate and delayed re-feed groups, respectively. Differences between groups in CK concentrations were *unclear* before (-0.4; ±0.9), after (-0.38; ±0.88), and 48-h after the simulation (-0.24; ±0.6).

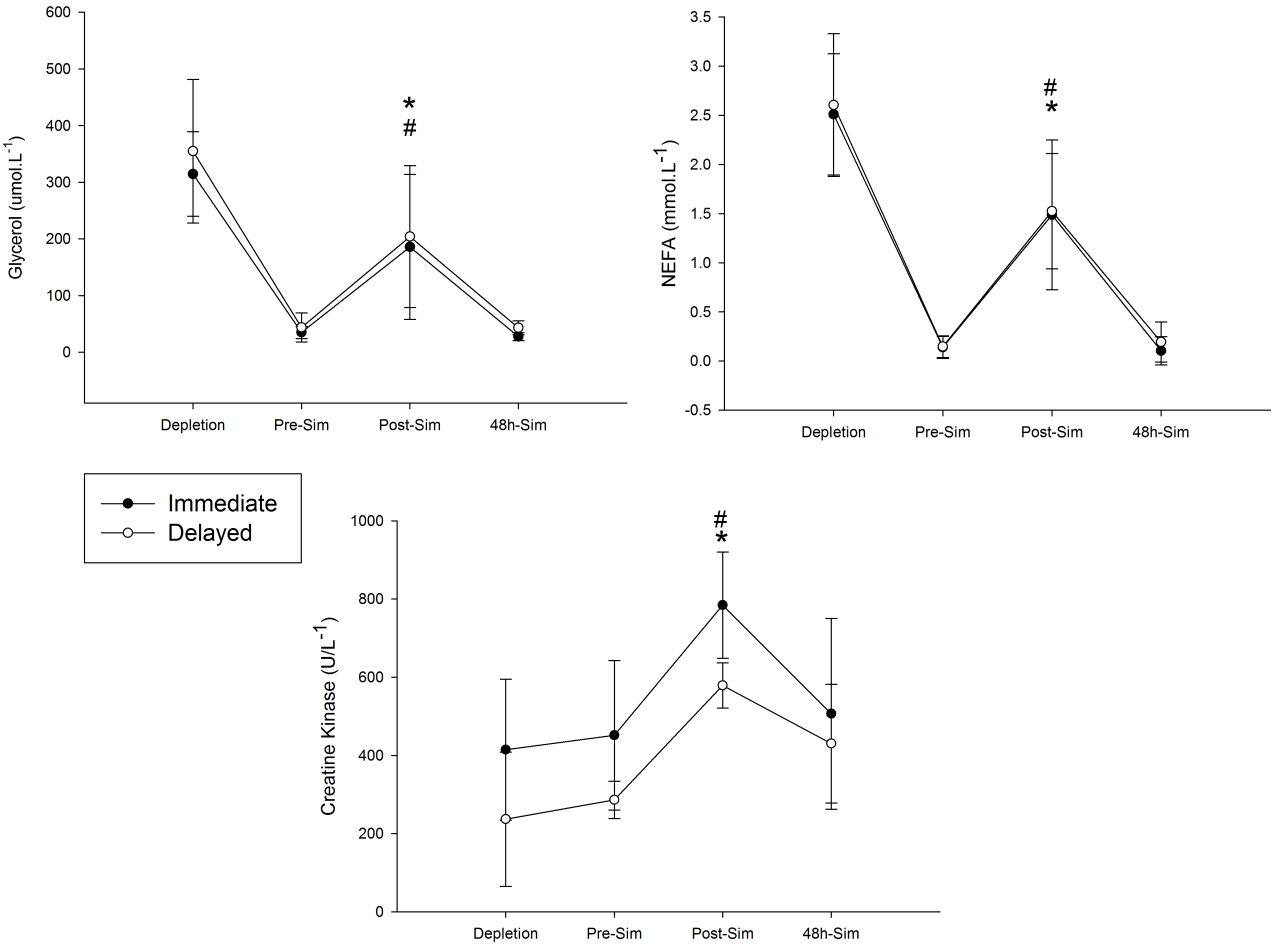

Figure 7.8 - Plasma glycerol, non-esterified fatty acid (NEFA), and creatine kinase (CK) concentrations at four time points: Depletion, Pre- and Post-Simulation, and 48-h Post-simulation presented in umol·L^{-1}, mmol·L^{-1} and U·L^{-1} respectively. * = difference in immediate group from previous time point. # = difference in delayed group fro previous time point.

7.5.4 Time to exhaustion test

TTE decreased from 671 ± 127 s to 586 ± 202 s (-14 ± 11%; -0.66; ±0.54, *likely*) in the immediate re-feed group and 611 ± 171 s to 564 ± 202 s (-10 ± 7% (-0.35; ±0.28, *likely*) in the delayed re-feed group. Differences in TTE between groups were *unclear* for the initial (-10 ± 19 %; -0.47; ±0.92) and follow up assessment (-11 ± 25 %; -0.34; ±0.85).

