Quantification of the demands of elite rugby union players and understanding the subsequent physiological and epigenetic responses – An applied approach towards prescription and individualisation

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Quantification of the demands of elite rugby union players and understanding the subsequent physiological and epigenetic responses – An applied approach towards prescription and individualisation

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Abstract

The development of effective training load monitoring tools has enabled key insights into elite rugby union players' training and game demands and significantly improved the physical management of players. Whilst the use of training load quantification methods is common practice in elite sport, in the scientific literature, little information is known about the training and periodisation strategies of professional rugby union clubs, and how this may change throughout a competitive season. Throughout a season, coaches and practitioners face frequent decisions on daily training sessions, team selection, and competition scheduling. Understanding the load imposed on players from training and competition informs and aids this decision making. These decisions are integral to the optimisation of repeated player performance throughout a season. Diagnostic tools provide another piece of the puzzle to support player management decisions.

The integration of both diagnostic measures and workload quantification aids the understanding of the dose-response relationship between fatigue and physiological adaptation. Building an effective individualised training load monitoring system is key within an elite rugby union club. Currently, practitioners are exposed to vast amounts of information that can be collected on individual athletes, slowing and limiting the ability to make effective choices on players' health and performance. There is a need to synthesise this process and optimise athlete monitoring without creating additional staff and player burden.

This thesis aimed to explore the multitude of factors that may influence the optimisation of elite rugby union players and explored the reasons for variation between individual players. The first part of this thesis quantified the external training load demands throughout a competitive season and evaluated the stress-induced effects of acute and chronic rugby training and match-play. Data from Chapter 4 demonstrated a strategical variation in training load prescription throughout a competitive season and showed key differences in external load metrics between position and the match status of players. Chapter 5 assessed changes in performance measures, biomarkers and subjective wellness throughout a full professional season. Certain periods of the season reported significant deviations from baseline measures and associations were observed between GPS variables and measures of performance and biomarkers.

The second part of the thesis explored novel circulating miRNA (ci-miRNA) biomarkers and their role in the observed variation between players in response to training. Chapter 6 revealed associations between specific ci-miRNAs and anthropometric and performance variables that are consistent with the current understanding of plausible biological mechanisms. Lastly, Chapter 7 revealed the variability in training-induced responses to preseason training and, reported associations between certain baseline ci-miRNA levels with the observed anthropometric and strength changes.

In summary, this thesis emphasises that even within a team sport, managing individual players is crucial to optimising performance; a one size fits all approach is not appropriate. A consistent and effective monitoring system needs to observe both external load and measures of fatigue, in order to inform and support the decision making of practitioners. Additionally, there is a great potential for the use of epigenetic information to inform practitioners of player management processes. However, this research in an elite sport setting is very much in its infancy and caution should be taken trying to implement this strategy.

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Contributions

Chapter 4, Quantification of Training and Match-Load across a Season in an Elite Rugby Union Team:

Study design – Joe Kupusarevic, Kevin McShane, Prof Emma Stevenson, Dr Tom Clifford, Dr Dan West

Data collection – Joe Kupusarevic, Kevin McShane

Study analysis – Joe Kupusarevic

Chapter 5, Monitoring biological, physiological and self-reported wellbeing measures in professional Rugby Union players throughout an entire season:

Study design – Joe Kupusarevic, Kevin McShane, Prof Emma Stevenson, Dr Tom Clifford, Dr Dan West

Data collection – Joe Kupusarevic, Kevin McShane, Newcastle University Sport & Exercise Science team

Study analysis – Joe Kupusarevic

Chapter 6, Circulating miRNA levels in elite male Rugby Union athletes and their associations with anthropometric and performance phenotypes

Study design – Joe Kupusarevic, Dr Colin Moran, Brian Coyle, Kevin McShane, Prof Emma Stevenson, Dr Tom Clifford, Dr Dan West

Data collection – Joe Kupusarevic, Kevin McShane, Newcastle University Sport & Exercise Science team

Study analysis – Joe Kupusarevic, Dr Colin Moran, Brian Coyle

Chapter 7, Can Baseline Plasma miRNA Levels Identify Individual Responsiveness to Preseason Training in Elite Male Rugby Union Players?

Study design – Joe Kupusarevic, Dr Colin Moran, Brian Coyle, Kevin McShane, Prof Emma Stevenson, Dr Tom Clifford, Dr Dan West

Data collection – Joe Kupusarevic, Kevin McShane, Newcastle University Sport & Exercise Science team

Study analysis – Joe Kupusarevic, Dr Colin Moran, Brian Coyle

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Chapter 1

1.0 Introduction

1.0 Introduction

Rugby Union (RU) is one of the most popular team sports in the world; recently a review from World Rugby, reported that the game is now played in more countries around the world than ever before, and currently has 3.5 million registered players (World Rugby, 2019). The game has vastly changed over the years and is now renowned for its fast-paced physical nature, believed largely due to the advent of professionalism in 1995. Since the sport has turned professional, players have become bigger, faster, stronger and involved in a greater number of collisions; this has resulted in increases in the pace and amount of physical contact within the sport (Duthie *et al.*, 2003; Quarrie and Hopkins, 2007). Professional RU requires players to exhibit a high level of position-specific skill as well as a high fitness level much greater than the general population (Scott *et al.*, 2003).

A RU team consists of 15 players that are part of 2 major positional groups; the forwards and the backs, within each positional group there are more specific roles. Forwards consist of two props, one loose-head (shirt number 1) and one tight-head (shirt number 3), the addition of a hooker (shirt number 2) makes up what is called the 'front row'. Added to these 3 positions are two locks (shirt numbers 4 & 5), these 5 positional groups together creates what is known as 'tight forwards' or the front-5 positional group. The final 3 positions within a forwards group are known as 'loose forwards' or the 'back row', these are blind-side flankers, open-side flankers and number 8 (shirt numbers 6, 7 & 8). Backs are made up of scrum-half (shirt number 9) and fly-half (shirt number 10), collectively known as 'half-backs'. Finally, inside centres (shirt number 12), outside centres (shirt number 13), left-wingers (shirt number 11), right-wingers (shirt number 14) and full backs (shirt number 15) complete 'the backs'. These positions together complete the 15 field positions needed for RU. Players positions are somewhat determined by possessing specific skill sets and physical attributes (Quarrie *et al.*, 2013).

RU match-play is intermittent in nature, as periods of low-intensity or rest, are interspersed with high-intensity activities (Roberts *et al.*, 2008; Jones *et al.*, 2015). Although all players are exposed to both collision and running demands, research has shown marked differences in the competition demands of forwards and backs. Typically backs perform higher locomotor activities when compared to forwards, as they cover greater distances and at higher speeds (Jones *et al.*, 2015). Forwards, however, perform a greater number of high-intensity activity efforts than backs, these are predominantly rucks and mauls, or static efforts such as scrums, (Duthie *et al.*, 2005). This requires players to possess a range of position-specific demands

such as repeated-sprint ability, speed, agility, lower-body power and strength (Brazier *et al.*, 2020).

Professional players in the modern era play competitively for approximately 40 weeks a year. As well as competitive games this also incorporates, field-based training sessions, gym sessions and non-rugby related requirements such as travel and media obligations (Quarrie *et al.*, 2017). The increased demands associated with an elite RU player have been linked to issues such as an increased injury incidence (Brooks *et al.*, 2008; West *et al.*, 2021a) and player burnout (Cresswell and Eklund, 2006; Hodge *et al.*, 2008). These concerns create several challenges for practitioners, as they are continually looking for methods to manage these increasing stressors on players throughout a season. Currently, the observation and quantification of accumulative load throughout an entire professional RU season has not been thoroughly investigated. Now more than ever, players need to be managed appropriately to ensure player welfare and performance. Therefore, research is needed to understand whether certain contextual factors influence a player's tolerability to training or whether certain times of the season present greater risk.

As well as understanding the contextual factors that may influence training loads, it is crucial to recognise individual responses so training prescription can be adjusted accordingly (Bourdon *et al.*, 2017). Research in the field of athlete personalisation is accelerating, the concept of utilising genetics for personalised nutrition (Guest *et al.*, 2019) and personalised athletic training (Jones *et al.*, 2016) possesses a great potential to optimise athletic performance. Recently, in the field of epigenetics, studies have shown circulating microRNAs (ci-miRNAs) responded specifically to different exercise protocols and associate with a variety of phenotypes (Sapp and Hagberg, 2019). Interestingly differential patterns of ci-miRNAs may be useful biomarkers of adaptation (Domańska-Senderowska *et al.*, 2019), but to date have not been explored in elite RU players. Research into this area may reveal the underlining biology of the individual response to exercise, helping to optimise individual training.

The overarching aim of this thesis was to understand the longitudinal demands of elite RU players to best optimise the training process and promote performance and player welfare. Additionally, a specific focus was on the individual responsiveness of training via the use of novel ci-miRNA methods. The thesis is presented in seven subsequent chapters. These chapters are detailed as follows:

Chapter 2: This chapter reviewed the current literature on the need for appropriate athlete load monitoring methods and the measures available to assess the response to training in elite RU. Furthermore, epigenetics is discussed and the role ci-miRNAs have in physiology and sport is explored.

Chapter 3: This chapter details the relevant materials and methods used to collect the data presented in the subsequent studies.

Chapter 4: This chapter presents a study investigating the external training demands of elite rugby players throughout an entire season. Players were grouped depending on their training group position and match starting status.

Chapter 5: This chapter presents a study investigating the associations between external training load and diagnostic markers in elite rugby players throughout an entire season. Players were grouped depending on their position and match starting status.

Chapter 6: This chapter presents a study investigating the potential regulatory role of ci-miRNAs in elite RU players.

Chapter 7: This chapter presents a study investigating associations between preseason performance changes and basal ci-miRNA abundance in elite RU players.

Chapter 8: This chapter discusses the findings presented in this thesis. Conclusions, practical applications, limitations and areas of future study are discussed.

Chapter 2

2.0 Literature review

2.1 Athlete load monitoring

Professional RU players are subject to a variety of rugby (physical loads) and non-rugby (interpersonal relationships, media demands) stressors. The term 'load' is defined as 'the total stressors and demands applied to the players' (Quarrie *et al.*, 2017). Representatives of the game at the highest level have recognised a duty of care to monitor and understand individual player loads (England Rugby, 2020, August 18; World Rugby, 2021). Within professional team sports, the collection and analysis of athlete monitoring data is a common and essential practice. The key aim of this process is to determine an athlete's responses to training loads, ensure adequate stress/recovery balance, and determine the relationship between training and performance (Halson, 2014; Mujika, 2017). Additionally, it allows the examination of performance potential, as well as minimizing the risk of injury and/or illness (Thornton *et al.*, 2019). Athlete monitoring is a continuous and cyclical process, which provides insights to facilitate the prescription of training (Figure 1). Effective monitoring systems provide objective evidence, to aid performance decision-making (Gabbett *et al.*, 2017; Robertson *et al.*, 2017). Daily decisions are made by coaches to determine the volume and intensity individual athletes will undertake for certain training sessions or time periods. These decisions are crucial to ensure an optimal workload that maximises performance, minimises fatigue and prevents the risk of injury.

As part of an athletic performance programme the terms training, testing/ screening, monitoring and adaptation are consistently used as part of the system to achieve the highest level of performance, whilst providing a duty of care to the athletes. It is important to consider how testing and screening differentiate from monitoring to inform the athlete monitoring process, this is predominately due to the frequency of the measure. Testing and screening is more an infrequent measure, to identify a deficiency, injury risk or the effectiveness of a training intervention. Monitoring is a higher frequency measure (daily, weekly) to assess ongoing recovery or readiness (Pedlar *et al.*, 2019).

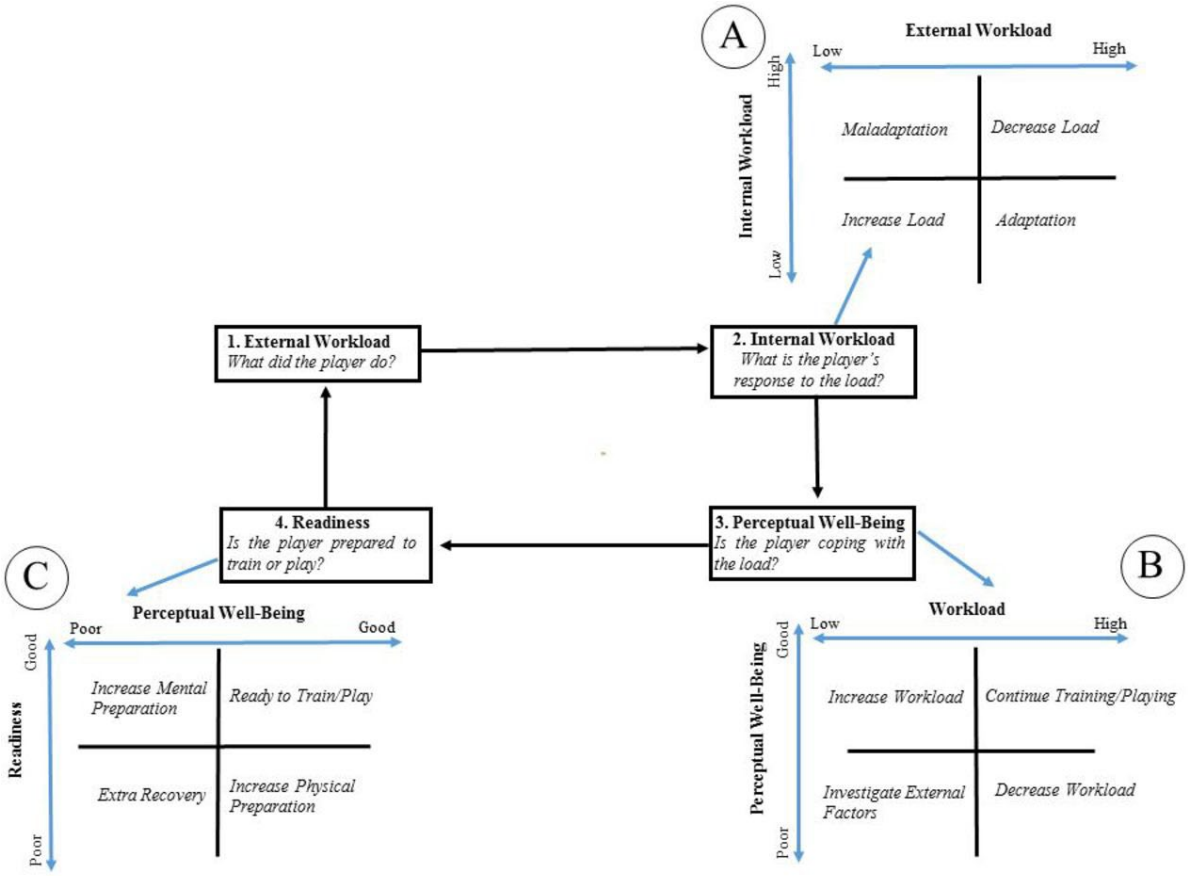

Figure 1. Gabbett *et al.* (2017), presents the athlete monitoring cycle process. This process aids the individual prescription of training (e.g. whether a player needs extra recovery time or training top-ups) to optimise performance and reduce the negative influences of fatigue.

2.1.1 Methods to quantify external training load

Currently, no single marker has been identified which can measure the fitness and fatigue responses to exercise or accurately predict performance (Borresen and Lambert, 2009), highlighting the need for an athlete monitoring system that can incorporate a variety of measures. The first part of this process is to quantify the training load that a player has undertaken. Globally training load reflects the internal and external loads imposed on an athlete (Thorpe *et al.*, 2017), for this section we are going to focus on the external load.

External load is commonly expressed as a function of distance, speed or time and refers to the total mechanical or locomotive work that is completed by a player during training or competition. Previously, the quantification of external training load has proved difficult, due to the random intermittent nature of RU activity (Duthie *et al.*, 2003) and the labour-intensive nature of video-based notational analysis (Roberts *et al.*, 2008). However, with the development of Global Positioning System (GPS) technology, these systems are now common

place in team sports (Malone *et al.*, 2017) and are regarded as the most important monitoring tool by English Premiership rugby teams (West *et al.*, 2019). The use of GPS within team sports focuses primarily on the quantification of an athlete's total distance, and the distances covered in various speed zones during training and competition (Aughey, 2011). The integration of inertial sensors within GPS devices has also allowed for the quantification of other key metrics such as accelerations and decelerations (Malone *et al.*, 2017). When GPS data is appropriately collected and interpreted, this data informs the decision-making processes regarding the planning and manipulation of training (Thornton *et al.*, 2019).

2.1.2 Methods to quantify internal training load

The internal load is a measure of the physiological and psychological stress imposed on an athlete. Whilst the external load is important to understand the work completed by an athlete, it is the internal training load that drives fitness outcomes. A combination of both may be important for training load monitoring (Halson, 2014). There are a variety of measures used to quantify internal training load but the most common in English Premiership RU teams is the use of S-RPE (session rating of perceived exertion) and heart rate (West *et al.*, 2019). In RU training load publications, S-RPE is the most commonly used due to its easy use and low cost. S-RPE provides a measure of internal training load that incorporates both the intensity and duration of exercise (Vanrenterghem *et al.*, 2017). Longitudinal studies in elite RU have primarily reported S-RPE in isolation with no understanding of the external load. This results in a reduced application of the findings as no context is provided as to what the training prescription was or how it may have changed throughout the season. Ideally, both objective and subjective measures should be used to ensure an equal balance between the athlete's perception and the quantifiable prescribed training (Bourdon *et al.*, 2017). Following a survey of Premiership RU clubs, only 28 out of 42 respondents used the S-RPE method, whereas all used HR in conjunction with GPS (West *et al.*, 2019), suggesting there may be a separation as to what is commonly used in the literature compared to what is used in practice. Although the applicability of the S-RPE method has been commended due to its ease of use, within a practical setting where a team has a high number of players and multiple training sessions a day its utility may not be as easy. In addition, S-RPE can often be misused by athletes looking to falsely influence subsequent training sessions (Bourdon *et al.*, 2017).