7.6 Discussion

The aim of the present study was to assess the metabolic demands and glycogen utilisation of a simulated RL match and assess glycogen resynthesis up to 48-h afterwards. This chapter provides novel data demonstrating that 1) simulated match-play utilises ~50% less muscle glycogen than previously reported for professional RL match-play. This is likely because of the difficulties in replicating physical collisions and exertions during the simulation; 2) a *very likely* increase in muscle glycogen repletion was observed when CHO was ingested immediately post-exercise compared with an *unclear* difference following the delayed re-feed; 3) the increase in muscle glycogen concentration occurred despite elevated plasma CK suggesting muscle damage has resulted from the simulated activity therefore implying that it is possible to acutely replete muscle glycogen in a damaged muscle following a simulated rugby performance.

Muscle glycogen was depleted by ~21% during the simulation, which is less than previously reported in RL match-play (~40%; Chapter 6). A lower glycogen depletion occurred despite heart rate (~83 $\%HR_{peak}$) and PlayerLoad (~7.7 $AU \cdot min^{-1}$) during the simulation being consistent with the loads reported in competitive matches (Waldron et al., 2011, Gabbett, 2015). Furthermore, analysis of blood metabolites revealed comparable

rates of lipid mobilization to those reported in elite RL match-play (Chapter 6), suggesting that the RLMSP successfully reflected the intermittent nature of the sport. That PlayerLoad was similar to actual match-play, despite the lack of true physical collisions in the simulation, is probably explained by the higher movement speeds in the simulation protocol (Sykes et al., 2013). The lower glycogen depletion in the simulation is likely a reflection of an inability to replicate the physicality of collisions in matches, with tackles and wrestling movements performed on a passive 30 kg tackle bag rather than an opposing player. Contacts sustained during RL match-play result in players experiencing a large magnitude of physical exertions (McLellan et al., 2011a, Twist et al., 2012), which are further compounded during the subsequent wrestle with an opposing ~100kg player. It is speculated therefore that these frequent high intensity contacts would result in significant muscle glycogen utilisation that was not reflected in this simulation. The smaller magnitude of increase in CK activity (2 to 3-fold, ~500-800 U/L) when compared to RL (~4-fold, >1000 U/L; Twist et al., 2012) and RU (~4-fold, 700-1200 U/L; Jones et al, 2014) match-play which are speculated to be in relation to numerous physical contacts, also suggest that the simulation was unable to replicate the collision. These findings reaffirm the difficulties in simulating physical contact (Norris et al., 2016) and, more importantly, highlight the large metabolic cost of the tackle in RL. Future study should now attempt to increase the physicality of the RLMSP to more accurately reflect the physical demands of rugby match-play.

The immediate re-feed elicited an *almost certain* increase (~53%) in muscle glycogen concentration 48-h after the simulated match, whereas the difference was *unclear* (~27%) in players who consumed the delayed re-feed. This large discrepancy highlights the importance of the short-lived non-insulin dependent phase for rapidly resynthesizing muscle glycogen after exercise in the presence of CHO (Jentjens and Jeukendrup, 2003b,

Price et al., 1994). Furthermore, while not reported in the literature, anecdotal evidence demonstrates that players struggle to consume food in the immediate period post-match and often do not compensate for this in the following days. The accumulative effect of this might lead to improper recovery and players dropping body mass throughout the competitive season, accentuating the importance of an appropriate nutrition strategy for recovery. Differences in glycogen repletion might also be because the delayed re-feed group consumed 180 g less CHO than the immediate re-feed group. Given that all players continued with the same high CHO diet for a further 48-h this is however unlikely, and highlights the importance of immediately re-feeding post-exercise for optimal muscle glycogen resynthesis. Despite raised CK concentrations suggesting muscle damage did occur, meaningful muscle glycogen resynthesis was possible in the immediate dietary re-feed group, suggesting that with appropriate feeding strategies it is possible to replenish a damaged muscle. It may be speculated however that given low glycogen utilisation rates were reported in this chapter when compared with elite RL match-play (Chapter 6), whether this re-feeding strategy would be appropriate to reload a more depleted muscle is currently unknown and warrants investigation.

The TTE performance test was used to assess the physiological consequences of the two dietary strategies 48-h after simulated match-play, and to mimic the schedule of professional rugby players (Chapter 5). A lower TTE 48-h after match-play for both groups is likely attributed to a reduction in force generating capacity from exercise-induced muscle damage (Clarkson et al., 1992) caused by the simulation protocol (Sykes et al., 2013). This decrease did not appear to be affected by manipulating CHO immediately after exercise. The lack of difference could be a result of the protocol used to assess performance, with a more prolonged rugby specific drill potentially resulting in differing results. Despite the lack of specificity to rugby, the use of the TTE performance measure

was necessary to avoid tissue damage that would be associated with load-bearing exercise. Moreover, it would be unlikely for players to perform prolonged rugby specific exercise 48-h post game and therefore current data suggest that the differing feeding strategies have no significant effects on light exercise performed in the days after a game.

7.7 Summary

It has been demonstrated for the first time that simulated RL match-play elicits lower muscle glycogen utilisation (21 *cf.* 40 %) despite similar player load and metabolic demands to a professional RL match. This may be attributed to the difficulties of replicating extensive structural damage and physical exertion from collisions during a simulation. Substantial muscle glycogen resynthesis was possible in the immediate dietary re-feed group despite evidence of muscle damage via increase blood proteins. This indicates that with appropriate feeding strategies, it is possible to replenish a damaged muscle with moderate glycogen depletion.

CHAPTER 8

SYNTHESIS OF FINDINGS

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

8. Synthesis of findings

The present chapter provides an initial overview of the findings of this thesis in relation to the aims and objectives presented in chapter 1. A general discussion is next presented where specific attention is given to how the research presented in this thesis has furthered our understanding of the nutritional requirements of elite rugby players during training and competition. Thereafter, the limitations and practical applications of this thesis together with future research directions are discussed.

8.1 Achievement of aims and objectives

The aim of this thesis was to quantify the physiological demands and nutritional practices of professional rugby players during the pre-season and in-season period, and furthermore, to identify the most appropriate feeding strategies for optimal performance and recovery post competition. If the aims of the thesis were achieved, it was proposed that data from this thesis would assist rugby players and coaching staff to make more informed decisions regarding nutritional intakes to improve performance and recovery. The aims of this thesis have been realised through a series of interlinked studies evaluated in sequence.

Objective 1 - ***To characterise the training demands of a typical twelve-week rugby pre-season using GPS technology as well as reporting the changes in anthropometry, markers of physical performance, and the typical macronutrient intakes including the use of supplements.***

Objective 1 was addressed in Chapter 4. At the time of publication, there was a lack of research into the training demands and nutritional intakes of elite rugby players during the pre-season, with no study combining all of the objectives stated. The findings of this research demonstrated that physical performance and players' anthropometrical profiles significantly improved over a 10-week pre-season period despite CHO consumption falling well below recommended intake alongside lower total energy intakes than expected. The findings summarised here suggested that elite rugby players do not necessarily need to consume large quantities of CHO or total energy intake as recommended to optimize training adaptations, however, the appropriateness of these intakes for competition next needing evaluating.

Objective 2 - ***To characterise the training demands of a thirty-six week rugby in-season using GPS technology whilst establishing the typical macronutrient and micronutrient intakes, and energy expenditure using SenseWear armband technology.***

Objective 2 was addressed in Chapter 5. At the time of publication, there were no studies showing the training demands and EIs and EEs of elite rugby players during the competitive season, with no previous study in rugby using micro-technology to report daily variations in EE. The findings of this research demonstrate that rugby players consume less energy than they expend during the first four-days of the training week, due to a low CHO intake which is suggested to be an apparent strategy to attempt to maintain or reduce body

fat. Players then increased CHO intake and total energy intake the day leading up to competition in an effort to load muscle glycogen, whilst simultaneously significantly reducing training load to avoid residual fatigue and promote competition preparation. Interestingly mean EI matched expenditure despite CHO consumption falling short of recommended guidelines (alongside no micronutrient deficiencies) suggesting that the current dietary practices of elite rugby players are sufficient to fuel training during the in-season providing.

Objective 3 - ***To establish the metabolic requirements of a competitive rugby match by assessing muscle glycogen and blood metabolites prior to and post-match using muscle biopsy and blood collecting techniques.***

Objective 3 was addressed in Chapter 6. Given that the metabolic demands of rugby competition were previously unknown and in order to devise appropriate nutrition strategies for optimal performance, it was important to ascertain these data. To this end, it was deemed appropriate to conduct the first ever muscle biopsy study around rugby competition with players following one of two dietary conditions (6 $g \cdot kg^{-1}$ or 3 $g \cdot kg^{-1}$ CHO) to a), ascertain pre-match muscle glycogen concentrations and the magnitude of depletion post-competition and b), to determine the most appropriate nutritional strategy for match-day performance. Muscle glycogen was found to deplete by ~40 % and performance variables did not differ between dietary conditions. However, further analysis showed more homogenous pre-match muscle glycogen concentrations in the higher CHO group. These data demonstrate that adequate muscle glycogen concentrations can be reached when ~600g CHO is consumed in the preceding 36-hours prior to match-play.

Objective 4 - ***To examine the metabolic demands of a simulated RL match and compare with previously published data from professional RL match play.***

Objective 4 was addressed in Chapter 7. Although the RLMSP had previously illustrated similar internal loads experienced and distances covered at varied speeds when compared with real match-play, the metabolic demands had never been examined. It was observed that simulated RL match-play elicits lower muscle glycogen utilisation (21 *cf.* 40 %) despite similar player load to a professional RL match. Reduced glycogen utilisation resulted from difficulties encountered whilst simulating the metabolic costs experienced by players during collisions. The RLMSP might still be used as a specific conditioning tool to condition or evaluate a player's readiness for match-play, but should be redesigned to try to improve replication of physical contacts.