2.1.3 The need for effective athlete monitoring

Understanding the relationships between training, injury, fitness and performance are critical within the field of sports science (Figure 2). Within elite sport, multidisciplinary teams work together to ensure optimal training loads are applied at an individual level to maximise performance potential and limit any negative consequences to training (Gabbett, 2016). Previous studies have aimed to assess the associations between athlete workloads and performance. Preseason is a crucial time period within team sports, as it is often the greatest opportunity for physical development over an entire playing season. In professional footballers, positive changes in aerobic fitness were associated with the time in a preseason spent > 90 % of maximal heart rate (Jaspers *et al.*, 2017). In professional rugby players, session rating of perceived breathlessness (sRPE-B) was used as a measure of internal training load throughout the preseason. Differences in weekly sRPE-B were observed between players who respond to training compared with players who do not (McLaren *et al.*, 2018). Superior physical attributes are associated with increased levels of rugby competition (Argus *et al.*, 2012), hence if you can maximise training time to facilitate adaptation, it may lead to better performances.

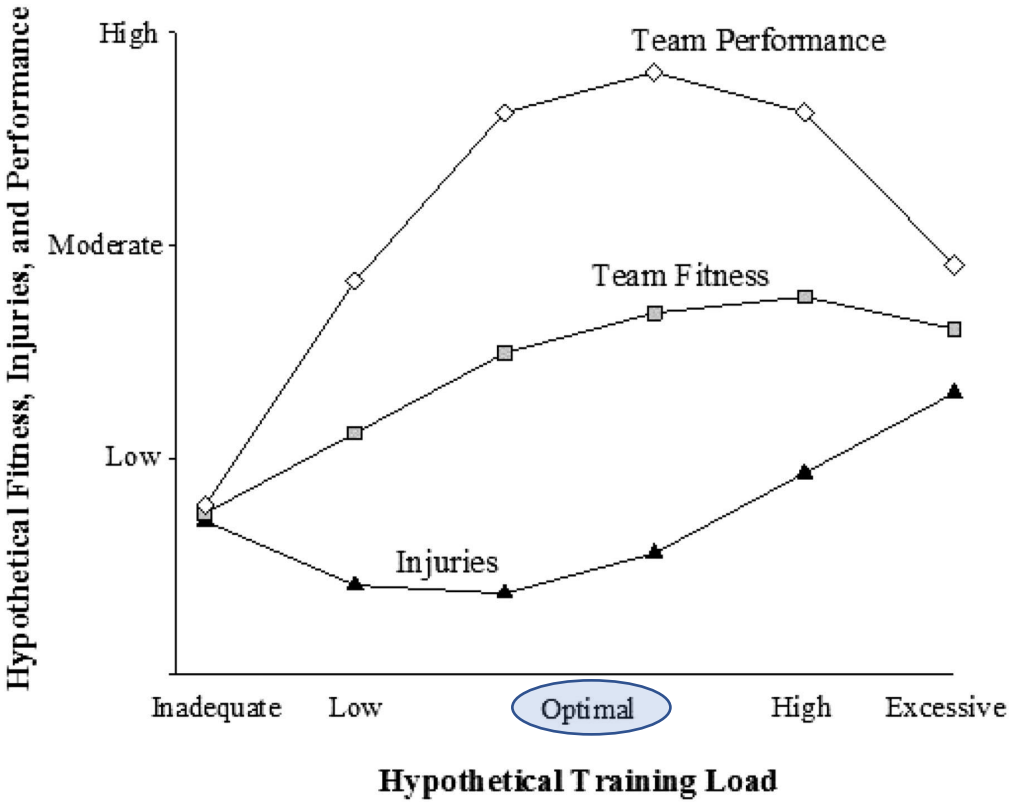

Figure 2. Graphic displaying the hypothetical relationship between training load, performance, fitness and injuries from Orchard (2012) and Gabbett (2016).

Relationships have also been observed between athlete workloads and injury. Currently in sport, competition calendars are becoming more demanding, this results in a greater requirement to perform regularly, thus a reduction in recovery time. Exposing athletes to these high loads with reduced recovery risks poor load management, which is a major risk factor for injury occurrence (Soligard *et al.*, 2016). This is significant for rugby performance, as higher injury rates reduce player availability, negatively influencing team performance (Hägglund *et al.*, 2013).

Cross *et al.* (2016) observed training load and time-loss injuries of 174 English Premiership players throughout a season, they reported that a high load and a large absolute change in load increases the risk of injury in professional RU players. All measures of load units were provided as arbitrary units obtained from session rating of perceived exertion (s-RPE) measures, this makes applicability to practitioners harder to apply as it is highly individual and club specific. GPS measures would provide additional data regarding external total training loads allowing greater implementation of this research. This method of quantification was potentially beyond the scope of the research question, however, there was also a lack of clarity on how training loads changed throughout the season and the impact this may have on injury occurrence. Data from Brooks *et al.* (2008) concluded that weekly training volumes of between 6.1 and 9.1 hours resulted in the lowest risk of match and training injuries for players. This data is slightly more applicable for practitioners as results allude to training hours rather than arbitrary units. Though, these findings still provide no indication of the intensity or specifically the distances of movement demands that could lead to injuries such as high speed running, sprinting, accelerations and decelerations.

As well as links to training load and injury, correlations have been reported between increased periods of training load and upper respiratory tract infections (URTI) (Cunniffe *et al.*, 2011a). An in-depth review and meta-analysis undertaken by Jones *et al.* (2017) concluded that immunosuppression occurs following large acute increases in training load making athletes much more susceptible to illness following a 7 - 21 day latency period. Individual characteristics have a significant impact on the internal training loads an athlete experiences. Keaney *et al.* (2021) reported no relationship between URTI risk and training load measures and suggested effective management of players influences this risk. It is suggested therefore, that longitudinal and consistent management of players can reduce the risk of injury and illness by reducing the occurrence of these sudden load increases.

2.1.4 Match activity profiles

Deciphering which external workload metrics to monitor needs to be both context and sport-specific (Gabbett *et al.*, 2017). It is essential therefore that the physical demands of the sport are understood. This understanding also facilitates practitioners and coaches' with the ability to prescribe and optimise training regimes that elicit individual physiological adaptations specific to playing position (Quarrie *et al.*, 2013). Additionally, with respect to performance, a greater understanding of game demands allows coaches to assess individual player contributions to a game (Jones *et al.*, 2015).

Rugby union is predominantly characterised as a sport in which intermittent periods of moderate to high-intensity activities (repeated running actions, static efforts and collisions) are interspersed with periods of lower intensity activity or rest (Cahill *et al.*, 2013; Quarrie *et al.*, 2013). There are a large number of published studies that have aimed to quantify the locomotor demands of a competitive RU match (Duthie *et al.*, 2005; Roberts *et al.*, 2008; Cahill *et al.*, 2013; Quarrie *et al.*, 2013; Jones *et al.*, 2015; Tierney *et al.*, 2021). However, methodological differences such as measurement method and quantification of metrics, have made locomotor and collision metrics difficult to compare. RU match activity has previously been assessed using time-motion analysis (Duthie *et al.*, 2005; Quarrie and Hopkins, 2007; Roberts *et al.*, 2008; Quarrie *et al.*, 2013) and has recently been superseded by GPS technology analysis (Cahill *et al.*, 2013; Jones *et al.*, 2015; Reardon *et al.*, 2015; McLaren *et al.*, 2016; Tierney *et al.*, 2021).

The disparity between study findings may arise due to the lack of consistency in terms of the threshold to which the various running intensities are quantified (West *et al.*, 2019). McLaren *et al.* (2016) defined a high-speed running distance between 15.0 – 19.9 km h^{-1} (4.4 – 5.5 $m \cdot s^{-1}$), whereas it has also been quantified as > 5 $m \cdot s^{-1}$ (Jones *et al.*, 2014; Tierney *et al.*, 2021). The number of participants is another important methodological consideration; Jones *et al.* (2015) reported a reduced total distance for match play when compared to Cunniffe *et al.* (2009). Consequently, this could be explained by Cunniffe *et al.* (2009) only conducting their analysis using 2 players (1 forward and 1 back), compared to the 33 professional players assessed in the other study. Findings have also shown large match movement characteristic variability between games (Forwards: 9 to 68 %, Backs: 9 to 44 %; dependent on metric) (McLaren *et al.*, 2016) and across different elite competition levels (Collision load-, the number

of collisions-, and high metabolic load efforts-per minute all increased at higher competition levels) (Tierney *et al.*, 2021).

Overall the total distance covered by forwards (4906 – 5581 m) during match-play is less than backs (5959 – 6127 m) (Roberts *et al.*, 2008; Cahill *et al.*, 2013; Jones *et al.*, 2015). Additionally similar findings between forwards and backs are observed when considering relative distance (77 ± 21 v 85 ± 10 $m \cdot min^{-1}$) (Lindsay *et al.*, 2015a). Backs also cover a greater majority of distance at high speed (> 5 $m \cdot s^{-1}$) (509 ± 150 m vs 231 ± 167 m) and total sprint distances (333 ± 122 m vs 121 ± 112 m) compared to forwards (Roberts *et al.*, 2008; Quarrie *et al.*, 2013; Jones *et al.*, 2015). Contrary to the above studies, research from Cahill *et al.* (2013) disputed that forwards actually undertook greater sprint distances. This discrepancy could be explained by methodological differences, as they used a relative percentage of max speed attained in games as opposed to absolute cut off totals e.g. >7 $m \cdot s^{-1}$. Additionally, max speeds were only obtained from match data and not in addition to data attained during training, this could lead to an underestimation of max speed and an overestimation of the distance forwards attain at max speed.

Research from Quarrie *et al.* (2013), Jones *et al.* (2015) and Cunniffe *et al.* (2009) additionally aimed to observe the impact of contact loads sustained during a match. Quarrie *et al.* (2013) used video and notational analysis to observe scrums, rucks, tackles and mauls whereas Jones *et al.* (2015) and Cunniffe *et al.* (2009) utilised the accelerometer-based metrics to gain an understanding of collisions. From these studies, it was concluded that although the locomotor demands of forwards may not be as high as backs, they are subjected to a greater amount of collision load. These findings have recently been further supported by Pollard *et al.* (2018), who reported collisions per minute for international forwards were significantly greater than international backs (Ball in play time: Forwards, 1.1 ± 0.2 vs backs, 0.5 ± 0.1).

2.1.5 Longitudinal Rugby union training and match load

As discussed, knowledge of the match demands is valuable to coaches and support staff in a training environment, as it ensures training specificity to facilitate match preparation and optimal player conditioning (Roberts *et al.*, 2008; Cahill *et al.*, 2013). On average, Premiership rugby players complete 6 hrs 48 min of training each week (West *et al.*, 2019) however, limited information on the volume and details of intensity demands of the training that is undertaken

currently exists (West *et al.*, 2021b). Additionally, very little information exists on how training load may fluctuate throughout a season. Longitudinal monitoring allows for an investigation as to how changes or accumulation in training load may be associated with performance or fatigue. This provides practitioners with objective data to help plan training over a full season, where the primary aim is to reduce the adverse effects of overtraining and prioritise performance (Jones *et al.*, 2017). Current training practices and load monitoring methods in elite team sports often remain unpublished, as there is an unwillingness to share data due to clubs wanting to retain a competitive advantage (Kelly *et al.*, 2020). Therefore the practices of elite teams and their approaches to season-long periodisation remain predominantly unexplored.

Limited studies have quantified the external training load of elite team sports using GPS throughout an entire professional season. Previous research has explored football (Malone *et al.*, 2015; Anderson *et al.*, 2016; Kelly *et al.*, 2020) and Australian Football (Moreira *et al.*, 2015a; Ritchie *et al.*, 2016). Prior research in RU, sevens and league has quantified aspects of preseason (Bradley *et al.*, 2015b; Daniels *et al.*, 2019; Grainger *et al.*, 2020; Tiernan *et al.*, 2020), microcycles (McLean *et al.*, 2010; Moreira *et al.*, 2015b) and intensified periods (Bouaziz *et al.*, 2016; Lacome *et al.*, 2018). These findings however are too short to display potential periodisation techniques throughout a full season. Furthermore, where intensified periods and preseason have been observed, this is not a fair reflection of the whole season, as in-season training prioritises recovery from and preparation for upcoming competitive games.

Table 1 displays the average weekly training and match totals from studies that have observed a professional rugby team throughout a season. The findings highlight that weekly training loads typically reflect the positional match activity differences that have previously been discussed. Backs complete greater weekly total distances, high-speed distances and more accelerations than forwards (Bradley *et al.*, 2015a; Dubois *et al.*, 2020a; Dubois *et al.*, 2020b). No differences in high impact collision demands were observed between forwards and backs (Dubois *et al.*, 2020a; Dubois *et al.*, 2020b), this was measured via inertial sensors rather than a direct count.

The quantification and distribution of training and match load across a full season in professional RU has only been described in two research papers Dubois *et al.* (2017) and Dubois *et al.* (2020b). Dubois *et al.* (2017) reported a progressive but significant reduction in

total distance, whereas moderate and high-speed running distances increased progressively and significantly throughout the season. This suggested that at the end of the season a low volume, high-intensity training methodology was prescribed. It is important to note, however, that this study only monitored 8 backs players, hence it remained unknown if forwards follow the same periodisation model. Dubois *et al.* (2020b) observed both professional forwards (n = 6) and backs (n = 8) throughout a full season. They found the highest total distances occurred in preseason compared to any in-season block. Unlike the previous study, they did not find a reduction in the total distance throughout the in-season period and they did not measure changes in high-speed running distances so this is not comparable. Although, they do report a significant reduction in heavy impacts towards the end of the season, which could suggest a reduction of contact practices in training. This could relieve some of the soreness stressors that occur with high levels of contact. These studies suggest that workload does change depending on the period within the season.

Position (forward vs back) has been the predominant contextual factor that studies have observed. Very limited studies exist where the player status (starter vs non-starter) has been considered with regard to training load. The weekly game is considered as a large proportion of the total weekly training stimulus, resulting in different individual loading patterns depending on whether a player starts or not (Kelly *et al.*, 2020). This creates difficulties for coaches trying to maintain squad physical fitness, as not all players are involved. Dubois *et al.* (2020a) monitored 14 professional players (6 forwards and 8 backs) throughout a full season. They reported no significant difference between the weekly total distance of starters and substitutes (12535 ± 3317 m vs 11769 ± 3168 m) but, did report a significant reduction in the high-speed running distance of substitutes (2012 ± 796 m) compared to starters (1614 ± 665 m). These findings suggest that substitutes should have their training practices manipulated to ensure comparable seasonal workloads.

Table 1. External training and match load values of professional rugby union players.

Participants	Total distance	Speed zone distances	Accelerometer-based metrics	Study
44 professional rugby union players (24 forwards, 20 backs) from a team in the European Rabo direct Pro 12 league	Forwards - 7827 ± 954 m Backs - 9572 ± 1233 m	**Jogging (4.4 – 5.6 m·s^{-1})** *Forwards - 665 ± 175 m* *Backs - 993 ± 196 m* **High-speed Running (5.6 – 7.5 m·s^{-1})** *Forwards - 194 ± 141 m* *Backs - 617 ± 232 m* **Sprinting(> 7.5 m·s^{-1})** *Forwards - 4 ± 17 m* *Backs - 40 ± 61 m*	**Maximal accelerations (> 5 m·s^{-2})** *Forwards - 15 ± 10* *Backs - 46 ± 15* **RHIE** *Forwards - 19 ± 8* *Backs - 15 ± 10*	(Bradley *et al.*, 2015a)*
8 professional backline rugby union players from a team in Rugby Pro D2	Backs - 19316 ± 2923 m	**Moderate and high-speed Running (> 13 km.h^{-1} / > 3.6 m·s^{-1})** *Backs - 3996 ± 701 m*		(Dubois *et al.*, 2017)
24 elite junior rugby union players in the u20 World Rugby Union Championship	High match play exposure time - 39030 ± 8061 m Low match play exposure time - 33923 ± 5797 m	**High-speed running distance (Individualised threshold according to maximal aerobic speed)** *High match play exposure time - 3427 ± 1865 m* *Low match play exposure time - 3260 ± 1416 m*		(Lacome *et al.*, 2018)**
14 professional rugby union players (6 forwards, 8 backs) from a French team in the Top 14	Forwards - 10866 ± 2419 m Backs - 13700 ± 3490 m	**High-speed running distance (> 14.4 km.h^{-1} / > 4.0 m·s^{-1})** *Forwards - 1425 ± 2419 m* *Backs - 2442 ± 724 m* **Very high-speed running distance (> 19.9 km.h^{-1} / 5.5 m·s^{-1})** *Forwards - 419 ± 157 m* *Backs - 1115 ± 428 m*	**Accelerations (> 2.5 m·s^{-2})** *Forwards - 125 ± 40* *Backs - 130 ± 53*	(Dubois *et al.*, 2020b) ***
14 professional rugby union players (6 forwards, 8 backs) from a French team in the Top 14	Forwards - 11482 ± 3239 m Backs - 14382 ± 4042 m Starters - 12535 ± 3317 m Subs - 11769 ± 3168 m	**High-speed Running (> 14.4 km.h^{-1} / > 4.0 m·s^{-1})** *Forwards - 1148 ± 468 m* *Backs - 2516 ± 785 m* *Starters - 2012 ± 796 m* *Subs - 1614 ± 665 m*	**New Body Load** *Forwards - 219 ± 71* *Backs - 321 ± 116* *Starters - 263 ± 107* *Subs - 232 ± 116*	(Dubois *et al.*, 2020a)

Note: * Is representative of only weekly training load, no game included. **Cumulated external workload throughout a 19-day tournament. ***Data representative of starting players. RHIE is defined as a cluster of three user-defined high-intensity efforts (contacts, accelerations or sprints) performed <21 s apart.

2.2 The training process

Due to the complex demands of RU competition, players are required to possess a broad range of well-developed physical qualities. Training is a process (Figure 3) they undertake whereby they are systematically and repetitively exposed to exercise stimuli to induce positive functional adaptations, underpinning physical performance (Impellizzeri *et al.*, 2019). It is common practice for practitioners to manipulate the measures of the fundamental principles of training (frequency, exercise intensity, volume & distribution over time), to apply a sufficient load to stimulate specific and individualised exercise-induced adaptations. Training sessions are designed to induce a specific physiological response, it is this response rather than the exercise task itself that provides the stimulus for adaptation (Booth and Thomason, 1991). Borresen and Lambert (2009) stated that the correlation between the imposed training load and changes in physiological variables is individually specific. The training response is dependent upon a multitude of factors that may influence a player's tolerance of an exercise load, suggesting that athletes may have a specific individual threshold. Individual athletes may respond differently to a given training stimulus and the required training load to promote adaptation may differ between them, requiring the need for individualised monitoring (Halson, 2014). To maximise the exercise-induced adaptive response coaches need to moderate the stress applied to athletes at an individual level. Within RU the one size fits all approach would not be appropriate, as it would reduce the likelihood all players will be subjected to a training load that is specific to their individual physiological requirements. However, continuously monitoring individual loads allows the ability to "boost" a player when his metrics suggest that he needs additional load or "regenerate" a player when he needs reduced load.

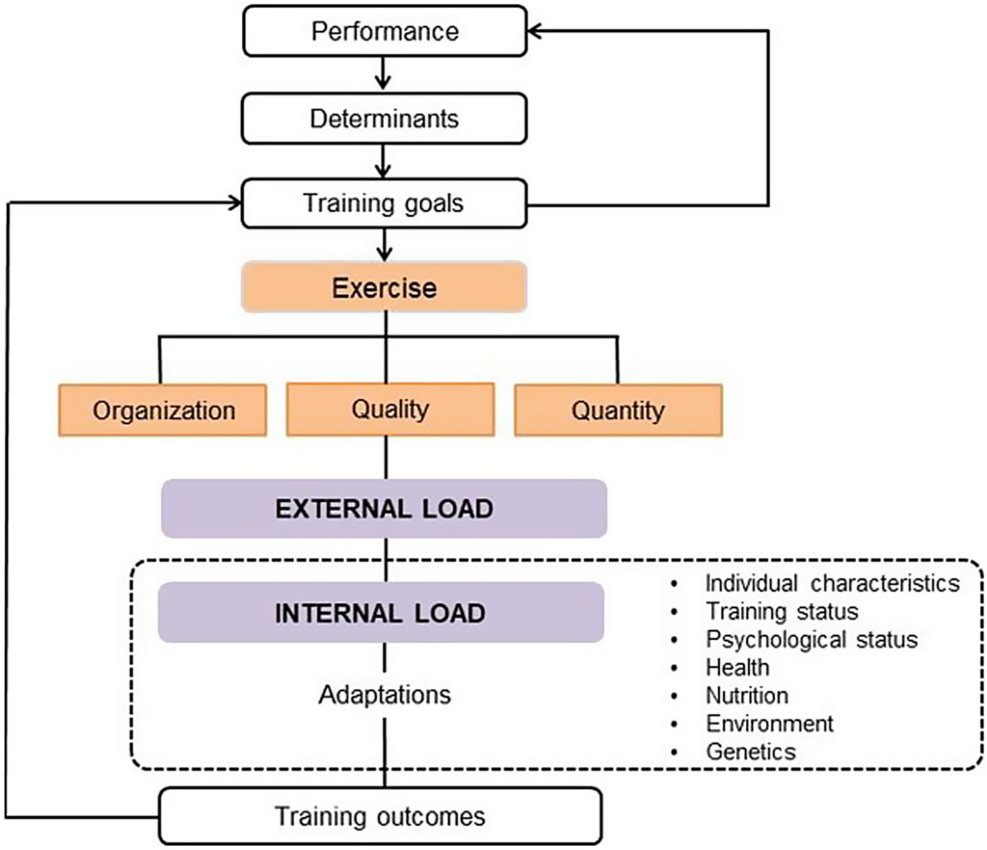

Figure 3. The training process as proposed by Impellizzeri *et al.* (2019).

2.2.1 The dose-response relationship

Identifying players 'optimal load' to elicit maximum performance levels is of significant interest to practitioners working in elite sport. The effectiveness of different training load prescription methods is dependent on the interactions between training load, 'the dose' and the subsequent training outcome such as, fitness, fatigue and performance, and 'the response'. Underpinning this relationship is the fitness-fatigue model proposed by Banister *et al.* (1975). This model stated that training results in two outcomes, a positive outcome (fitness) and a negative outcome (fatigue). The fitness response is the positive physiological adaptation to long-term training, this will result in an increase in performance. The fatigue response is the shorter term negative consequence of training (e.g. neuromuscular fatigue), resulting in a decrease in performance (Banister *et al.*, 1975). Improperly managed loads could lead to accumulated fatigue, which results in negative responses e.g. illness, injury and non-functional overreaching (Figure 4A) (Halson, 2014). However, where appropriate recovery follows intensified training, this process enables the necessary stimulation for positive physiological adaptation (Figure 4B) (Hacker *et al.*, 2021). This supports the need for monitoring the dose-

response relationship to attain consistently high performance and avoid the negative consequences of fatigue. As elite rugby players are consistently training and in a constant flux between fitness and fatigue, it is likely the dose-response will not always produce a uniform adaptation when continually repeated, hence coaches need to be adaptable and utilise continuous athlete monitoring to explore the relationship to maximise an individual training effect (Smith, 2003).

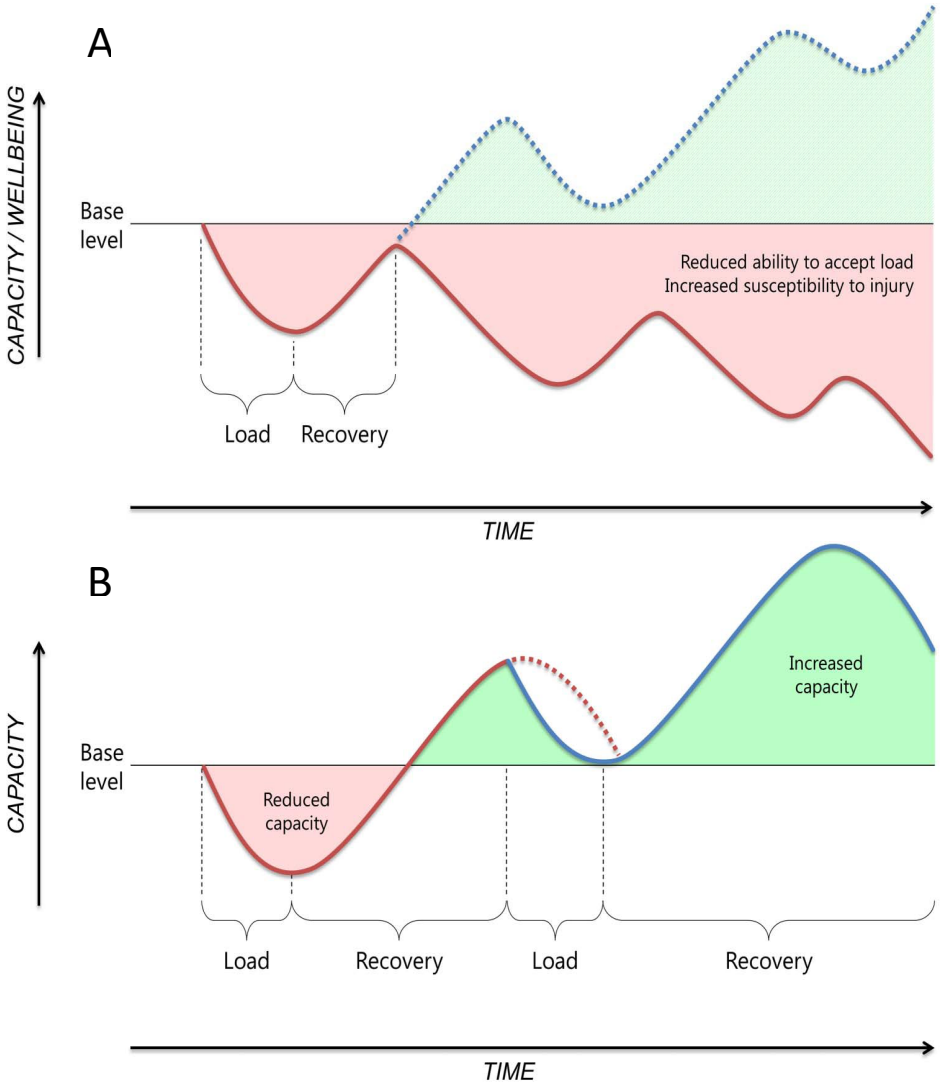

Figure 4. Soligard *et al.* (2016) presents the relationship between the training and match 'dose' and the athlete 'response'. These responses can result in a maladaptation (A) or positive physiological adaptation (B).

2.2.2 Variable responses to training

Physiological adaptations to training programs are prone to significant inter-individual variability. Hubal *et al.* (2005) examined the responses of 342 women and 243 men following

12 weeks of resistance training. They reported a wide range of responses to resistance training; 1 RM strength change ranged between 0 to 250 % and ~ 10 % of the individuals did not exhibit any muscle hypertrophy changes. McLaren *et al.* (2018) investigated the inter-individual fitness responses to an 8-week preseason in professional RU players participating in the English rugby championship. The proportion of individual responders to a variety of fitness tests ranged from 37 to 82 %. They also found large inter-individual differences in the fitness response to preseason training, as 18 to 63 % of players showed no meaningful change above what is observed during the off-season period. This highlights the heterogeneous response to exercise even among a homogeneous group of high-level RU players. There is a substantial genetic influence on an individual's trainability to exercise, supported by heritability estimation research (Bouchard, 2012; Mann *et al.*, 2014), although current knowledge is limited with regards to the links between genetics and inter-individual variation in response to exercise training (Vellers *et al.*, 2018). Conversely, the study by McLaren *et al.* (2018) also provides an area for thought, as it is being assumed that all players received the correct and therefore optimal dose to promote an adaptation. The study does not provide a measure of external load or provide detail as to how individual training load was prescribed. Due to the nature of team sports training, players will likely receive unequal doses of training which also could be the cause of the measured heterogeneous response.

A myriad of intrinsic and extrinsic factors can influence an individual's response to exercise (Figure 5). Participant compliance and the intensity of a training program are often reported in research papers and can provide some insight into observed responses. Little information is known though about how the rate of compliance may influence the response and often there appears to be no reported cut off on individuals who don't complete every testing session. Additionally, standardised programs based on intensity and duration are often provided to participants. A participant's individual variation of homeostatic stress, associated with each training session would result in a different exercise stimulus. This would contribute to varied adaptive responses to training programs (Mann *et al.*, 2014).

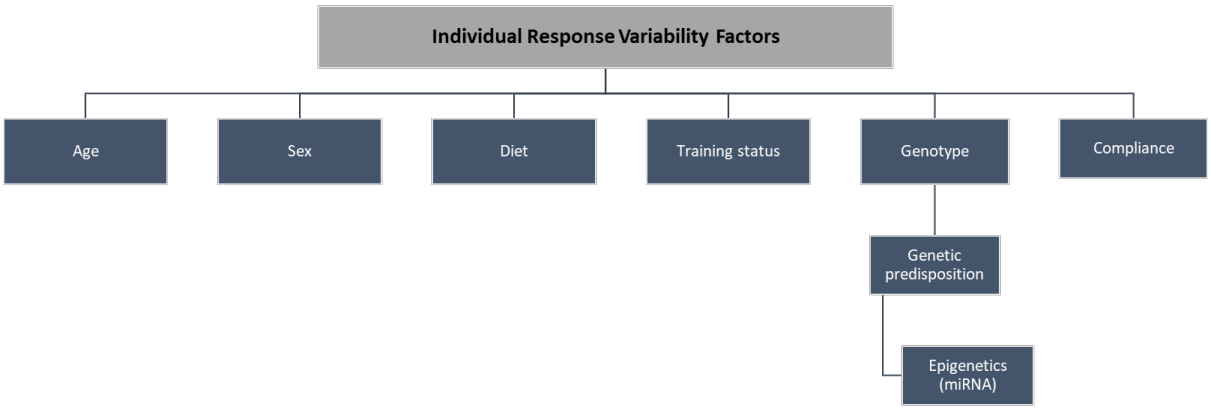

Figure 5. Various intrinsic and extrinsic factors which can influence an individuals training response.

2.3 The relationship between physical characteristics and rugby union performance

Rugby union is a unique sport where player roles differ distinctly as each position requires specific technical and physical qualities (Duthie *et al.*, 2003). The advancement of GPS technology and its usage in matches allows for an accurate and non-invasive measure of the movement characteristics required in RU. This has helped elucidate the positional differences in physical and metabolic demands (Quarrie *et al.*, 2013; Jones *et al.*, 2015). Broadly, professional RU requires a well-developed aerobic and anaerobic system, as well as strength, speed and power qualities.

Backs in attack are required to beat the opposition using a combination of speed, agility and strength. They are also required to continuously reposition themselves in defence (Nicholas, 1997). When compared to forwards, backs tend to cover greater total distances. A larger proportion of this distance is at higher speeds and closer to their maximum velocity (Roberts *et al.*, 2008). These movement demands suggest a highly developed anaerobic metabolism and also a greater proportion of fast-twitch fibres (Heffernan *et al.*, 2015). Forwards are known as the 'ball winners', as they have an important role in contesting possession at breakdowns and set pieces (Nicholas, 1997). Thus, the forwards game requires them to be involved in more static and impact-related exertions such as scrums, line-outs, rucks and mauls (Cahill *et al.*, 2013). This requires forwards to possess a high degree of strength and additionally, have an enhanced ability to recover between high-intensity and static bouts of activity (Heffernan *et al.*, 2015).

A good understanding of match demands is needed to inform training practice, as this allows practitioners to prescribe optimal programs to players that elicit appropriate and specific adaptations needed to perform in the game (Austin and Kelly, 2013). The positional specific phenotypes have been discussed in a study by Brazier *et al.* (2020), who reported the differences in anthropometric and physiological characteristics between elite rugby players. Both forwards and backs body mass has increased over time, Sedeaud *et al.* (2013) showed that over a 20 year period (1989 – 2009) elite French backs and forwards have become heavier by 12 kg and 12.3 kg. The increase in mass can be attributed to its importance in generating and tolerating greater forces in tackles and greater superiority in collisions (Gabbett *et al.*, 2008). Furthermore, for forwards, a heavier individual mass is associated with a greater individual ability to produce force in the scrum (r = 0.54; 95% CI = 0.27± 0.73) (Quarrie and Wilson, 2000). With respect to body fat, forwards tend to have a greater fat mass as backs rely on a greater lean mass relative to the total body mass ratio (Heffernan *et al.*, 2017). This is reflective of the higher power-to-body mass ratio which is desirable for the strength, speed and power needed for performance (Duthie *et al.*, 2003).

Rugby union is an intermittent sport, where long distances at low speeds are interspersed with periods of high-intensity activity, hence a high maximal aerobic power is important for all players. This allows a consistently high level of required skill execution to be undertaken on top of the required work demands (Nicholas, 1997). An improved capacity of the aerobic system will also enhance the recovery between the bursts of high-intensity exercise (Johnston *et al.*, 2014). Typically it has been indicated that backs possess a higher level of endurance fitness than forwards (Duthie *et al.*, 2003). Cunningham *et al.* (2018) measured the endurance fitness of forwards using the Yo-Yo intermittent recovery test. They reported positive correlations between the scores of this test and with effort-based match activities such as the number of tackles (r = 0.717) and effective attacking rucks hit (r = 0.651). They also observed significant positive correlations for performance-based match activities, such as the percentage of caries made over the gainline (r = 0.610) and the number of successful turnovers (r = 0.518), thus highlighting the importance of physical qualities that are associated with successful key performance indicators.

The energy contributions required for periods of high activity in rugby such as repeated high-speed efforts (> 5 m·s) and efforts requiring tackling, hitting rucks and scrummaging are primarily anaerobic in nature. Within a game backs are required to sprint multiple times above

7 m·s (Eaton and George, 2006), highlighting that repeated sprint ability is an important attribute. Smart *et al.* (2014) used the Rugby-Specific Repeated-Speed test (RS^2) to measure anaerobic performance and repeated sprint ability in elite RU players, they found that forwards with a slower average time of 12 × 20m sprints performed a lower activity rate in games and a low activity rate was also observed for backs who had a high repeated sprint fatigue score.

Possessing high levels of muscular strength and power is also required for elite rugby performance. Distinct differences in strength with regards to squat and bench press have been reported, with professional forwards being notably stronger than backs (Smart *et al.*, 2013; Bradley *et al.*, 2015b). The relationship between strength and power is an important factor; strength is defined as the maximum force produced by a muscle or group of muscles at any given speed, and power is the product of strength and speed (Baker and Nance, 1999). Baker and Nance (1999) showed that in rugby players, maximal strength is highly related to maximal power. Relationships have also been shown between strength and power qualities and key match performance indicators (Smart *et al.*, 2014; Cunningham *et al.*, 2018). The positives of stronger players have also been found to benefit other physical qualities needed to perform in rugby such as sprinting (Comfort *et al.*, 2012; Furlong *et al.*, 2021).

Speed testing highlights distinct differences between forwards and backs (Duthie *et al.*, 2003; Brazier *et al.*, 2020) and reflects required match demands, as backs players require speed to evade opponents and accelerate through contact (Nicholas, 1997). As typical sprint distances in rugby are no greater than 20 m (Duthie *et al.*, 2006; Austin *et al.*, 2011), the development of acceleration capabilities is of primary importance. The importance of speed is highlighted by Smart *et al.* (2014) who revealed that faster professional players were more evasive with ball in hand, made more line breaks and broke more tackles.

Elite RU players, therefore, embody a unique phenotype where distinct physiological qualities are required for a successful performance. It is recognised that regular training promotes advantageous physiological adaptations however, less is known about how these adaptations may differ in accordance with an individual's genetic predisposition and heritability. Greater knowledge of the mechanisms that govern the exercise-induced adaptive pathways is important to understand when training for athletic performance (Coffey and Hawley, 2007).

2.4 Fatigue in rugby union

Fatigue is a complex phenomenon that can be appreciated as an exercise-induced reduction in the ability to generate maximal voluntary muscle force (Gandevia, 2001). The mechanisms underlying fatigue are numerous. Origins of muscle fatigue can be central (proximal to the neuromuscular junction) (Taylor *et al.*, 2016) or peripheral (distal to the neuromuscular junction) (Boyas and Guével, 2011). It has been found that in central fatigue the recruitment of the new motor units and/or firing frequency of the active units is lowered (Green, 1987) and that peripheral fatigue takes place primarily in the contractile processes (Bigland-Ritchie *et al.*, 1986). The peripheral and central components of muscle fatigue are intrinsically related since the recruitment of motoneurons depends on the descending drive from supraspinal sites, and the central drive is controlled through a combination of excitatory and inhibitory reflex inputs from muscles, joints, tendons and cutaneous afferents (Millet *et al.*, 2011). Buckthorpe (2014) summarised that muscle fatigue negatively impacts muscle performance and therefore hinders the ability for an athlete to produce maximal and explosive force.