Objective 5 - ***To assess the efficacy of an immediate or delayed carbohydrate re-feed on muscle glycogen resynthesis in damaged muscle after a simulated rugby match.***

Objective 5 was addressed in Chapter 7. From the data obtained in Chapter 6, it was possible to devise a pre-competition nutrition strategy that was appropriate for rugby players. However, appropriate strategies to recover and refuel post rugby match-play were still unknown, with dietary practices following recommendations informed by data from other team or related endurance sports. For the first time, dietary intakes post rugby match-play were manipulated to ascertain the most appropriate method of re-feeding to maximize muscle glycogen resynthesis. Immediately re-feeding with CHO post match-play showed a significantly greater magnitude of muscle glycogen replenishment after 48-h when compared with a 2-h delayed re-feed, although both groups significantly increased after 48-h. The findings in this study are novel and demonstrate that rugby players can replenish

muscle glycogen to an appropriate degree even when delaying their post-game re-feed, however, for optimal replenishment, CHO should be consumed immediately post-match-play.

8.2 General discussion

Prior to commencing this research, there was a paucity of research on the demands of rugby training, player nutritional intakes, or nutritional strategies to prepare for and recover from rugby competition. Of the limited studies undertaken, a number of interesting findings have been reported. In particular quantifying the movement and physiological demands of elite rugby match-play (Waldron et al., 2011). However, most of the limited studies undertaken have been descriptive, and no study has investigated fuelling strategies for rugby competition. Similarly, the most suitable refuelling strategies to opitmize recovery and muscle glycogen replenishment post rugby match-play were unknown.

The four studies undertaken in this thesis provide novel data for the literature on rugby. Importantly, there are a number of novel findings that have been highlighted, particularly relating to the characterization of a rugby season (pre-season and in-season), the daily energy balance of rugby players, the metabolic demands of elite rugby competition, and the development of nutritional guidelines to fuel and recover from competition (Table 8.1). A schematic representation of the main findings of the thesis can be seen in Figure 8.1.

Physiological demands of rugby training

Running volumes and training loads (sRPE) experienced by elite RU players were significantly greater during the pre-season (10.6 km, 3200 AU respectively; Table 4.3) than the in-season (8.6 km, 1660 AU respectively; Table 5.3), owing to emphasis placed on different training outcomes for both training periods and illustrating periodization of the season. Significantly greater training loads were experienced during the pre-season through increased gym based sessions (pre-season – 4 x 1-hr sessions, *cf.* in-season 2 x 1-hr sessions) alongside the addition of 3 x 45-min conditioning sessions per week (See Tables 4.2 and 5.2 for an overview of training) in an attempt to stimulate physiological adaptation as previously reported in professional RU by Argus et al, (2009). It was then observed that training loads decreased significantly moving in to the in-season where emphasis shifted towards the preparation for and recovery from competition. Reductions in training load may result from increased rest days (generally one day preceding and one day following competition), meaning that training only comprised a maximum of 4 days per week during the in-season as opposed to 5 days per week in the pre-season. Interestingly however, players completed on average more high-speed sprints per week (>7.5 $m \cdot s^{-1}$; 12 *cf.* 20) alongside greater numbers of RHIE's (14.7 *cf.* 17.5) during the in-season compared with pre-season. This suggests that alongside manipulating training load throughout the season, periodization of exercise activity may also occur to incorporate more match-play specific activities during training in preparation for competition during the in-season. Furthermore, despite lower training loads reported in-season in this thesis, analysis of in-season match-play, which would considerably contribute to overall all weekly running volume and player loads sustained (Austin et al., 2011, Cahill et al., 2013, Duthie et al., 2005, Jones et al., 2015, Roberts et al., 2008), was not conducted, and data should therefore be interpreted with a degree of caution.

Energy balance of professional rugby players

Improvements in player anthropometry and performance markers during the pre-season, despite CHO intakes falling well below textbook recommendations (Burke et al., 2011), demonstrate that it may not be necessary to ingest large quantities of CHO to attain desired training adaptations for rugby. In fact, the low CHO and high PRO intakes observed may be beneficial in the promotion of fat loss and other adaptions to training such as increased mitochondrial biogenesis (Hawley and Morton, 2014). Furthermore, measurements of energy balance during the in-season revealed that players habitually ingest less energy than expended during most of the training week, with CHO intake only increasing above EE the days before and after a match in order to load and replenish muscle glycogen respectively. This cycling of CHO intake might be a suitable way of maintaining weekly energy balance yet still allowing sufficient CHO intake as to increase muscle glycogen and thus enhance match day performance. Moreover, current guidelines clearly state that CHO intakes should be designed to meet the fuel requirements of the training programme (Burke et al., 2011). It is therefore proposed that players are attempting to match carbohydrate intakes with training demands such that CHO intakes of 4-6 $g \cdot kg^{-1}$ body mass are not '*low*' and are in fact '*appropriate'* for rugby players, providing CHO intake is increased in the day before and after a game.