Rugby athletes often complete multiple training sessions daily and on consecutive days to ensure both rugby-specific and physical performance needs are achieved. The high density of the weekly schedule can lead to high fatigue accumulation. Participation in rugby training and competitive games is associated with enhancing a fatigued state. Players are subjected to neural types of training (high load/ velocity of contraction) in the gym or on the field, as well as the impact that occurs during training and competition. These events result in exercise-induced muscle damage and the consequent inflammation which plays a primary role in the decreased capability to perform (Tavares *et al.*, 2017). It is important to understand, however, that fatigue does not just manifest itself as a reduction in muscle performance. Fatigue can reduce both physical and cognitive function as a result of exercise-induced impairment of performance and sensations of tiredness and weakness (Enoka and Duchateau, 2016). This demonstrates the multisystem nature of fatigue and why it is important to take a multivariate approach to monitor fatigue in elite RU players.

2.5 Monitoring tools in rugby union

As part of athlete monitoring, it is common practice to have a system in place to detect and monitor the development of fatigue in players (Halson, 2014). Monitoring markers have proved critical to understanding the dose-response relationship. These tools enable the

identification of individuals who may not have had adequate recovery and maybe show early signs of non-functional adaptations (Coutts *et al.*, 2007). To date however, no one marker can accurately quantify the response to a bout of training (Bourdon *et al.*, 2017). Therefore, an array of markers is required to provide coaches with sufficient knowledge to make evidence-based decisions regarding how best to optimise an individual's performance. It has previously been recommended that a multi-dimensional approach to blood biochemistry, psychological and performance parameters are needed to quantify stress and recovery (Meister *et al.*, 2013). It is important to not just understand how markers change week to week but also throughout a season. This provides insights as to how players may be tolerating fatigue as a result of the accumulating training load and matches. Residual fatigue could have built up over the course of the season combined with inadequate recovery time between weekly competition and rugby training demands. To ensure an optimised approach to prescribing training, subjective and objective information is crucial to ensure performance.

2.5.1 Physical performance measures

As previously mentioned in section 2.3, professional RU players, require a broad range of physical capabilities and superior physical adaptations are associated with key performance indicators (Cunningham *et al.*, 2018). Therefore, throughout a competitive season, it is critical to enhance and ensure players are performing optimally. Strength and power have previously been observed throughout a full English Premiership season, results show that the greatest opportunity for physical development occurs in the preseason and that maintenance or slight decreases define the latter stages of the season (Gannon *et al.*, 2016). The decrease in physical qualities at the latter stages of the season could reflect the diminishing likelihood of positive physical adaptations, due to the association with accumulated fatigue. Additionally, periods of intense competition have been associated with reductions in leg explosive power and speed variables (Meister *et al.*, 2013). This requires careful management and planning throughout a season to limit these effects.

Declines in performance measures can indicate impaired neuromuscular performance (Hills and Rogerson, 2018). Primarily within the literature, the counter movement jump (CMJ) has been the most utilised measure to assess lower-body performance and the impact of neuromuscular fatigue. Studies observing the acute impact of a rugby game on neuromuscular

performance have highlighted players may be compromised for up to 48 hours following a game (West *et al.*, 2014; Shearer *et al.*, 2015). Twist *et al.* (2017) measured CMJ flight time throughout an intensified fixture schedule (4 matches in 22 days) in professional rugby league. They reported a non-linear reduction in CMJ performance across the intensified period, indicative of the accumulated neuromuscular fatigue. This highlights to practitioners that they need to be aware of the negative performance outcomes associated with cumulative fatigue and the need to measure throughout a season.

Changes in performance measures have also been assessed across a competitive season in elite RU players (Table 2). Throughout a 13-week season, Argus *et al.* (2009) reported moderate increases in box squat strength performance (9%) with a small decrease in jump squat power performance (-3%). Although Gannon *et al.* (2016) reported increases in peak force (4%) performed via an isometric squat and power (3%) performed via a hack squat from baseline at the start of preseason to the end of the season, they did report decreases in both measures from mid-season to end of the season (-1% for both measures). These findings match with acute changes in CMJ following a rugby league match, where power was reduced, but the force-producing capacity was maintained (McLellan *et al.*, 2011b). Interestingly, from a longitudinal perspective, the reduction in measures from mid-season to the end of the season suggests this period is crucial when attempting to optimise the performance of players.

Studies from Dubois *et al.* (2020b) and Hills and Rogerson (2018) both tracked CMJ performance throughout a competitive RU season. Dubois *et al.* (2020b) reported no significant changes in CMJ performance throughout the season, whereas Hills and Rogerson (2018) reported a significant reduction in CMJ velocity from baseline across the 12-week competitive period. The differences between the two findings may be firstly due to the sampling times. Hills and Rogerson (2018) recorded CMJ performances the first day back into training, whereas the cohort in Dubois *et al.* (2020b) study didn't report CMJ performances until 2 days before a match and following 24 hours of recovery. Hence the players in the first study may still be exhibiting fatigue at the start of the week. Also, there is a difference in the CMJ metrics collected between the studies. Dubois *et al.* (2020b) observed only flight time whereas Hills and Rogerson (2018) used a linear positional transducer to observe a variety of CMJ variables incorporating a velocity component. It has previously been reported that CMJ parameters involving a velocity measure are more sensitive to neuromuscular fatigue (Johnston *et al.*, 2014).

Within RU, no studies to date have implemented measures to see the impact of high or low training and game exposure on performance parameters. Meister *et al.* (2013) conducted a study on elite footballers (N = 19, age: 19.7 ± 2.8 years, weight 75.3 ± 8.3 kg) to observe the effect of 3-week periods of high versus low match exposure on CMJ and drop jump performance, throughout the season. Results showed no significant changes in either of these tests, which could suggest players were able to tolerate the higher game exposures as no signs of neuromuscular fatigue were evident. A possible limitation of this study is that players could be rotated and change exposure groups throughout the season, it therefore may not assess the impacts of players who had consistently high (the regular starters) training load demand.

To summarise, the limited available literature looking at performance changes and the impact of neuromuscular fatigue throughout a professional rugby season, has highlighted that athletes do experience training and match induced fatigue for substantial parts of the season. This is particularly evident following periods of intensive training and at the end of the season period. Additionally, the changes in performance have been associated with training load, perceived wellness, and the number of heavy contacts (Table 2), highlighting the importance of sensitive monitoring markers to assess the dose-response relationship and enable an optimised approach to prescribing training.

Table 2. Studies investigating longitudinal performance changes throughout a rugby union season

Subject characteristics	Measures	Protocol	Findings	Study
32 professional rugby union players from a super 14 team (age = 24.4 ± 2.7 years, body mass = ; 104.0 ± 11.2 kg)	**Upper-body measures:** Bench press Bench throw **Lower-body measures:** Box squat Jump squat	Players were assessed on a minimum of 2 and up to 5 occasions throughout a 13-week competition phase, following a 7-week preseason phase.	Throughout the competitive season: Bench press *trivial* ↓ (-1.7 kg) Bench throw *trivial* ↓ (-40 W) Box squat *small* ↑ (16 kg) Jump squat *small* ↓ (-175 W)	Argus *et al.* (2009)
16 professional rugby union players from an English Premiership team (age = 23 ± 4 years, body mass = ; 109.0 ± 11.4 kg)	**Lower-body measures:** Isometric squat Explosive hack squat	Players were assessed at the start of preseason (T1), post-preseason (T2), mid-way through the competitive season (T3) and at the end of the competitive season (T4).	*Very likely* ↑ in force at 50 ms and 100 ms between T1-T2 (isometric squat). *Likely* ↑ in power between T2-T3 (explosive hack squat). *Likely* ↓ in force at 50 ms and 100 ms between T3-T4 (isometric squat).	Gannon *et al.* (2016)
8 professional rugby union backs from a Rugby Pro D2 team (age = 25.8 ± 4.2 years, body mass = ; 88.4 ± 3.1 kg)	**Lower-body measures:** Drop jump	Players performed 3 maximal drop jump tests 2 days before each match on an Optojump. Tests were measured throughout the competitive season (n = 26).	Drop jump power index (DJPI) was significantly ↓ in block 0 compared to all other blocks throughout the season. Session-Rating Perceived Exertion (s-RPE) was highest at this time point. The % change in DJPI was significantly correlated to total distance (r = -0.49) and subject training load (r = -0.40).	Dubois *et al.* (2017)
37 professional rugby union players from an English Championship team (age = 25.9 ± 4.1 years, body mass = ; 103.8 ± 13.7 kg)	**Lower-body measures:** CMJ with 20 kg barbell CMJ	Over 12 weeks of a competitive season, players performed 4 CMJ tests weekly. Tests were taken on the 1st training day back of each week and 48 hours after a game.	CMJ velocity reduced significantly from baseline across the 12 weeks (P < 0.05). Large positive associations were observed between wellness, time to peak velocity (r = 0.74) and peak velocity (r = 0.67).	Hills and Rogerson (2018)
14 professional rugby union players from a French team in the Top 14 (age = 26.9 ± 1.9 years, body mass = ; 97.6 ± 13.2 kg)	**Upper-body measures:** Bench press **Lower-body measures:** CMJ **Fitness measures:** YYIRT	Players performed 3 YYIRT throughout the season. Maximal strength tests were assessed 5 times throughout the season. CMJ measures were taken in the strength sessions after 24 hours of full recovery and 2 days before a game (n = 14).	No significant changes observed throughout the overall season for CMJ performances or upper-body strength measures. Chronic workload of conditioning sessions (s-RPE) moderately influenced the $\dot{V}O_{2max}$ estimated from YYIRT (r = 0.46). The number of heavy impacts was slightly negatively associated with bench press performance (r = -0.29). Chronic weekly workload was negatively associated with CMJ performance (r = -0.36).	Dubois *et al.* (2020b)

Note: CMJ = Counter movement jump; YYIRT = Yo-Yo intermittent recovery test; $\dot{V}O_{2max}$ = Maximal oxygen consumption

2.5.2 Blood-based biomarker measures

Evaluating training or match responses via the consistent use of maximal-strength testing is not applicable within an elite environment due to the further fatigue-inducing factors performing these tests can cause. Blood-based biomarkers could be a viable method to assess exercise response with minimal interference on training or games (Hacker *et al.*, 2021). Assessing a variety of these biomarkers can provide a powerful tool to identify the balance between training and recovery (Lee *et al.*, 2017). There are a variety of biomarkers that provide information about an athlete's preparedness and recovery status, such as indicators of muscle damage (creatine kinase, myoglobin, C-reactive protein), inflammation (interleukin 6) and health status (full-blood count, immunoglobulin A) (Lindsay *et al.*, 2015c; Lee *et al.*, 2017; Burden *et al.*, 2019). Previous biochemical analysis has provided key insights into the individual nature of an ability to recover week-to-week from the competition and training of professional rugby (Lindsay *et al.*, 2015b). Understanding elite rugby players' recovery responses from a competition provides key information to aid practitioners and coaches when designing the following week to prepare players for subsequent bouts of competition.

A primary measure of muscle damage, and likely the most common biomarker investigated in RU and league literature is creatine kinase (CK) (Takarada, 2003; McLellan *et al.*, 2010; McLellan *et al.*, 2011a; McLellan *et al.*, 2011b; Jones *et al.*, 2014). Prior research has revealed, that in response to a match, subsequent changes in CK are associated with the number of impacts, collisions, high-speed running distance and acceleration demands (Takarada, 2003; McLellan *et al.*, 2011a; Oxendale *et al.*, 2016). Similarly, studies have also observed the acute effects of competitive matches on subsequent responses to measures of endocrine function (Cunniffe *et al.*, 2010; West *et al.*, 2014; Lindsay *et al.*, 2015c) and inflammatory markers (Cunniffe *et al.*, 2010). This information provides crucial information on the time-course responses following competitive games and helps to guide recovery protocols and structure the following training week. However, limited studies observe the longitudinal impact and the accumulative impact of games on biochemical disturbance. For relevance to this thesis, this section will be focusing on longitudinal measures of full-blood counts and CK.

Limited studies have monitored either CK or haematological profiles in RU players throughout a full season (Table 3). Banfi *et al.* (2006) was the first paper to observe changes in blood haematological markers across a competitive RU season. They recruited 19 players from the Italian national team training camps, 13 players provided samples for all 4 camps and 6 players

from 3 camps. They reported significant changes in haemoglobin (HB), haematocrit (HCT) and red blood cells (RBC) throughout the season. Specifically, values of all measures significantly reduced at time-point 3 (before the Six Nations tournament) compared to time-point 1 which was at the start of the training period. Additionally, RBC and Hct were also significantly reduced at time-point 4 (end of the national championships) compared to time-point 1. They concluded a reduction in HB and HCT was associated with the increased physical demands, although no training volumes were reported. Without this information, it is hard to confirm if the variability of the haematological parameters during the season is linked to different training and competition workloads.

A recent study by Dubois *et al.* (2020b) also tracked biomarker measures throughout a professional RU season. They obtained blood samples at 3-time points throughout a competitive season from 14 professional players. Unlike the study by Banfi *et al.* (2006), they only observed a significant change in HCT, which decreased between the middle and end of the competitive season. In this study, external training load was quantified, yet no significant change in total distance was observed between these time points. Thus, this makes it difficult to confirm the impact of training and competition load on haematological variables, but towards the end of the season, it is important to take into account the length of time players have been training and competing. They also observed associations between acute heavy impacts, acute competitive workload and CK. CK was moderately lower during the second sampling point compared to the first (489.9 ± 361.9 $U \cdot L^{-1}$ vs 849.2 ± 725.3 $U \cdot L^{-1}$). This finding is generally in agreement with the conclusions of previously published studies, which report an increase in CK during the first 3– 5 weeks of a season, followed by a reduction (Alaphilippe *et al.*, 2012). Importantly both of these studies did not measure a true baseline as players on both occasions were in-season. Understanding a true baseline for players needs to be undertaken before any competition or training (Lee *et al.*, 2017). The best occasion to do this is following the off season and prior to preseason.

Within football, it has been much more common to employ routine blood sampling throughout a season (Meyer and Meister, 2011; Heisterberg *et al.*, 2013; Owen *et al.*, 2018). Similar to the findings in RU studies, they have also reported significant changes in HCT and CK (Meyer and Meister, 2011; Heisterberg *et al.*, 2013) as a result of competition and training. However, similar to the prior studies observing longitudinal blood sampling in RU, the findings from Heisterberg *et al.* (2013) are difficult to interpret due to the lack of a baseline, as samples

were obtained during periods of training and competition. It is very difficult to understand the impact of an accumulated physical demand, but a very interesting study from Owen *et al.* (2018) aimed to explore the association between post-seasonal haematological profiles and seasonal minutes players played. They observed significant associations between the total minutes played and RBC (R^2 =0.34), mean corpuscular volume (MCV) (R^2 =0.34) and mean corpuscular haemoglobin (MCH) (R^2 =0.33). This data suggests the haematological parameters are significantly impacted in players with greater minutes accumulated across the playing season versus players with less playing time. Only one study to date has reported on the association between game time and blood based biomarkers in rugby union (Alaphilippe *et al.*, 2012). They observed significant associations between game time and CK (r = 0.46).

In summary, limited longitudinal observational studies in professional RU exist, this makes it difficult to understand if and how blood-based biomarkers may fluctuate throughout a season. Previous papers have displayed methodological issues whereby samples were not collected during a true rested baseline. Instead, typically comparisons are made comparing to either the start of the competitive season or during the preseason. These time periods are already associated with greater training stressors making observations into change difficult (Lee *et al.*, 2017). However, studies have supported the need to observe a variety of biomarker measures throughout a season. The regular analysis of these samples could provide important insight into the optimisation of training prescription and periodisation. It is also essential that this analysis is used on an individual basis as this may help coaches identify 'at-risk' players throughout the season (Heisterberg *et al.*, 2013). Currently, very little research has observed the potential differences that regular starters may experience compared to other match statuses such as bench and non-squad players. A further limitation to the majority of research to date is the lack of physiological or physical data that is used in conjunction with biomarker data to contextualise the results. This would enable a greater understanding of the process.

Table 3. Studies investigating longitudinal biomarker changes throughout a rugby union season

Subject characteristics	Measures	Protocol	Findings	Study
19 international rugby union players from an English Premiership team (age = not provided = ; 100.7 ± 14.1 kg)	**Haematological parameters:** WBC, RBC, HB, HCT, Plts, MCH, MCHC, Ret	Venous blood sample measures were collected throughout the season 1st blood sample - Start of the training period (T1) 2nd blood sample - After the training meeting and before the start of the Italian and French championships (T2) 3rd blood sample - Before the Six Nations tournament (T3) 4th blood sample - End of the national championships (T4)	↑ levels of HB and HCT at the start of the season and a reduction when physical demand is higher. WBC and Plts are stable throughout the season. RBC ↓ towards the end of the season when training loads were increased.	Banfi *et al.* (2006)
17 professional rugby union players from a French team in the Top 14 (age = 27.0 ± 3.4 years, body mass = ; 94.1 ± 10.0 kg)	**Haematological parameters:** Neutrophils, monocytes **Muscle damage Markers:** CK	Venous blood sample measures were collected throughout the season 1st blood sample - Beginning of the competitive period (T1) 2nd blood sample - Most important period of competition (T2) 3rd blood sample - Beginning of the season (T3) 4th blood sample - Beginning of the competitive period (T4)	Neutrophils present significantly ↓ in T2 compared to T3. T1 and T4 monocyte values were significantly ↑ than T3. CK was significantly ↓ in T2 compared to T3. The most intense periods of training were in T1 and T4. The lowest training volume occurred at T2.	Finaud *et al.* (2006)
12 professional rugby union players from a French team in the Top 14 (age = 20.5 ± 0.9 years, body mass = ; 100.1 ± 11.4 kg)	**Muscle damage Markers:** CK	Capillary blood sample measures were collected throughout the season. Samples were taken every 2-3 weeks on a Monday, 48 hours following a game (n = 14)	Significant time effects were observed for CK. Game time, overtraining score and number of physically difficult training sessions were significantly associated with CK (r = 0.46, r = 0.30 & r = 0.22).	Alaphilippe *et al.* (2012)
8 professional rugby union backs from a Rugby Pro D2 team (age = 25.8 ± 4.2 years, body mass = ; 88.4 ± 3.1 kg)	**Haematological parameters:** WBC, RBC, HB, HCT, Ret **Muscle damage Markers:** CK	Venous blood samples were obtained at 3-time points throughout the season. 1st blood sample - In training block 2 after a no game week (T1) 2nd blood sample - In training block 4, during a no game week (T2) 3rd blood sample - In training block 6, before the last 3 games of the season (T3)	Significant ↑ in RBC and Ret concentrations during T3 compared to T1 and T2. Significant ↑ in Ht concentrations during T3 compared to T1 and T2. No significant differences were observed in HB throughout the season. No significant differences were observed in CK throughout the season.	Dubois *et al.* (2017)
14 professional rugby union players from a French team in the Top 14 (age = 26.9 ± 1.9 years, body mass = ; 97.6 ± 13.2 kg)	**Haematological parameters:** WBC, RBC, HB, HCT, Ret **Muscle damage Markers:** CK	Venous blood samples were obtained at 3-time points throughout the season. 1st blood sample - In 1st competitive block (T1) 2nd blood sample - In middle competitive block (T2) 3rd blood sample - In the final competitive block (T3)	Significant ↓ in Ht between T2 and T3 Significant ↓ in CK between T1 and T2 No significant differences were observed in HB, WBC, Ret throughout the season. Acute heavy impacts and acute competitive workload were significantly associated with CK (r = 0.46 & r = 0.56). Acute heavy impacts were significantly associated with RBC (r = -0.44).	Dubois *et al.* (2020b)

Note: CK = Creatine kinase; HB = Haemoglobin; HCT = Haematocrit; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; Mean cell haemoglobin corpuscular (MCHC) Plts = Platelets; WBC = White blood cells; Reticulocytes = Ret. ↑**:** significant increase, ↓significant decrease

2.5.3 Other biomarker sampling measures

The use of biomarkers derived from blood is considered the gold standard due to their ability to accurately provide information on exercise-induced physiological stress (Lindsay and Costello, 2017). The collection of plasma and serum via a blood sample enables an ability to profile a wealth of training load associated biomarkers (Pedlar *et al.*, 2019). However, limitations about the invasive nature of blood sampling have been raised as well as the ability to obtain samples frequently (Lindsay and Costello, 2017). Capturing and quantifying the physiological stress placed upon elite RU players can also be obtained using biomarkers obtained from other bodily fluids such as saliva and urine.