Metabolic demands of rugby match play

Progressing from previous match-play research (Twist et al., 2014, Waldron et al., 2011), the metabolic requirements of rugby match-play were assessed under two dietary conditions; relatively high (6 $g \cdot kg^{-1}$; HCHO) or low (3 $g \cdot kg^{-1}$; LCHO) CHO diet. Muscle biopsy analysis revealed a ~40 % utilisation rate of muscle glycogen with no mean

difference between groups, although pre-match concentrations were more homogenous across the HCHO group, and higher rates of FFA availability were observed in the LCHO group. This is an important finding given that current nutritional guidelines recommend ~8-10g.kg CHO leading up to a match (Burke et al., 2011), and considering player loads and distances covered during this study were comparable to elite rugby match-play, may make players gain unwanted fat. This clearly raises the point that nutritional guidelines must be individualized to the sport, and that further research must be conducted to refine these findings. Additionally, this research has shown that muscle biopsies can be taken from elite athletes around competition and in the field, enhancing the scope of future research.

Muscle glycogen replenishment

Following competitive match-play, nutrition comprises one of the most important components of player recovery. It was shown that despite both groups increasing muscle glycogen to above pre-match-play values, immediately re-feeding with CHO post-RLMSP elicited a far greater magnitude of muscle glycogen repletion when compared with delaying this re-feed by 2-h in damaged muscle. It could be argued that this discrepancy is a direct result of the immediate re-feed group consuming greater total CHO (180g in the 2-h post-exercise period), however, given that all players continued with the same high CHO diet for a further 48-h (6 $g \cdot kg^{-1}$ CHO), this is unlikely. This research has revealed that there is a diminishing window of opportunity to re-feed with CHO and capitalize on the non-insulin dependent phase of muscle glycogen synthesis post muscle-damaging exercise, which may have a profound impact on the devising of nutrition strategies for rugby competition. It was also found that the RLMSP used for this research elicited comparable player loads, total distance covered, and distance covered at varied speeds to elite rugby

match-play, however, the RLMSP was unable to accurately reflect the physical exertions experienced during the tackle in rugby which significantly reduced muscle glycogen utilisation when compared to elite match-play. The RLMSP may be used as a specific conditioning tool to condition or evaluate a player's readiness for match-play, although future research should look to improve the tackle component of the RLMSP.

Table 8.1 – Proposed CHO requirements of rugby players determined from the findings of this thesis for; Training (pre-season and in-season) and competition (Glycogen loading and glycogen replenishment)

	Suggested CHO intake	Instruction	Rationale
		Training	
Pre-season	~3-4 $g \cdot kg^{-1}$	Consume relatively low CHO throughout the week. Increase CHO when necessary e.g. before and after exceptionally hard training sessions.	Physiological adaptation and body composition improvements achieved.
In-season	~4-6 $g \cdot kg^{-1}$	Consume lower CHO intake during the beginning of the training week, increase CHO intake in the days leading in to competition.	EI exceeded EE, players able to perform at elite playing intensities.
		Competition	
Glycogen load for match-play	~600 g	Begin ~36-h prior to KO, all meals to contain CHO.	Mean starting muscle glycogen ~450 $mmol \cdot kg^{-1}$d.w. Low variability between players in ~6 $g \cdot kg^{-1}$ group.
Post-match-play	~90 g - Immediate ~90 g - 1-h ~6 $g \cdot kg^{-1}$ - 2-h onwards	Immediately re-feed with CHO for optimal glycogen replenishment.	Greater magnitude of muscle glycogen repletion with an immediate CHO re-feeding strategy.