Cunniffe *et al.* (2011a) and Lindsay *et al.* (2015b) both used saliva to measure markers of inflammation and immunity status and assess the associations with illness and injury. Cunniffe *et al.* (2011a) were the first to observe these measures in elite RU, they measured a squad of 31 players across a competitive season. To collect a good baseline sample, they tested at the start of the season following a 4-week rest, following this samples were taken, end of pre-season, midseason following the start of Europe, end of midseason, after a period of intense competition, the penultimate stage of the season and before the start of the following season. This permitted a comprehensive overview of the season to be observed. Upper respiratory tract infections and training load monitored using S-RPE were also taken throughout the season, to uncover relationships between these and biomarkers, specifically immunoglobulin A, cortisol and saliva lysozyme. Combining these markers of immunity provides a greater mechanistic understanding of why changes in training loads may impact illness. To standardise collection conditions this study took samples on either a Monday or a Tuesday depending on the day of the preceding game. Measures of training load showed June was significantly lower than all months except August and May with peak loads occurring during February. Illness data showed 92% of players experienced at least one URI during the season. The number of players reporting a URI increased during August and March, with a large peak during December. One potential limitation is that players self-reported URI illness rather than a doctor reporting URTI occurrence, which could lead to an over or underestimation of occurrences. Higher mean immunoglobulin A concentrations were seen in forwards when compared to backs making them more susceptible to infection, conversely, as the load was provided using S-RPE it makes it hard to distinguish whether this could have been due to positional differences in training and or match demands. Results showed clusters of infections after intense training periods

and reduced game time, particularly during December, August and March however normal seasonal effects of illness occurrence could have contributed to these observed trends in the markers investigated, especially during the winter months.

Lindsay *et al.* (2015b) also used saliva and urine samples to observe immunity and inflammatory and psychophysiological stress responses throughout a season. They gained samples from 37 professional players, who were competing in the super 15 competition. They analysed neopterin, cortisol and immunoglobulin A to determine the effects of competition on stress, immunity and inflammation. The authors believed that the few papers that have used biochemical analysis throughout the season simply didn't undertake enough sampling points and therefore failed to observe trends or patterns in the data. So to avoid this they sampled more frequently than the previously discussed studies, totalling 13 time-points. However, sampling always took place on a Monday so unlike Cunniffe *et al.* (2011a) who sampled always match day plus 2 which allowed for a standardised sample, the results although more were taken could be influenced by acute changes following a match that could have been played the day before. Results showed no significant differences between forwards and backs at any time points and also as a squad throughout a season when analysing cortisol and neopterin. Immunoglobulin A showed again no differences between forwards and backs, however, were shown to increase during the competition with significant differences measured following an international break. Although not many group changes were observed, large inter-individual variations for all markers were observed. They concluded that professional RU does not cause significant changes in psychophysiological stress however, this is when results have been analysed as a group. This masks individual changes as the large inter-individual variation highlight some players are more susceptible to fatigue and inflammation throughout the season as a result of suppressed immunity and sustained activation of inflammatory response. Unlike Cunniffe *et al.* (2011a) they did not provide a measure of load which makes it difficult to distinguish potentially what load demands may be making it difficult for these certain individuals.

2.5.4 Subjective wellness measures

Subjective measures are one of the most common monitoring tools utilised in high-performance sports to assess athlete well-being (Taylor *et al.*, 2012). A variety of different subjective measures have been implemented to help identify early signs of players experiencing high levels of physical strain and stress (Coutts and Reaburn, 2008; Saw *et al.*,

2016). The Recovery-Stress Questionnaire for Athletes (RESTQ-S) and the Brief Assessment of Mood (BAM) are popular tools within team sports to measure mood states and the recovery-stress balance to training stressors (Hills and Rogerson, 2018). Significant decreases in mood have previously been demonstrated in professional players following a RU match but have returned to baseline after ~ 60 hours (West *et al.*, 2014; Shearer *et al.*, 2015). However, limited studies have observed how wellness responds to training and recovery throughout the week or season. These questionnaires also raise logistical issues to implement in a practical setting due to the significant time they take to complete (Twist and Highton, 2013). As a result it is now also common practice that teams implement their own shorter questionnaire to understand the perceived wellness of players (Taylor *et al.*, 2012).

Dubois *et al.* (2020b) provided professional rugby players with the RESTQ-S questionnaire at 10 time-points throughout a full season. They reported psychological stress remained relatively high during the season and observed significant associations between individual Z scores of recovery-stress with measures of workload, obtained via the S-RPE method. Specifically, the acute workload was related to changes in stress (r = 0.30) and the chronic competitive workload was negatively associated with recovery indicators (r = 0.27). The high level of observed stress throughout the season demonstrates the importance of monitoring players to avoid non-functional adaptation and presents the psychological fatigue that results from competition and training.

Hills and Rogerson (2018) implemented and observed a custom-made wellness questionnaire during a professional rugby season. They identified a trend of under-recovery, as represented by a reduction in wellness from baseline over the 12-weeks. Interestingly though, they did observe increased levels of wellness following a no-game week, suggesting the psychological importance of rest weeks and rotation for players. The consistent reduction in wellness change from baseline throughout the season is in contrast to the findings of Dubois *et al.* (2020b) who reported no change in scores but a consistently high level of stress. This discrepancy could be due to the differences in methods to obtain perceived wellness, or due to when the players completed the questionnaire. Players in the Hills and Rogerson (2018) study completed the questionnaire following 48 hours of a game on the first day back to training whereas, players completed the REST-Q in the training week following a day off in the other study.

In summary changes in wellbeing and mood are influenced by a match, however, little research in rugby has examined how this may fluctuate throughout a week and a season. Differences in the methods used to understand player wellbeing make comparisons difficult, but practically a custom-made questionnaire appears to be more commonly used within team sports and rugby. Maximising opportunities to unload players to ensure sufficient recovery, via methods of bye weeks and squad rotation could be an effective way to alleviate the psychological pressures of competition that are carried into the training week. However, the disassociation between wellbeing recovery and neuromuscular recovery (Hills and Rogerson, 2018) confirms the need for a multidimensional approach to player monitoring.

2.6 Epigenetics

The development of elite rugby players is underpinned by both genetic predisposition and environmental influences. Rugby union performance is one of the most complex phenotypic traits (Massidda *et al.*, 2019), influenced by both anthropometric and physiological properties (Brazier *et al.*, 2020), as well as by appropriate training and diet (Denham *et al.*, 2014; Moran and Pitsiladis, 2017). Elite athletic performance is thought to be the product of a high genetic potential actualised through an optimised training approach (MacArthur and North, 2005). Understanding the interaction between genetics and training-induced adaptation could be exploited to further enhance performance (Coffey and Hawley, 2007).

2.6.1 The environmental influence

To understand how potential genetic variations relate to sporting performance, it is important to understand the relationship between genetic predisposition and human performance variation. Familial and twin studies provide a platform for unearthing the genetic and environmental factors that contribute to the magnitude of an individual's inherent performance determinants. A pioneering study by Bouchard *et al.* (1998), comprising 481 sedentary individuals, examined $\dot{V}O_{2max}$ trainability in response to a 20-week training program. Results estimated approximately 47 % variance in $\dot{V}O_{2max}$ scores could be explained by genetic predisposition, i.e. the percentage of this performance trait that is heritable. Additional studies have also sought to determine the heritability of physical performance (Peeters *et al.*, 2005) and athlete status (De Moor *et al.*, 2007). A crucial finding of these studies is that although there is a significant element of genetic predisposition, there remains a major proportion of unaccounted variation, primarily due to environmental influences. Recently

epigenetic research has presented a plausible explanation for the variation in individual responses to various stimuli.

Epigenetics was first termed by Professor Conrad Waddington in the 1930s. The term aimed to link the fields of developmental biology and genetics together (Holliday, 2006). Due to a lot of uncertainty and lack of clarity within the field, it wasn't until 1987 that a publication by Professor Robin Holliday provided a critical paper, which led to the study of epigenetics being as we understand it today (Holliday, 1987). Epigenetics (epi: in addition, genetics: genes) is the study of changes to gene expression patterns, without changes to the DNA base sequence (Jacques *et al.*, 2019). Epigenetic modifications are defined as a source of heritable variation but one that is malleable within an individual (Moran and Pitsiladis, 2017). Research describes epigenetics as a specific fine-tuning mechanism that modulates the latter stages of transcriptional response, allowing for rapid responses to stress and demands imposed on the body (Bianchi *et al.*, 2017). Their involvement at a molecular level therefore is key, as they act to enhance or prevent transcription, although to date, this interplay is not fully understood (Denham *et al.*, 2014).

Epigenetic processes regulate gene expression via transcription machinery, these various regulatory mechanisms include DNA methylation, histone modification and MicroRNAs (miRNAs) regulation (Denham *et al.*, 2014). For this thesis, the emphasis will be on miRNA regulation.

2.7 MicroRNAs

MicroRNAS (miRNA) are noncoding RNAs approximately 22 nucleotides in length that regulate gene expression at a posttranscriptional level (Ambros, 2004). Ever since the discovery of non-coding miRNAs in 1993 (Wightman *et al.*, 1993), a significant amount of research has aimed to uncover their exact cellular functions and understand the role they may play in both medicine and sport. The first miRNA, Lin-4, was discovered in the laboratories of Dr Ambros and Dr Ruvkin. They were the first to report the regulatory potential of miRNAs, as they discovered Lin-4 regulates Lin-14 mRNA translation via an antisense RNA – RNA interaction (Lee *et al.*, 1993).

To date, almost 2,000 human miRNAs have been identified in a variety of tissues and fluids (Griffiths-Jones_Lab, 2018). It is estimated that miRNAs can bind to approximately 60% of all protein-coding genes (Lewis *et al.*, 2005). MiRNAs also have the ability to target multiple

messenger RNAs (mRNAs), enabling a robust control over complex cellular processes (Stefani and Slack, 2008). This capability highlights their complex but formidable role as gene regulators for physiological processes. MiRNAs have previously been shown to be important regulators of a variety of cellular processes via a multitude of signalling pathways (Domańska-Senderowska *et al.*, 2019).

2.7.1 MiRNA biogenesis

MiRNA biogenesis has been discussed in detail in previous papers (Finnegan and Pasquinelli, 2013; Ha and Kim, 2014) and is illustrated in Figure 6. The process requires multiple steps and starts in the cell nucleus. Briefly, a primary miRNA (pri-miRNA) is transcribed via RNA polymerase II (Bartel, 2004). The formed pri-miRNA is then cleaved by the enzyme Drosha, which has been guided into position by its cofactor DiGeorge Syndrome Critical Region 8 (DGCR8) to form a 70-100 nucleotide hairpin structure, the precursor miRNA (pre-miRNA). Pre-miRNA is then exported from the nucleus into the cytoplasm by Exportin 5 (Chen *et al.*, 2012), where it is again cleaved by a secondary dicer to form a much shorter, ~22 nucleotide double-chain miRNA. This is also known as the miRNA / passenger miRNA duplex, each duplex consists of a mature miRNA and a passenger/complementary strand. The duplex miRNA is de-natured by a helicase, which releases the mature functional strand (Bartel, 2004). Together the mature miRNA with an Argonuate protein is transferred into the RNA-induced silencing complex (RISC) forming a miRSC complex, where it can now start to regulate translation. The passenger strand is usually degraded, however in some instances it could also be active in gene regulation (Ha and Kim, 2014).

MiRNAs incorporated within the RISC regulate gene expression via two distinct mechanisms. These are dependent upon where the miRNA guides the RISC to the target site. MiRNA's typically target the 3-UTR or 5-UTR regions, and the outcome is dependent upon the degree of base pairing complementarity. Full mRNA target complementarity leads to the cleaving of the mRNA molecule and its degradation. Whereas, if the target is only partially complementary, this interaction leads to suppression of mRNA translation (Fernández-Sanjurjo *et al.*, 2016; Domańska-Senderowska *et al.*, 2019). The resultant effect of either one of these mechanisms is the reduced expression of the targeted mRNA transcript.

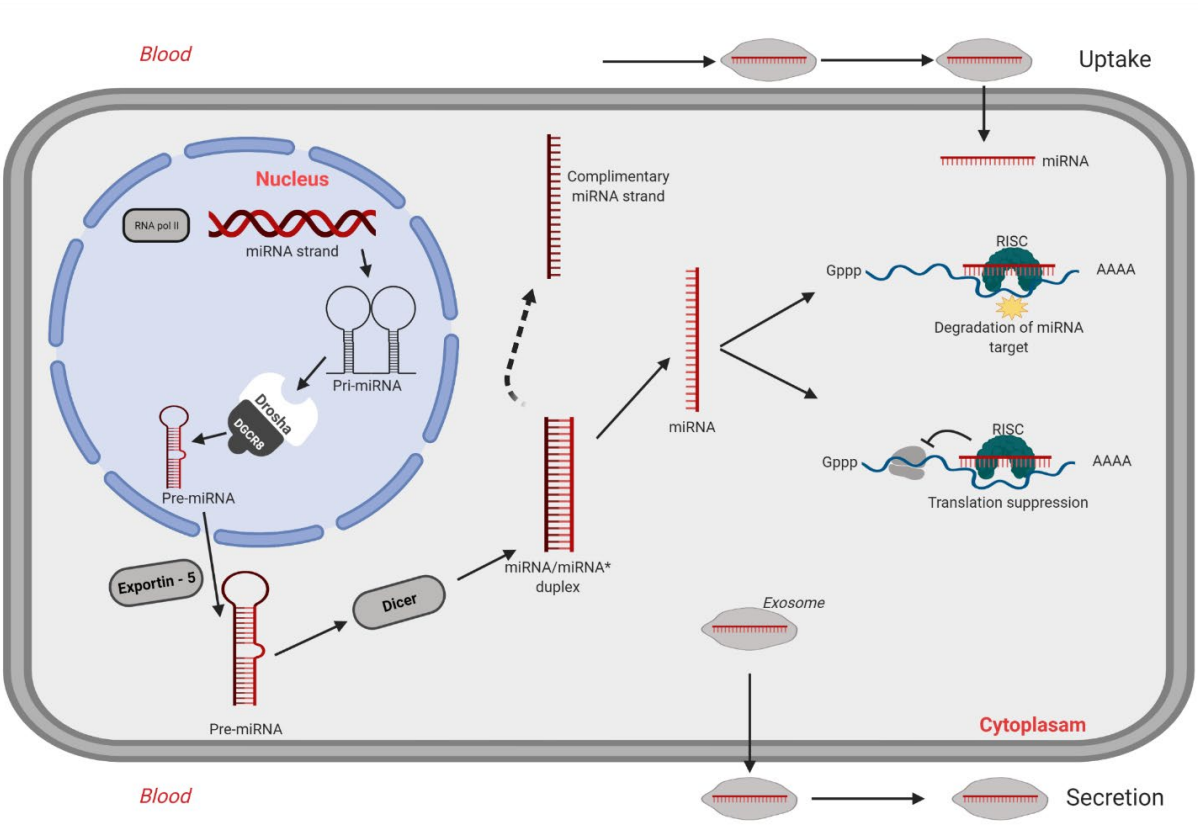

Figure 6. Figure adapted from Aoi and Sakuma (2014) and Joshi *et al.* (2011). miRNA biogenesis begins within the cell nucleus. Following transcription and processing, the pre-miRNA is exported into the cytoplasm via Exportin-5 protein. The mature miRNA strand is synthesised and upon binding to an mRNA strand results in a translational suppression or degradation. This causes a reduction in protein expression of the gene encoded by the mRNA strand.

2.8 Circulating microRNAs

MiRNAs were first only considered to exert their function on mRNAs within the tissue. However, in 2008 researchers demonstrated stable miRNAs within the circulation, known as circulating miRNAs (ci-miRNAs) (Chen *et al.*, 2008; Mitchell *et al.*, 2008). Their presence within human plasma, serum and other bio-fluids have enhanced their application and feasibility within sport science, as previously miRNA responses could only be observed via a muscle biopsy sample. This method of collection would be very difficult to obtain from elite sports teams. Studies have shown a unique characteristic of ci-miRNAs, is that they can resist degradation under tough conditions such as freezing and thawing, long term storage and physical disturbance (Glinge *et al.*, 2017), highlighting their potential as non-invasive diagnostic and predictive biomarkers.

2.8.1 Ci-miRNA secretion

Ci-miRNAs are thought to be able to regulate various interactions between tissues and reflect physiological states (Aoi and Sakuma, 2014). It is believed that upon stimulation, their presence within circulation is primarily due to both active (secretion) and passive (membrane leaking) mechanisms in response to a range of stimuli (Siracusa *et al.*, 2018) (Figure 7). It has been reported that miRNAs can be released passively due to cell damage or necrosis such as the release of miR-1 in response to myocardial infarction (Chen *et al.*, 2012; Sapp *et al.*, 2017). However, increasing evidence is reporting a deliberate packaging and active release of specific miRNAs. Recent research has supported this specific active release and suggested the role of ci-miRNAs as cell-to-cell communicators acting in a paracrine/endocrine and autocrine fashion (Sapp and Hagberg, 2019). Hereby suggesting that ci-miRNAs are physiological mediators of systemic responses to exercise, rather than just being passive markers of cellular processes (Siracusa *et al.*, 2018; Sapp and Hagberg, 2019).

The precise mechanisms of secretion, packaging and transport are still as yet unknown. However, previous studies have supported the function of ci-miRNAs as cell-to-cell communicators (Ma *et al.*, 2018). Ci-miRNAs contained in either exosomes, microvesicles, high-density lipoprotein or bound to a protein carrier (Argonaute 2) are released and circulated (Silva *et al.*, 2017), where they can be selectively taken up by neighbouring cells. This allows ci-miRNAs to exert their specific functions of gene expression regulation. The carrying via intracellular small vesicles prevents the degradation of RNases (Vickers *et al.*, 2011). The specific release mechanism of ci-miRNAs during and following exercise is also still not completely understood, but it is suggested to be stimulated via methods of laminar shear stress of endothelial cells, hypoxia, inflammation, muscle contraction and cell damage (Sapp and Hagberg, 2019).

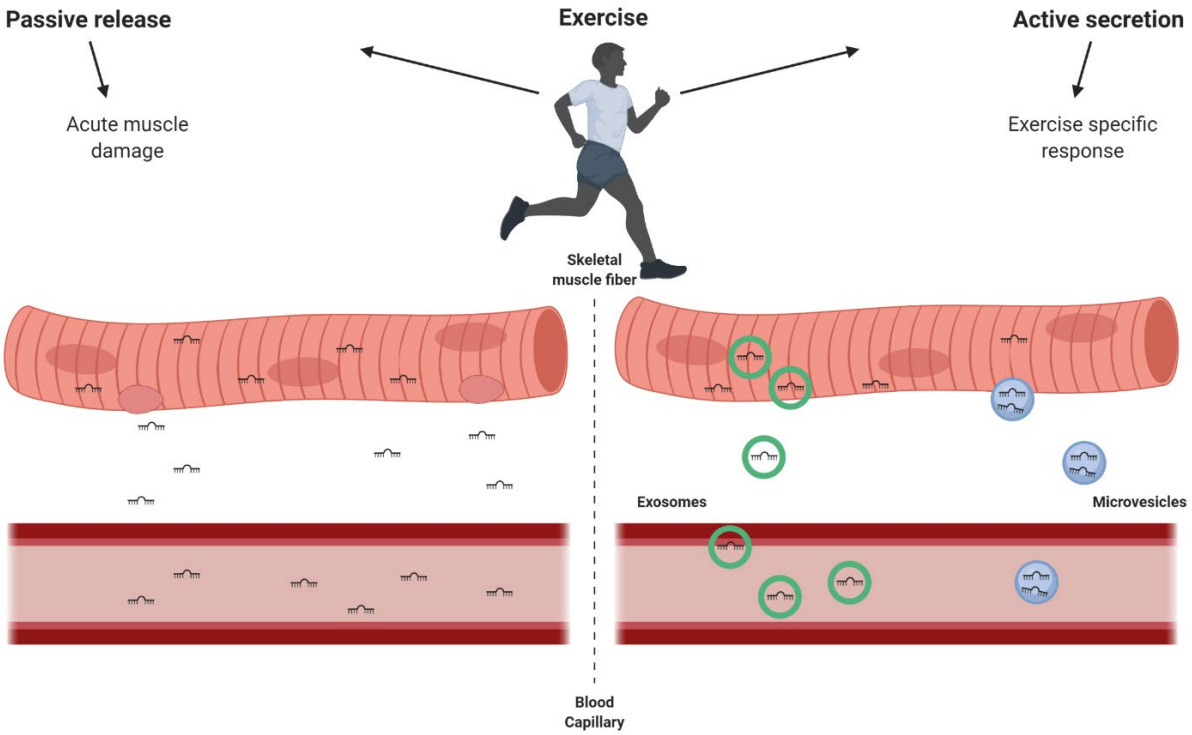

Figure 7. Figure adapted from Siracusa et al. (2018) on the possible release of miRNAs into circulation from skeletal muscle fibres. The mechanisms of miRNA release into an extracellular environment are believed to occur in both an active and passive fashion. Myofibre necrosis via acute muscle damage is a cause of the passive release of miRNAs into the blood. Increased shear stress along the endothelium could be responsible for the stimulation of specific miRNA secretion. Ci-miRNAs could then be absorbed by other tissues, where they can exert their fine-tuning effects via regulating gene expression.

2.8.2 Ci-miRNA response to exercise

Acute and chronic exercise can transiently and adaptively change ci-miRNA baseline measures and expression patterns. It is these pattern changes in response to exercise training, which provide insight into the processes associated with exercise-induced adaptations in response to varying stimuli. An overview of altered ci-miRNA expression profiles in response to a variety of exercise stimuli can be found in Table 4. Additionally, prior reviews have discussed in detail ci-miRNA responsiveness to exercise (Fernández-Sanjurjo *et al.*, 2016; Polakovičová *et al.*, 2016; Sapp *et al.*, 2017; Silva *et al.*, 2017; Sapp and Hagberg, 2019). The findings of these studies promote the role of ci-miRNAs in governing the acute responses to exercise and physiological adaptations.

The research to date has been dominated by studies primarily focusing on aerobic exercise and endurance training. Only a few studies have also explored the role of ci-miRNAs in the

response to, and adaptation from, a resistance training stimulus. This leaves a gap to explore ci-miRNA responses among team sports players. The study findings in Table 4 display very heterogeneous responses, which could be due to different exercise modes or athlete levels, but it makes it difficult to apply specific ci-miRNA findings to RU players. To date, only two studies have used team sports players where concurrent training methods are required. Following two months of competitive soccer training, the abundance of serum exosomal ci-miR-27b increased (Domańska-Senderowska *et al.*, 2017). No changes were found in ci-miR-133 across the time period unlike Nielsen et al. (2014) who reported a decrease after a sustained cycling training period. Additionally, after three months of basketball training, the abundance of ci-miR-208b decreased and ci-miR-221 increased. It remains unknown however if the same findings will be found following a period of RU training.

It is well documented that sustained training can modify the basal circulating expression levels of ci-miRNAs and that these levels differentiate between the levels of training. Baggish *et al.* (2014) reported resting levels of ci-miR-126 are higher in trained athletes and Bye *et al.* (2013) found levels of ci-miR-210, ci-miR-222 and ci-miR-21 (male group only) were higher in the low $\dot{V}O_{2max}$ group compared to those with a high $\dot{V}O_{2max}$. This suggests there could be considerable variability in ci-miRNA resting levels and patterns due to training/sporting experience. These findings make application to an elite population difficult and currently no studies in elite RU players exist.

Table 4. Circulating microRNA (ci-miRNA) profiles in response to various types of acute and chronic training.

Type of Exercise	Study Design	Subject characteristics	Sample Time points	Sample Type	ci-miRNA Responses Compared to a Baseline	Study
Endurance						
1. Acute trial: Exhaustive incremental cycling exercise test, before and post-training 2. Chronic training; 90 days of sustained rowing training	Acute & Chronic	10 male student-athletes; 19.1 ± 0.6 years	1. Pre, immediately post & 1 h following the acute exercise trial 2. Pre & post sustained training period	Plasma	miR-146a, -222, -21, -221 **↑ : immediately post exercise** miR-146a, -222, -21, -221, 20a **↑ : At rest following sustained training** miR-146a, -222, -21, 221, 20a **↑ : immediately post exercise after sustained training**	Baggish *et al.* (2011)
1. Acute trial: Cycling at 70% $\dot{V}O_{2max}$ for 60 min 2. Chronic training: Cycling at 70% of $\dot{V}O_{2max}$ for 30 min, 3 days/ wk for 4 wk	Acute & Chronic	10 healthy untrained males; 21.5 ± 4.5 years	1. Pre, immediately post, 3 and 24 h following acute exercise 2. Pre and 48 h post-cessation of training	Serum	miR-486 **↓ : Immediately post-exercise** miR-486 **↓ : 48 h after exercise training**	Aoi *et al.* (2013)
Marathon	Acute	14 moderately trained endurance males, 42.8 ± 6.0 years	Pre, immediately post and 24 hours following a marathon	Plasma	miR-1, -133a, -206, -208b, -499 **↑ : Post marathon** miR-1, -133a, -206 **↑ : 24 h post marathon**	Mooren *et al.* (2013)
Running at 80% of $\dot{V}O_{2peak}$ for 30 minutes	Acute	8 national ski level athletes; 21.7 ± 2.6 years	Pre, immediately post, 30 mins & 1 h following exercise	Whole Blood	miR-24-2-5p, -27a-5p, -181a-5p **↑ : Post exercise**	Tonevitsky *et al.* (2013)
Marathon	Acute	21 healthy male runners; 51.8 ± 1.4 years	Pre, post and 24 hours following a marathon	Plasma	miR-1, -126, -133a, -134, -146a, -208a, -499-5p **↑ : Immediately post marathon**	Baggish *et al.* (2014)
1. Acute trial: 60 min cycling at 65% Pmax 2. Chronic training: cycling 5 times a week for 12 weeks	Acute & Chronic	13 healthy males; 28 ± 8 years completed the acute trial, a sub set of 7 healthy males; 28 ± 5 years completed the chronic trial	Pre, immediately post, 1, 3 h post-acute exercise and at rest following a sustained training period	Plasma	miR-106a, -221, -30b, -151-5p, let-7i, - 146a, - 652, -151-3p **↓ : Immediately post-exercise** miR-338-3p, -330-3p, -223, -139-5p, -143, -145, -424 **↑ : 1 h post exercise** miR-1, -424, -133a, -133b **↑ : 3 h post exercise** miR-342-3p, let-7d, -766, -25, -148a, -185, -21, -148b, -133a, -92a, -29b **↓ : At rest following sustained training** miR-103, - 107 **↑ : At rest following sustained training**	Nielsen *et al.* (2014)
10 - km race Marathon (M)	Acute	9 active middle-aged males; 39.1 ± 2.2 years	Pre, immediately post and 24 hours following each trial	Serum	miR-150-5p **↑ : Post 10 km race** let-7d-3p, let-7f-2-3p, miR-125b-5p, -132-3p, 143-3p, -148a-3p, -223-3p, -223-5p, -29a-3p, -34a-5p, -424-3p, -424-5p **↑ : Post 10 km race**	de Gonzalo-Calvo *et al.* (2015)

1. Acute trial: Marathon race 2. Chronic trial: 10 weeks of running specific training	Acute & Chronic	30 male marathon runners grouped based on km/week; Elite group (n = 15) 40.0 ± 1.7 years & Non-elite group (n = 15) 40.1 ± 1.4 years	1. Baseline, immediately and 24 h post marathon 2. Baseline and following a 10-week training program	Plasma	miR-1, -133a, 30a **↑ : post-marathon (elite & non elite groups)** miR-26a **↓ : post marathon (elite group)**	Clauss *et al.* (2016)
Half-marathon race	Acute	28 middle-aged recreational athletes (11 women and 17 men); 46 years	Pre and immediately post the race	Plasma	miR-133a, 206 **↑ : Immediately post-race**	Danese *et al.* (2018)
10-km race Half marathon (HM) Marathon (M)	Acute	9 trained amateur runners; 39.1 ± 6.7 years	Pre, immediately post, 24 and 72 hours following each race	Serum	miR-132-3p, -150-5p **↑ : IM post 10 km race** miR-139-5p, -103a-3p **↓ : IM post 10 km race** miR-590-5p **↓ : 24 h Post 10 km race** miR-21-5p, -27a-3p, -29a-3p, -30a-5p, -34a-5p, -126-3p, -142-5p, -195-5p,199a-3p **↑ : IM post M race** miR-25-3p, -29b-3p, - 30b-3p, - 106b-5p, -107, -497-5p **↓ : 24 h Post M race** miR-103a-3p, -375 **↓ : Post & 24 h Post M race**	de Gonzalo-Calvo *et al.* (2018)
Intensity cohort: constant distance of 5 miles, ran at 3 different intensities (6, 7 and 8 miles/h) Duration cohort: constant speed of 7 miles/h at different durations (30, 60, 90 min)	Acute	26 healthy men Intensity cohort (n=12), 21 ± 1 years Duration cohort (n=14), 22 ± 3 years	Pre and immediately post each trial	Plasma	miR-24, miR146a, -133a, -222, -1 **↑ : Intensity cohort, 6 miles/h** miR-1 **↑ : Intensity cohort, 7 miles/h** miR-133a, -222 **↑ : Duration cohort, 30 minutes** miR-24, -146a, -1, -133a, -222 **↑ : Duration cohort, 60 minutes** miR -133a **↑ : Duration cohort, 90 minutes**	Ramos *et al.* (2018)
3 cycling sessions a week for 20 weeks. Exercise sessions progressed throughout the training period. Intensity varied between exercising at 55 and 75 % $\dot{V}O_{2max}$ and for a duration between 40 and 50 minutes	Chronic	10 sedentary males & 10 sedentary females	Baseline and following a sustained training period	Serum	miR-142-3p, -221-3p, -126-3p, -146a-5p, -27b-3p **↑ : Post sustained exercise training** miR-486-5p, let-7b-5p, -29c-3p, let-7e-5p, -93-5p, -7-5p, -35-3p, -92a-3p, -29b-3p **↓ : Post sustained exercise training**	Barber *et al.* (2019)
High-intensity interval & sprint training						
Two 30 s all-out cycling sprint efforts against a predetermined load of 7.5% of body weight. 4 min active recovery was performed between them	Acute	18 healthy males; 20.23 ± 0.97 years	Pre and immediately post the exercise trial	Plasma	miR- 1, -133a, -133b, -122, -16 **↓ : Post sprint efforts**	Cui *et al.* (2015)

High-intensity interval exercise (HIIE) trial: 7 x 4 mins running at 85 - 95% of HR max, interspersed with 2 min active recovery Vigorous-intensity continuous exercise (VICE) trial: Work matched to HIIE trial whereby distance covered matched that to the distance covered in the previous trial	Acute	26 healthy males; 20.38 ± 0.12 years	Pre and immediately post-exercise trials	Plasma	miR-1 **↑ : in VICE compared to HIIE** miR-1, -133a, -133b, 206, 485-5p, 509-5p, 517a, 518f-3p, -520f, -522, -553, -888 **↑ : Post HIIE and VICE**	Cui *et al.* (2016)
10 × 60 s cycling intervals at a predetermined peak power output. 75 s rest intervals separated each effort	Acute	10 healthy males; 24.6 ± 4.0 years	Pre, immediately post and 4 h following the exercise trial	Plasma	miR -134-3p **↓ : Post** miR -378a, -486-5p **↓ : 4 h** miR -1-3p, -16-5p, -107 **↓ : Post & 4 h** miR -146a-5p **↑ : Post** miR -21-5p, -126-3p, -221-3p, -222-3p **↑ : 4 h**	D'Souza *et al.* (2018)
1. Acute trial: 4, 30 s all-out maximal sprint efforts on a bike separated by 4 min rest 2. Chronic trial: 6 weeks sustained training program, 4 - 6 30 s maximal cycling efforts were completed three times per week. 4 min rest between each set and efforts were progressed 1 a week every 2 weeks	Acute & Chronic	28 healthy males; 35.5 ± 11.1 years completed the acute trial a sub set of 10 healthy males; 33.3 ± 10.9 years completed the chronic trial	1. Pre and 30 min post the exercise trial 2. Pre and 2 - 4 days following 6 week training intervention	Whole Blood	miR-1-3p, -133a-3p, -133b-3p, -486-5p **↓ : Following 6 week sprint interval cycling training**	Denham *et al.* (2018)
EE Day 1 - Cyclists completed 30 min at 73 % of $\dot{V}O_{2max}$, 5 min rest, intervals of 20 minutes at 73% $\dot{V}O_{2max}$ until exhaustion, 5 min rest, repeated intervals of 60 s at 90% of $\dot{V}O_{2max}$ until voluntary exhaustion EE Day 2 - Following around 18 hours of recovery cyclists completed a 10 s sprint, 30 min at 73 % of $\dot{V}O_{2max}$ a time trial effort, 10 s sprint TT - Cyclists completed 30 min at 73 % of $\dot{V}O_{2max}$ followed by a 60 minute time trial	Acute	Exhaustive exercise cohort (EE) = 8 elite level male cyclists; 22.9 ± 1.2 years. Time trial cohort (TT) = 13 elite-level male cyclists ; 27.7 ± 1.2 years	EE cohort - Day 1 - baseline and 2 h following voluntary exhaustion and trial completion EE cohort - Day 2 - Pre-warm-up and immediately following trial completion TT cohort - Pre exercise, immediately post, 2 & 5 h following the exercise trial	Plasma	miR-193a-5p, -29a-3p **↑ : in EE cohort 2 hours post exercise** miR -99b-5p, -151a-3p **↓ : in EE cohort next day pre exercise** miR -142-3p, -29a-3p, -141-3p, -150-5p, 424-5p, 423-3p, let-7g-5p **↑ : in EE cohort post day 2 exercise** miR -106b-5p, -30d-5p, 23a-3p **↓ : in EE cohort post day 2 exercise** miR -106b-5p, 155-5p **↓ : in TT cohort post exercise** miR -29a-3p , -193a-5p **↓ : in TT 2 h post exercise**	Håkansson *et al.* (2018)
Repeated sprint training consisted of eighteen 15 m maximal sprints with 17 s of passive recovery between each effort. Training was performed 3 times a week for 8 weeks	Chronic	9 healthy males; 24.3 ± 3.77 years	Baseline, week 4 and 24 h following the 8-week training intervention	Plasma	miR-23a-3p, 24-3p **↓ : Post 4 & 8 weeks of sprint training** miR-122-5p, -125-5p, -148-3p **↓ : Post 4 weeks of sprint training** miR-100-5p **↓ : Post 8 weeks of sprint training** miR-93-5p **↑ : Post 4 weeks of sprint training**	Sansoni *et al.* (2018)