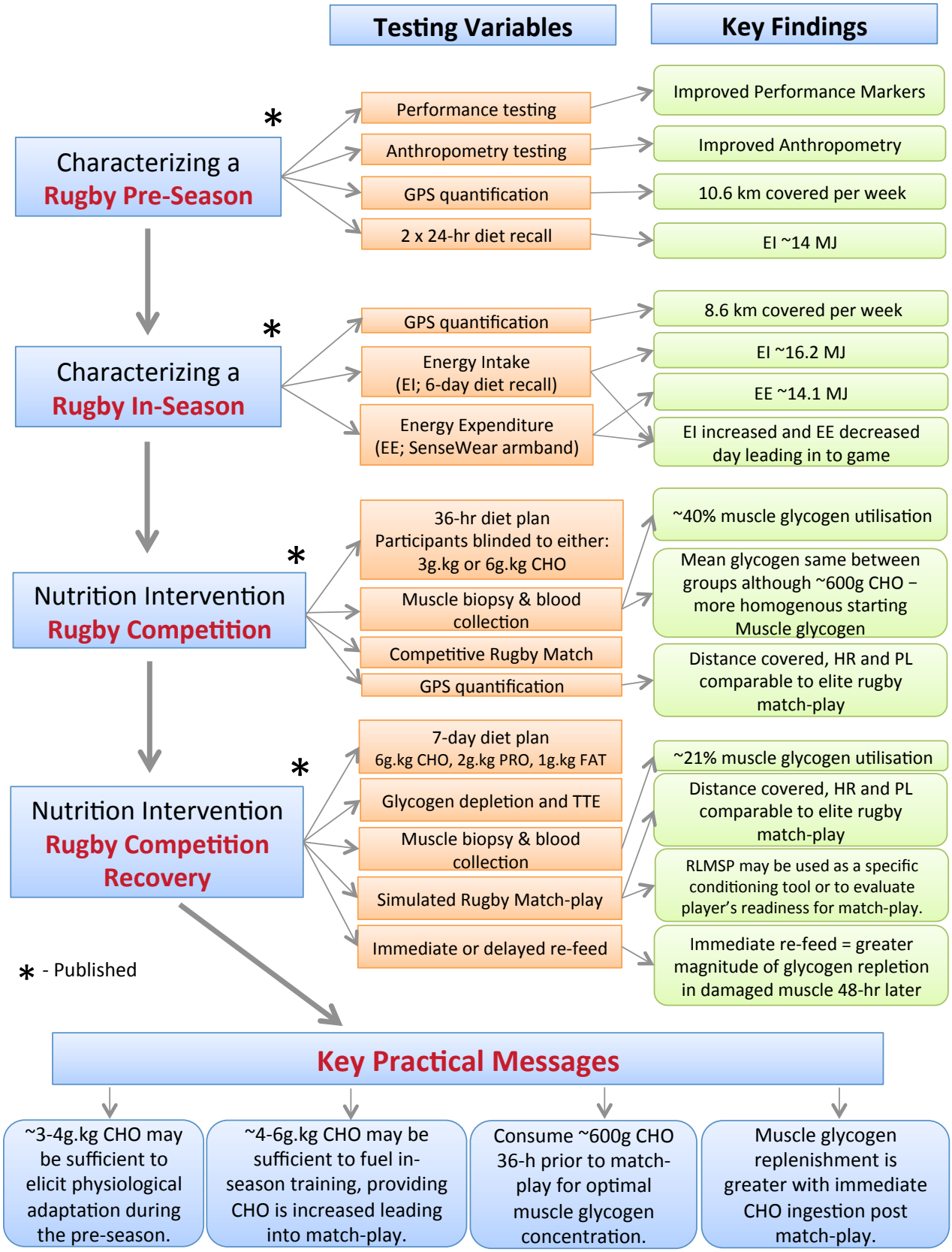

Figure 8.1: Schematic representation of the main findings of this thesis. After characterising a rugby pre-season (Chapter 4) and a rugby in-season (Chapter 5), it was necessary to understand the metabolic demands of rugby competition (Chapter 6) in order to formulate appropriate nutrition strategies to fuel rugby competition, followed and the most optimal nutrition strategy to refuel after rugby competition (Chapter 7).

8.3 Limitations of thesis

Despite providing novel data for the literature, the present thesis is not without limitations, many of which are a direct consequence of collecting data from elite athletes outside of the controlled laboratory environment. These limitations will now be addressed in turn.

Sample size

It is important to highlight that the findings from studies 1 and 2 are from a single professional rugby team, study 3 a single professional rugby academy, and study 4 a single university rugby team, which may not accurately represent every rugby club. Future studies might therefore choose to collect data from a variety of teams.

GPS

At the time of data collection for Chapters 4 and 5, specified speed zones within GPS were absolute, and well reported in the literature (Roberts et al., 2008, Austin et al., 2011, Cummins et al., 2013, Jones et al., 2015). Emerging research suggests that the use of relative speed zones may provide a clearer picture of the positional running demands of rugby players (Dwyer and Gabbett, 2012, Gabbett et al., 2013, Reardon et al., 2015). Preliminary data on players running abilities would be required to formulate these relative thresholds however, which in some cases may not be a possibility. For example, Chapter 6 of this thesis where only a short period of time was permitted around the clubs training regime for the extraction of valuable data during this unique opportunity. Furthermore, it is

still possible to use data collected for distance covered at a variety of speeds relatively alongside frequency of RHIE bouts, to inform rugby training.

Muscle biopsy

To assess individual muscle fibres for glycogen concentration, a greater yield of muscle would be required, possibly using either the Bergstrom needle or Conchotome technique (Dietrichson et al., 1987). Moreover, a large cross sectional area of a sample is obtainable utilising these methods, allowing for detailed histological analysis (Dietrichson et al., 1987). Due to the physical nature of rugby however, these methods were deemed unsuitable and the less invasive micro-biopsy technique was utilised. It is important to note that the *vastus lateralis* is composed of both fast and slow twitch muscle fibres, permitting the extraction of single muscle fibres from either fibre type which was unknown during this research. Histochemical analysis of individual skeletal muscle fibres would provide more clarity regarding muscle glycogen concentration in these fibres.

During study 3, post-match biopsies were collected over a 40-minute period rather than at precisely the same time due to logistical reasons (i.e. only having four qualified biopsy takers). Future studies might wish to take measures from fewer players but over a series of matches to facilitate a more rapid removal of tissue as well as consider a half-time muscle biopsy to provide a more detailed analysis of muscle glycogen utilisation during competitive rugby match-play.

During study 4, ethical constraints did not permit the extraction of four muscle biopsies from the same leg hence the final study adopting a contra-lateral limb biopsy design where in a randomised order the dominant or non-dominant leg was biopsied twice (post-

glycogen depletion and post-simulation) and the opposite leg was biopsied twice (pre-simulation and 48-hours post-simulation). This approach was reliant upon the assumption that muscle fibre type composition was similar between both legs. Such an assumption could not be validated with histochemical analysis due to lack of tissue (Dietrichson et al., 1987). Resultantly, the possibility that fibre type differences between legs influenced certain findings cannot be discounted.

Energy Intake

Given that there is no gold standard assessment of energy intake, any method employed is subject to error (Hackett, 2009). Future studies should look to validate a specific method for use with elite athletes and in the meantime data collected on energy intake must be treated with some degree of caution (Magkos and Yannakoulia, 2003).

During Study 2, energy intake was not assessed on game day which lead to the use of a 6-day food diary. Whilst game day information would have been valuable, given the elite standard of the participants it was deemed necessary to avoid adding to player's game day stresses and potentially disrupting normal practices. The 6-day food diary has since been reported in the literature by Morehen at al, (2016), who assessed the energy balance of elite RL players during two in-season training weeks.

Energy Expenditure

The measurement of energy expenditure in contact sports is somewhat difficult given that many of the tools available such as the SenseWear armband technology used in Study 2 would not be suitable during physical collisions, either through danger to the athlete or to

the equipment. Perhaps the best method would be to use DLW over the course of a training week as employed by Morehen et al, (2016) in professional RL, and future studies should certainly consider this despite the obvious financial implications.

Rugby league match simulation protocol (RLMSP)

To ensure that exercise time, player loads and distances covered were standardised throughout testing, an adapted version of a RLMSP was used in place of a competitive game. This meant that forwards who usually play for ~44 mins, (Waldron et al., 2011) exercised for ~92 mins, which may have had bearing on player load and muscle glycogen utilisation. Furthermore, replicating contacts with a tackle bag was necessary to control the number and intensity of the collisions, but means the blunt force trauma experienced by players was much lower compared to match-play (Norris et al., 2016). Future studies may wish to better replicate contact situations, possibly performing the RLMSP with pairs of participants who take it in turn to tackle one another. Furthermore, given the discrepancy in muscle glycogen utilization between the RLMSP and rugby match-play, it may be pertinent to assess these reloading strategies using muscle biopsies from a real game.

Time to exhaustion test (TTE)

Despite the lack of specificity to rugby, the use of the TTE performance measure was necessary to avoid tissue damage that would be associated with load-bearing exercise (Clarkson et al., 1992, Clarkson et al., 1999). Moreover, it would be unlikely for players to perform prolonged rugby specific exercise 48-h post game. That the effect of manipulating CHO immediately after exercise on subsequent TTE performance was

unclear, suggests that a larger sample was required or a more sensitive measure of performance was needed to resolve the uncertainty.

Switch from union to league

Despite Chapters 4 and 5 of this thesis being conducted in RU, two important reasons justified the progression to RL in Chapters 6 and 7 and the amalgamation of both codes in to thesis; i) A unique opportunity arose consenting the extraction of muscle fibres from professional RL players around a competitive match, the first and only study of it's kind to be proposed in elite rugby and 2), both codes of rugby have been shown to poses similar physiological demands (Duthie et al., 2003, Gabbett et al., 2012, Twist et al., 2014) and nutritional behaviours (Morehen et al., 2016, Chapters 4 and 5 of this thesis). However, it must be acknowledged as a limitation as there will be some metabolic differences between the two sports.

Statistical analysis

Chapters 4 & 5 of this thesis were published using traditional null hypothesis significance testing, whereas Chapters 6 & 7 were published using MBI after a collegaue (Statistician) provided advice and education on the appropriateness of their use in applied research. For consistency, Chapters 4 & 5 were reanalysed using MBI and the rationale for this change was as follows:

1) P values and in turn, study conclusions, are sample-size dependent, irrespective of the size of the effect, whereas MBI provide a standardized means of analysis.

2) Significance testing doesn't permit information on the magnitude of an effect, yet for the applied practitioner magnitude is of upmost importance. With a sample size great enough, even very small, trivial or non-practical effects can become significant.
3) MBI allows the smallest meaningful effect to be standardised across different testing protocols (using either recommendations from research, or creating own best fit) improving interpretation.
4) MBI is easily interpreted for researchers and applied practitioners alike, and supported by spreadsheets freely available on the internet.