1. Acute trial: Participants completed either 4 sets of 30 s sprinting with 30 s rest or 4 sets of 30 s sprinting with 180 s rest. 2. Chronic trial: Participants undertook 4 weeks of either 4 x 30: 30 or 4 x 30: 180 HIIT. Sessions were performed twice a week	Acute & Chronic	50 moderately trained students consisting of 30 females; 23.0 ± 3.0 years and 20 males; 23.3 ± 3.1 years	1. Pre and immediately post the acute exercise trial. 2. Pre and immediately post-exercise trial following a 4 week training period	Whole Blood	miR-24, -96-5p, -126, -143 **↑ : post-exercise (4 x 30 : 180 group)** miR-96-5p **↓ : Following 4 weeks training (4 x 30 : 30 group)** miR-126 **↑ : Following 4 weeks of training (4 x 30: 30 group)** miR-96-5p **↓ : Following 4 weeks training (4 x 30 : 180 group)** miR-24, -126, -143 **↑ : Following 4 weeks training (4 x 30 : 180 group)**	Schmitz *et al.* (2019)
Strength / Resistance training						
5 sets of 10 reps at 70% 1 RM, Bench press & bilateral leg press	Acute	12 healthy males; 29.9 ± 1.2 years	Pre, immediately post-exercise, 1 h post, 1-day post and 3 days following exercise trial	Serum	miR-149* **↑ : 1 day after** miR-149* **↑ : 3 days after** miR-146a, -221 **↓ : 3 days after**	Sawada *et al.* (2013)
3 sets of 10 reps at 80% 1 RM, Leg extension & bilateral leg press	Acute	18 sedentary males; Young, 22 ± 1 years (n= 9), Old, 74 ± 2 years (n= 9)	Baseline (24 h pre-trial), immediately post and 6 hours post exercise	Serum	miR-17-5p, 19a-3p, 19b-3p, 20a-5p, 26b-5p, 93-5p, 106-5p, 143-3p, 195-5p **↑ : 6 hours post**	Margolis *et al.* (2016)
Bench press, squat, pulldown, overhead press, standing dumbbell curl, all exercises performed in that order SE: 3 sets of 16-20 reps at 40% 1 RM MH: 3 sets of 12 reps at 70% 1 RM MS: 4 sets of 6 reps at 90% 1 RM	Acute	45 males; Strength endurance (SE), 19.36 ± 1.2 years, Muscular hypertrophy (MH), 19.72 ± 0.20 years, Maximum strength (MS), 18.87 ± 0.30 years	Pre, immediately post exercise, 1 h post, 24 h following exercise trial	Plasma	miR-532 **↑ : 1 h & 24 h post (SE)** miR-208b **↓ : Immed. Post & 24 h post (MH)** miR-133b **↑ : Immed. Post to 24h post (MH)** miR-206 **↑ : Immed. Post to 1 h post (MH)** miR-206 **↓ : Immed. Post (MH)** miR-21 **↑ : Immed. Post to 1 h post (MH)** miR-181a **↑ : 1h post (MH)** miR-181a **↑ : Immed. Post to 1 h post (MH)** miR-181a **↓ : 1h to 24 h post (MH)** miR-221 **↓ : Immed. Post to 1 h post (MH)** miR-181a **↑ : 1h to 24 h post (MH)** miR-133a **↓: Immed. Post (MS)** miR-133b **↑ : Immed. Post to 1 h post (MS)**	Cui *et al.* (2017)
6 sets of 8-10 reps at 80% 1 RM: Horizontal leg press 8 sets of 8-10 reps at 80% 1 RM: Seated leg extension	Acute	9 resistance trained males; 24.6 ± 4.9 years	Pre, 2 h & 4 h following exercise	Plasma	miR-133a, 149 **↑: 4 hours post**	(D'Souza *et al.*, 2017b)

Resistance & Endurance training						
6-day training period specific for the strength or endurance cohort. Both consisted of 2 training sessions a day except for day which only had 1	Chronic	29 well trained male athletes; 15 well-trained cyclists & 14 strength athletes]	Pre & post a 6-day simulated training camp for strength and endurance-trained individuals	Plasma and whole blood	Not validated by qRT-PCR	Hecksteden *et al.* (2016)
All training was performed 3 times a week for a total of 8 weeks. EXPL: 4 sets of 8 reps of barbell jump squats at 90% of maximal power HYP: 3 sets of both back squat and leg press at 75% HIIT: 30 running efforts at 90% maximum heart rate, 15 s on 15 s rest	Chronic	30 males; 22.5 ± 4.1 years. Divided into 3 groups, Explosive strength training (EXPL), Hypertrophic strength training (HYP), High-intensity interval training (HIIT)	Baseline, week 5 and week 8 of the training program	Plasma	miR-93 ↑ **: 5 weeks (HIIT)** miR-16 ↓ **: 8 weeks (HIIT)** miR-222, -16 ↓ **: 5 weeks (EXPL)** miR-16 ↓ **: 5 weeks (EXPL)** miR-93, - 16, -222 ↑ **: 5 weeks (HYP)**	Horak *et al.* (2018)
Eccentric vs Concentric						
Uphill (high concentric component) and downhill (high eccentric component) walking. 30 min at 1 m/s with a grade of 25% and an additional weight of 12% of body weight.	Acute	9 recreational active men, 27 - 36 years	Pre, immediately post, 2, 6, 24-, 48 and 72 h following exercise trial	Plasma	miR-1 ↑ **: 2 h post downhill walking** miR-1, -133a, -133b, -208b ↑ **: 6 h post downhill walking** miR- 181b, -214 ↑ **: Immediately post uphill walking**	Banzet *et al.* (2013)
Sport Specific Training						
An 8-week long in-season soccer training cycle	Chronic	22 youth soccer players; 17.5 ± 0.7 years	Baseline, after 1 week & 2 months of competitive in-season training	Serum exosomes	miR-27b, -29a ↑ **: 1 week of training** miR-27b ↑ **: 2 months of training**	Domańska-Senderowska *et al.* (2017)
3 months basketball training	Acute & Chronic	10 amateur basketball players; 25.9 ± 4.95 years	Baseline, immediately post-exercise test and 3 months following a training period	Serum	miR-208b ↓**: 3 months of training** miR-221 ↑ **: 3 months of training** miR-221, -21, -146a, -210 ↓**: post exercise test**	Li *et al.* (2018)

2.8.3 MiRNAs and physiological adaptation

As research into this area progresses, relationships are being uncovered between certain ci-miRNA profiles and specific signalling pathways that are initiated via exercise (Domańska-Senderowska *et al.*, 2019). Research has supported their role in mediating a wide range of physiological processes such as muscular hypertrophy (Diniz and Wang, 2016), angiogenesis (Wang *et al.*, 2008) and inflammation (Urbich *et al.*, 2008).

Some of the most researched and best-characterised miRNAs have been found specifically in cardiac and skeletal muscle; they have been termed 'myomiRs' (miR-1, -133a, -133b, -206, -208a, -208b, -486 and -499). This group of miRNAs are primarily known due to their fundamental roles in skeletal muscle development and remodelling (Kirby and McCarthy, 2013; Polakovičová *et al.*, 2016). Specifically, miR-133a is expressed in both heart and skeletal muscle and miR-206 is strictly skeletal-muscle specific (Kirby and McCarthy, 2013). Additionally, miR-208b and miR-499 play a dominant role in muscle fibre type specification (van Rooij *et al.*, 2009).

MyomiRs have a significant role to play in skeletal muscle development due to their regulatory influences on myogenesis. They have integral governance in modulating skeletal muscle proliferation and differentiation (Kirby and McCarthy, 2013). miR-133a promotes proliferation via repression of serum response factor (SRF) which is known to block cell proliferation (Chen *et al.*, 2006). Downstream of this process miR-206 promotes myogenic differentiation via regulation of the *Pax 7* protein. *Pax 7* is a direct regulatory target of miR-206, upregulation of miR-206 inhibits *Pax 7* and halts proliferation, this timely downregulation is important to assist the transition of primary myoblasts from proliferation to differentiation (Figure 8). Human rhabdomyosarcoma cells display reduced expression levels of miR-206, highlighting inhibition of miR-206 reduces its function to inhibit cell proliferation and reduces the capacity to differentiate (Hanna *et al.*, 2016). Together both these miRNAs play clear roles in muscle regeneration.

More evidence is supporting the role of a multitude of miRNAs involved in physiological adaptation via signalling pathways. For example, miR-126-3p is enriched in the vascular endothelium (Uhlemann *et al.*, 2014) and suppresses the negative regulation of SPRED-1, which subsequently enhances the pro-angiogenic actions of vascular endothelial growth factor (VEGF) (Wang *et al.*, 2008; da *et al.*, 2012). Additionally, miR-100-5p is a member of the

miR-99 family (Sun *et al.*, 2011) and is known to inhibit the mammalian target of rapamycin (mTOR) pathway. The mTOR pathway is critical for muscle protein synthesis (Zacharewicz *et al.*, 2014) and pre-adipocyte differentiation (Kim and Chen, 2004).

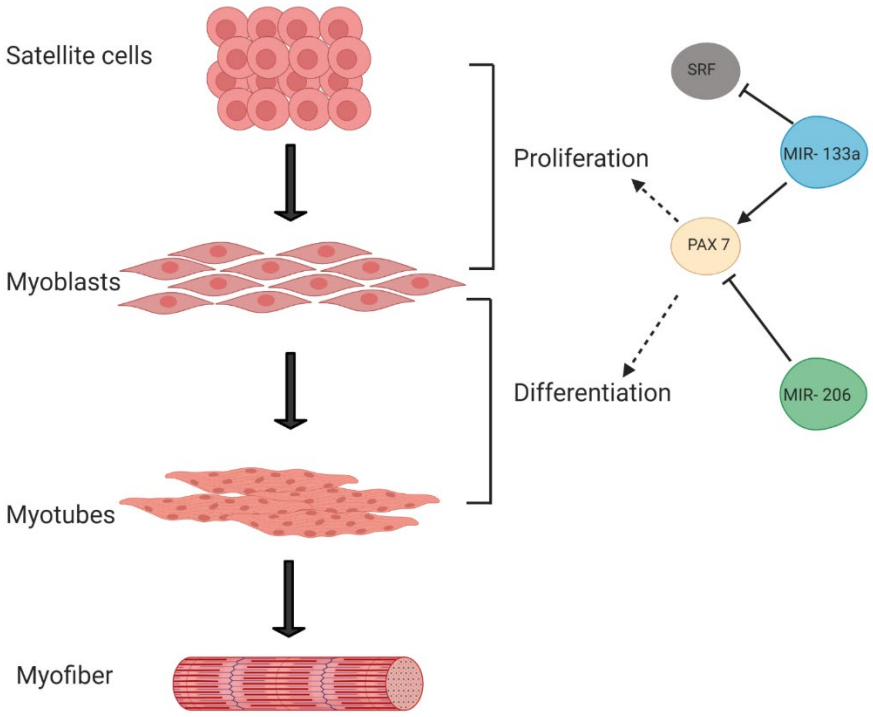

Figure 8. This diagram highlights the important roles of miR-133a and miR-206 in proliferation and differentiation as part of the myogenesis process.

2.8.4 MiRNAs associations with fitness measures, phenotypes and trainability

A considerable amount of literature has been published surrounding ci-miRNAs in a medical capacity. The ever-growing body of research has highlighted their capacity as predictive and diagnostic markers in response to disease or illness. Early research has shown their potential as predictive markers in the early detection of cancers (Wozniak *et al.*, 2015), as well as heart failure following a myocardial infarction (Matsumoto *et al.*, 2013). Previous studies have applied this methodology within sport and explored the application of using miRNAs as biomarkers of exercise capacity and adaptation (Table 5). Primarily studies have observed the association between ci-miRNAs and important performance phenotypes in non-athletic populations. This includes the studies that have investigated the role of miRNAs in team sports (Domańska-Senderowska *et al.*, 2017; Li *et al.*, 2018), where a broad range of physiological

qualities are required. No research to date has examined potential associations in elite rugby union players.

Table 5. Studies reporting associations with circulating microRNAs and measures of exercise and fitness

Subjects	Sample Type	Performance metric associations	Study
10 male student-athletes; 19.1 ± 0.6 years	Plasma	Exercise-induced increase of miR-146a: **$\dot{V}O_{2max}$**	Baggish *et al.* (2011)
14 moderately trained endurance males, 42.8 ± 6.0 years	Plasma	Exercise-induced increase of miR-1, -133a, -206: **$\dot{V}O_{2max}$** Exercise-induced increase of miR-1, -133a, -206: **Speed at individual anaerobic lactate threshold**	Mooren *et al.* (2013)
10 healthy untrained males; 21.5 ± 4.5 years	Serum	Exercise-induced increase of miR-486: **$\dot{V}O_{2max}$**	Aoi *et al.* (2013)
Low $\dot{V}O_{2max}$ group: 19 male & 19 female; 45.6 ± 3.2 years High $\dot{V}O_{2max}$ group: 19 male & 19 female; 45.4 ± 3.3 years	Serum	Baseline miR-210, - 21, -22 : **$\dot{V}O_{2max}$**	Bye *et al.* (2013)
18 healthy males; 20.2 ± 1.0 years	Plasma	Exercise-induced increase of miR-133b: **Peak power** Exercise-induced increase miR-122: **Peak power ratio**	Cui *et al.* (2015)
Masters athletes	Serum	Baseline miR-21-5p, -146a-5p: **Knee flexion and bench press strength** Baseline miR-146a-5p : **60m sprint time**	Kangas *et al.* (2017)
22 youth soccer players; 17.5 ± 0.7 years	Serum exosomes	Post-training miR-29a: **Post-training $\dot{V}O_{2max}$**	Domańska-Senderowska *et al.* (2017)
10 amateur basketball players; 25.9 ± 5.0 years	Serum	Post-training miR-221: **Post-training anaerobic threshold, peak workload & creatine kinase**	Li *et al.* (2018)
79 older postmenopausal women; 69.6 ± 5.6 years	Serum	Baseline miR-125-5p: **CMJ velocity and power**	Chen *et al.* (2019)
50 healthy middle-aged men; 48.8 ± 4.5 years	Plasma	Baseline miR-146a, -451a : **Lean leg mass** Baseline miR-222, -361: **Cross-sectional thigh muscle area**	D'Souza *et al.* (2019)

Note: $\dot{V}O_{2max}$ = Maximal oxygen consumption

Established baseline epigenetic modifications have been shown to affect the baseline physiological status of tissues. This is reflected by the differential basal signature of miRNAs when comparing specific groupings of participants (Table 6). Interestingly the signature of tissue miRNAs has been shown to differ between elite power-lifters and controls (D'Souza *et al.*, 2017a). In addition, Wardle *et al.* (2015) compared the ci-miRNA profiles of strength and endurance athletes. They reported specific ci-miRNAs (miR-222, miR-21, miR-146a and miR-221) are associated with specific training-related performance phenotypes. As previously detailed in section 2.3, RU requires certain positional specific physiological qualities that are integral for sporting performance. Specific genetic variations exist between playing positions

in elite rugby athletes (Heffernan *et al.*, 2017), but to date, no observations have been made on the potential ci-miRNA differences between positions.

Table 6. Studies reporting specific microRNA and circulating microRNA patterns dependant on the group

Subjects	Methodology	Sample Type	microRNA expression pattern alterations	Study
Low $\dot{V}O_{2max}$ group: 19 male & 19 female; 45.6 ± 3.2 years High $\dot{V}O_{2max}$ group: 19 male & 19 female; 45.4 ± 3.3 years	Assessed whether ci-miRNAs are associated with $\dot{V}O_{2max}$ level	Plasma	ci-miR-21 (male only), -210, -222 **↑ Increased levels found in low $\dot{V}O_{2max}$ group**	Bye *et al.* (2013)
10 Strength-trained males; 22.2 ± 2.1 years 10 Endurance-trained males; 22.6 ± 3.7 years 10 Age-matched non-exercising controls; 24.0 ± 2.8 years	Examined ci-miRNA profile differences between participants involved in long term training of either a strength or endurance modality training	Plasma	ci-miR-222, -21, -146a, -221 **↑ Increased levels found in endurance cohort** **↓ Decreased levels found in the strength cohort**	Wardle *et al.* (2015)
61 controls; 28.7 ± 10.6 years 67 endurance-trained; 33.9 ± 10.8 years	Examined ci-miRNA profile differences between endurance-trained and healthy controls	Plasma	ci-miR-1, -486, and -494 **↑ Increased levels found in endurance cohort**	Denham and Prestes (2016)
13 recreationally active young students; 24.3 ± 1.8 years 15 elite Norwegian Powerlifters; 23.5 ± 3.1 years	Examined ci-miRNA profile differences between elite level power lifters and healthy controls	Muscle biopsy (Vastus lateralis)	miR-15a, -16, -23a, -23b, -30b, -206, -451 **↑ Increased levels found in elite power lifters** miR-1, -126, -133a, -486, -499a **↑ Increased healthy controls**	D'Souza *et al.* (2017a)
10 sedentary males 14 male mountain ultra-trail non-professional athletes	Examined ci-miRNA profile differences between sedentary and trained participants	Plasma	ci-miR-1, -148b-3p, -28-3p, -29b-3p, -29c-3p, -335-3p, -374a-5p, -502-3p **↑ Increased levels found in trained participants** ci-miR-101-3p, 140-5p, -181a-5p, -199a-5p, -29a-3p, -378a-3p, -424-5p **↑ Increased in sedentary participants**	Faraldi *et al.* (2019)

Note: $\dot{V}O_{2max}$ = Maximal oxygen consumption

A pioneering study by (Davidsen *et al.*, 2011) indicated that miRNAs may play a role in phenotypic change and the observed variation in resistance exercise responsiveness in untrained individuals. Ogasawara *et al.* (2016) reported that 17 miRNAs obtained from skeletal muscle at baseline were differentially expressed between the top 5 high and top 5 non-hypertrophic responders. These findings present great promise of miRNA utility within an elite sport environment as only one sample is required to predict exercise responsiveness.