8.4 Practical Implications

The findings of this thesis have important practical implications.

- A rugby union pre-season can be fueled (and significant improvements in body composition and performance can be made), on CHO intakes that fall below what has been previously been suggested as adequate for a rugby type training programme.
- Given the surprisingly low EI and CHO intakes of rugby players during the pre-season, it seems important to quantify training loads to inform dietary intakes. Furthermore, strength and conditioning professionals and coaching staff must attempt to match the nutritional requirements to their own specific training demands rather than simply adopting standard guidelines.
- During a rugby union in-season, energy intake matched expenditure for both forwards and backs despite CHO consumption falling short of recommended guidelines, suggesting that these intakes may be appropriate for rugby players providing CHO is increased leading in to competition.

- Muscle biopsies are possible before and after a competitive rugby league match proving a framework for future research in this sport.
- Approximately 40 % of muscle glycogen appears to be used during a competitive rugby league match, although the effects on individual fibres remain unresolved.
- There were no noticeable differences in high intensity running capability between ~6 or ~3 g.kg CHO dietary groups, suggesting that the lower CHO condition had no gross effects on performance.
- Notwithstanding the limitations of interpreting movement characteristics of a single match, it is proposed that a diet comprising ~600 g CHO 36-h before a match could be recommended to ensure all players commence the match with appropriate muscle glycogen concentrations.
- It would appear that CHO consumption in the week leading into a rugby match is a major contributor to pre-match muscle glycogen concentration, rather than the CHO content of the diet in the preceding 36-h and therefore this should be monitored by coaches and players.
- Simulated rugby match-play elicits a similar internal load, but the metabolic demands are lower than during professional RL match-play.
- Collision events in rugby are speculated to present a large metabolic cost to the player and are difficult to simulate in training and research settings
- The RLMSP might be used as a specific conditioning tool to condition or evaluate a player's readiness for match-play.
- Despite high CHO consumption in the 48-h after RL match-play, immediately re-feeding with CHO seems to be a major contributor to muscle glycogen resynthesis, highlighting the importance of the insulin-independent phase of muscle glycogen replenishment.

Recommendations for future research:

Based upon the findings of this thesis, future research should look to further titrate these studies to improve understanding of the metabolic requirements of elite rugby training and competition, and also the optimal nutritional strategies to facilitate this. The following recommendations are made for future research:

1. Repeat studies 1 and 2 in a larger cohort of athletes and teams to provide a more accurate reflection of the training and dietary practices of elite rugby players. The findings from studies 1 and 2 clearly demonstrate that the current nutritional practices of rugby players from one elite professional team do not meet the textbook guidelines for CHO intake. Anecdotal evidence suggests that these practices are commonplace across rugby teams. Logically, therefore, such a study should now be repeated in a large cohort to validate these findings. Weekly measures of sRPE and GPS should be collected to aid in the determination of fatigue and injury risk over the course of the season.

2. Repeat study 3 using fewer players, but over a series of matches to facilitate a more rapid removal of tissue as well as consider a half-time muscle biopsy to provide more detail regarding muscle glycogen utilisation before, during, and after rugby match-play. Alongside, analyse CK and collect subjective markers of muscle soreness for up to 72-h post-match-play to assess muscle damage and analyse the relationship between CK and muscle glycogen utilisation – does a more depleted muscle reveal greater muscle damage?

3. Repeat study 3 to assess muscle glycogen utilisation in individual muscle fibre types. Given that muscle passes were analysed as a whole homogenate in this thesis, it is impossible to report the glycogen depletion of individual fibre types which may vary greatly as seen in soccer (Krustrup, et al., 2006). If collected, these data have the potential to significantly improve the literature and allow nutritional strategies to be refined further optimising muscle glycogen loading and refuelling strategies. Furthermore, segmenting and analysing match-play in 10-15 minute sections would allow greater detail in the assessment of temporal fatigue between dietary conditions.

4. Repeat Study 4 to assess muscle glycogen replenishment in individual fibre types after an immediate or delayed re-feed. Furthermore, given the large metabolic cost of collision events in match-play, contacts must be better replicated in the RLMSP. It is speculated that the best way to maintain a standardised methodology and to improve the ecological validity of the tackle componenet, participants should pair up and tackle one another on alternate cycles complete with a 4 s wrestle as seen in RL match-play. Thereafter, assessment of EIMD using a subjective marker of muscle soreness alongside analysis of CK concentrations over the following 72-h is warranted. This study would more accurately reflect the metabolic demands of rugby competition in a controlled setting, validating the RLMSP for use in future research, and furthermore provide greater insight in to the subsequent replenishment of muscle glycogen after damaging rugby type exercise.

In conclusion, and taking into consideration the limitations, this applied thesis has successfully clarified some confusion around the current dietary and training practices of rugby players, alongside the quantification of the metabolic demands of elite rugby match-play, and postulating the most appropriate dietary intakes around competition. These data are already influencing practice in elite RU and RL teams.

CHAPTER 9

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