Nevertheless, a muscle biopsy is still required which will be very difficult to obtain from professional players. As can be seen from Table 7 however, recent research has shown ci-miRNAs obtained from one blood sample also have a potential diagnostic ability to predict a magnitude of training response. Yet to date, this research is very much in its infancy and has never been trialled in elite sports people following designated training programmes.

Table 7. Studies reporting associations between a pre/post change and a baseline abundance of microRNAs and circulating microRNAs with performance changes

Subjects	Sample Type	Performance metric change associations	Study
Untrained subjects 8 high responders; 22 ± 1 years 9 low responders; 23 ± 1 years	Muscle biopsy (vastus lateralis)	Δ miR-378, -29a, -26a, -451: **High responder status** Δ miR-378: **Δ muscle mass**	Davidsen *et al.* (2011)
3 older males and 4 older females	Muscle biopsy (vastus lateralis) & plasma	Δ miR-133a, -133b, 206: **Δ Knee strength** Δ ci-miR-499: **Δ Knee strength** Baseline ci-miR-499: **Δ Knee strength**	Zhang *et al.* (2015)
18 healthy males; 21.4 ± 1.4 years	Muscle biopsy (vastus lateralis)	17 mi-RNAs expressed at baseline: **High responder status (1RM)**	Ogasawara *et al.* (2016)
33 obese older adults; 69.3 ± 3.6 years	Plasma	Baseline ci-miR-181a-5p: **Δ Gait speed** Δ ci-miR-92a-3p: **Δ Gait speed**	Zhang *et al.* (2017)
30 young male collegiate athletes; 22.5 ± 4.1 years	Plasma	Baseline levels of ci-miR-93: **High responder status (isometric leg extension)**	Horak *et al.* (2018)
50 breast cancer surviving females; 50.7 ± 8.7 years	Serum	Δ ci-miR-133a-3p, -133b-3p: **Δ Leg press strength** Δ ci-miR-370-3p: **Δ Non-surgical arm strength**	Hagstrom and Denham (2018)

Note: Δ = Change

2.9 Summary of experimental aims

The key aim for coaches in elite sport is that the team performs in the game at the weekend and maintains this high level of performance throughout a full season. The training week is designed to ensure players are tactically and physically prepared for the task ahead. The challenge is for coaches to adopt an individualised approach that maximises performance, minimises fatigue and reduces injury risk in large squads. Appropriate load monitoring, measuring the response to training and understanding inter-individual variability is crucial in team sports, in order to maximise performance over an entire season (Figure 9). The thesis also provides a unique opportunity to understand how epigenetics and specifically microRNAs could integrate with an elite sport setting and how this information could enhance sporting performance. The overarching aim of this thesis was to understand how these approaches

work within an elite RU setting, and how best to optimise the training process to promote longitudinal performance. The aims for each of the studies are detailed below:

Chapter 4:

Currently, very limited research has reported on the training and match load demands of professional RU players (Table 1). Additionally, it is not well known how training practices may be manipulated over the course of a full rugby season (section 2.1.4). Therefore, this study aimed to quantify the external training and match loads to determine the influence of both position and match status. Secondly, the aim was to observe the distribution of load throughout an entire season in the English Premiership.

Chapter 5:

This study sought to understand more about monitoring the training response in elite RU players. The aim of this study was to (a) examine potential changes in performance, biomarkers and subjective wellness throughout a professional RU season and (B) assess the influence external training load measures may have on these responses.

Chapter 6:

Ci-miRNAs present a novel approach to understanding individual phenotypes, however to date no studies have examined them in elite RU players. The aim of this study was (a) to examine if ci-miRNA profiles were different between positions and (b) to observe associations between baseline ci-miRNA profiles with anthropometric and performance variables.

Chapter 7:

Ci-miRNAs may also provide a novel approach to understanding individual responsiveness to training, although studies to date are limited and have not been undertaken in an elite sporting population. The aim of this study examined the association between ci-miRNA abundance and the magnitude of change in performance variables associated with rugby performance.

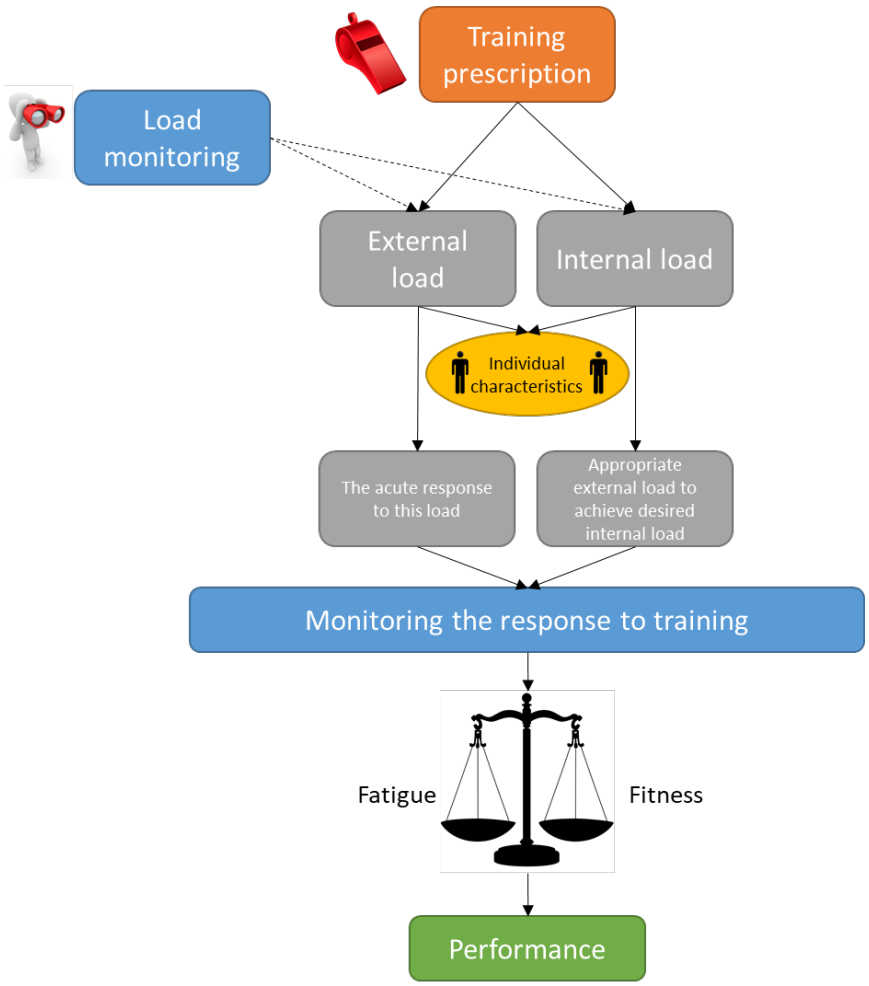

Figure 9. Optimising training prescription to enhance performance requires effective methods of 'quantifying training load' and 'monitoring the response to training load'. These processes are common and essential practices in elite team sports. Both of these monitoring areas are needed to inform future training prescription (Saw *et al.*, 2016; Thornton *et al.*, 2019).

Chapter 3

3.0 General methods

3.1 Introduction

The methods described in this chapter provide an overview of the assessments and analysis, which have been repeated and employed consistently throughout the experimental investigations within this thesis. In instances where the methods employed were specific to an individual investigation, details can be found in the methods section of the respective chapter.

3.2 Ethical approval

Prior to data collection, institutional ethical approval was obtained from the Newcastle University ethics committee. All testing was carried out in accordance with the declaration of Helsinki. In all experimental chapters, participants were provided with information sheets detailing the purpose of the study (Appendix A). Participants were also informed of the potential benefits and risks of taking part. All participants willing to take part signed the informed consent (Appendix B) and completed a health screening questionnaire (Appendix C). This research programme was undertaken in collaboration with Newcastle Falcons RFC. To maintain both professional and ethical standards, all study procedures were discussed and agreed upon with the head of athletic performance and director of rugby prior to commencing.

3.3 Participants

All participants were volunteers from a professional Premiership Rugby Union club, playing in the top English and European competitions. All volunteering players were regarded as first-team or senior academy professionals and were involved in international, Premiership and European matches. Participants had all previously performed the measures outlined in this chapter. Therefore, they were all familiarised with the experimental procedures prior to commencing the testing. All testing was undertaken at the club's training facility.

3.4 Training and match load quantification

Measures of external training load were calculated for every pitch-based training session and each match played during the experimental periods. The various methods used are explained below. An overview and description of the external load variables used in this thesis can be found in Table 8.

Table 8. Description of external training load variables via global positioning system (GPS) technology.

GPS Variable	Description
Total distance (TD)	Total distance covered in training or match play
Total low-speed running (TLSR)	Distance covered at or above 2.0 $m \cdot s^{-1}$ to 4.9 $m \cdot s^{-1}$
Total high-speed running (THSR)	Distance covered at or above 5.0 $m \cdot s^{-1}$
High-intensity running (HIR)	Distance covered at or above 5.0 $m \cdot s^{-1}$ to 6.9 $m \cdot s^{-1}$
Very high-intensity running (VHIR)	Distance covered at or above 7.0 $m \cdot s^{-1}$
PlayerLoad™ (PL)	Provides an overall external load
Total acceleration and deceleration efforts (AD Efforts)	The number of accelerations and decelerations is quantified as efforts ± 2 $m \cdot s^{-2}$
Individualised sprint efforts (IS Efforts)	The number of sprints is quantified as efforts above 70% of individual max speed with a minimum time of 0.4 second

3.4.1 Global positioning system

External load was quantified using micro-technology devices (Optimeye X4 devices, Catapult Sports, Melbourne, Australia). To determine an individual's locomotive characteristics and external forces, a GPS unit with a 10 Hz sampling rate and an in-built 100 Hz tri-axial accelerometer was worn. To avoid inter-unit error, before any experimental testing each participant was assigned a specific unit to wear for each session throughout the testing period (Jennings *et al.*, 2010). For training sessions and matches, players wore specialised jerseys or vests to house the device in a fixed position between the participants' shoulder blades (Figure 10A). Prior to use, GPS units were turned on 20 minutes before an activity; This ensures the adequate acquisition of satellite signals in advance (Waldron *et al.*, 2011). Live GPS data for each training session was collected in the team's stadium (Figure 10B). Following each activity, training and match data were checked for any irregularities using the manufacturer's software (Catapult Openfield, Catapult Sports, Melbourne, Australia). The checking of data required removing any instances of 'spikes' in player GPS traces and ensuring the players were in the correct time periods and drills. Data was subsequently downloaded and organised into a spreadsheet (Microsoft Excel, Microsoft Corporation, Redmond, WA).

GPS has been shown to provide valid and reliable estimates of constant-velocity movements during linear and rugby-specific high-intensity activities (Jennings *et al.*, 2010; Reardon *et al.*, 2015). Research has also shown valid and reliable measures for instantaneous velocity during acceleration and deceleration movements (Varley *et al.*, 2012).

Figure 10. These pictures highlight the placement of GPS units being placed in the players' jerseys (A) and where the live GPS data were collected within the stadium (B).

3.5 Physical performance testing

3.5.1 Counter movement jump

Countermovement jump (CMJ) performance was measured from flight time scores using a timing mat (Just Jump System, Probotics, Huntsville, Alabama, USA). Each participant performed three maximal efforts on every required testing occasion. Participants were instructed to perform each jump with maximal effort and jump as high as possible. Each jump was separated with sufficient rest to allow for a full recovery. To perform a valid jump, participants were instructed to place their hands on their hips, descend rapidly to a self-selected depth, usually ~ 90° knee joint angle, and then jump vertically with maximum force. Standardised verbal encouragement was provided throughout. Jumps were void if the participants' hands left their hips to allow an arm swing, or knee and/or hip flexion occurred during the flight phase. The best height achieved from the three repetitions was used for data analysis and the inter-day coefficient of variation for this protocol was calculated as <2.5%.

3.5.2 Weighted squat jump

A linear velocity transducer (LVT) was used to determine the mean and peak velocity during a weighted squat jump (GymAware, Kinetic Performance Technology, Canberra, Australia). An LVT is both valid and reliable at recording peak and mean measures of velocity (García-Ramos *et al.*, 2016; O'Donnell *et al.*, 2018). Following a warm-up participants performed 3 jump squats with an external load of 40 kg. This required them to firstly unrack the 20 kg barbell with an additional 20 kg weight and place it on their upper trapezius just below C7. They then used a self-selected foot position and performed a countermovement jump as detailed above. Again depth of the jump was self-selected but they were advised to keep this consistent throughout each jump. Participants performed 3 jumps and the peak scores were used for data analysis. The intra-individual reliability of mean velocity returned a coefficient of variation of 2.5% and 2.0 % for peak velocity.

3.5.3 Drop jump

The reactive strength index (RSI) was determined via the completion of a drop jump test. Participants were instructed to step forward off a 30 cm box with hands placed on their hips. On contact with the jump mat (Just Jump System, Probotics, Huntsville, Alabama, USA) participants were instructed to jump 'as high as possible and as quickly as possible'. To ensure a measure of standardisation and a successful jump, participants ground contact time had to record below 200 ms, confirming this test is assessing the fast portion of the stretch-shortening cycle (Young *et al.*, 1995). Additionally, no knee and/or hip flexion was allowed during the flight phase. The reactive strength index ($cm \cdot s^{-1}$) was calculated as jump height (cm) divided by ground contract time (s). All participants were given three attempts at each jump with two minutes provided between jumps to ensure adequate rest. The best RSI score generated across the three repetitions was used for subsequent data analysis.

3.5.4 One Repetition Maximum Strength Testing

For both the squat and bench press 1 repetition maximum (1RM) tests, the following procedures were undertaken. As a warm-up, each athlete was required to perform three submaximal sets (50%, 70% and 90% efforts) of 5 - 2 repetitions with progressively heavier loads. The test commenced following the completion of the warm-up, where the aim was to achieve at least an individually prescribed 1RM goal. This goal was predetermined by the lead strength and conditioning coach and based on previous testing results. Adequate rest was provided between each set and maximum attempt. If the athlete successfully achieved this

goal, they were allowed another attempt at completing a 1RM lift. Once both the coach and athlete were happy with a successful 1RM attempt, the athlete's score was recorded. If however, they failed the further attempt the previous successful lift was given.

For both exercises, a trained strength coach visually assessed the 1RM tests. For a successful full squat attempt, athletes used a self-selected foot position and were required to descend until the top of the thigh was parallel with the floor and then return to a standing position. For the bench press, athletes used a self-selected hand position and were required to lower the bar to the chest before pressing the bar back up in a vertical movement so that the arms were fully extended. The bar could not be bounced off the chest.

3.6 Acute exercise stimulus

3.6.1 England anaerobic endurance test

The England Anaerobic Endurance Test (EAET) was developed by the RFU to measure a player's ability to perform repeated bouts of high-intensity exercise. The test is widely used within Premiership Rugby Union clubs and at an international level as a measure of rugby-specific fitness. The test is designed to mimic specific running actions and demands in Rugby Union such as distances and work-rest ratios. Research has shown repeat sprint tests mimicking Rugby Union demands are informative to coaches as they correlate to match performance demands (Beard *et al.*, 2019). The EAET is separated into two distinct tests: one specific for each of the two positional groups (forwards & backs). Position-specific tests are important as Scott *et al.* (2003) showed physiological differences between forwards and backs, indicating that position should be taken into account when testing and evaluating aerobic fitness. This test was chosen because it was a requirement of the coaching staff, and was integrated into their usual training routine. The Bronco test is an additional test that is used commonly within Rugby Union (described specifically in Chapter 6). A simple regression analysis of in-house data highlighted that the EAET correlates well Bronco performance (All; $P < .001$, $r = 0.76$; Forwards, $P < .001$, $r = 0.72$, (Figure 11A); Backs, $P < .001$, $r = 0.72$, (Figure 11B).

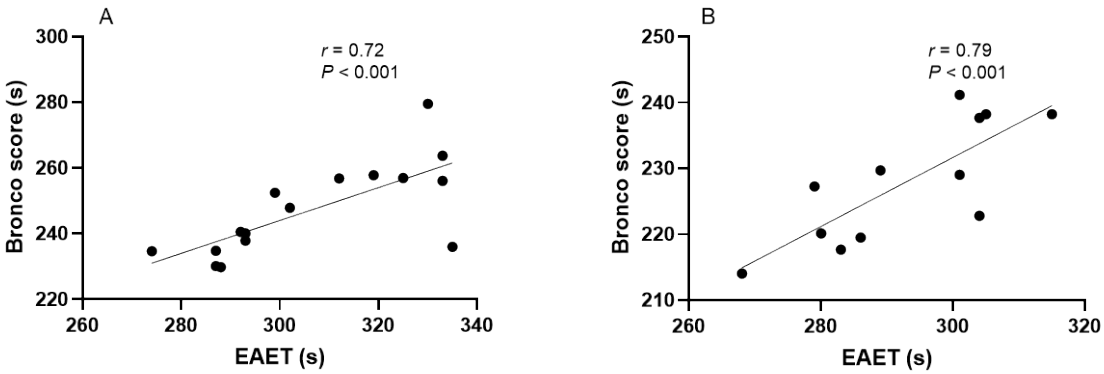

Figure 11. Significant correlations were observed between the forwards (A) and backs (B) Bronco test times and performance in the England anaerobic endurance test (EAET).

Performance in the EAET is determined via the total time taken to complete the test and also the fatigue index between the first and last repetitions. The forwards test as seen in Figure 12A requires players to start on their chest, sprint out 5m and return running backwards to the start line where they then again drop down to their chest. They then sprint out and perform return shuttles to the 10m and 20m cone, dropping down to their chest on each return to the start line. The test format follows, 1 x 1 repetition, 1 x 2 consecutive repetitions, 1 x 2 consecutive repetitions, 1 x 4 consecutive repetitions and finally 1 x 1 repetition. A schematic for the backs test can be seen in Figure 12B, backs also start on their chest and sprint out to the 5m line and return running backwards to the start line where they again drop to their chest, this is performed twice. They then get back up and sprint out and around the outside of the 10m pole, on to and around the outside of the 20m pole, on again to the 30m pole, and then back to the start line. The test format for backs is 1 x 1 repetition, 1 x 1 repetition, 1 x 2 consecutive repetitions, 1 x 2 consecutive repetitions, 1 x 2 consecutive repetitions and finishing with 1 x 1 repetition. For both tests, the recovery time between each set is based on the running clock and the time taken to complete the required repetitions, i.e. the start of each section is fixed on a running clock so that the faster the completion time, the more rest the player receives.

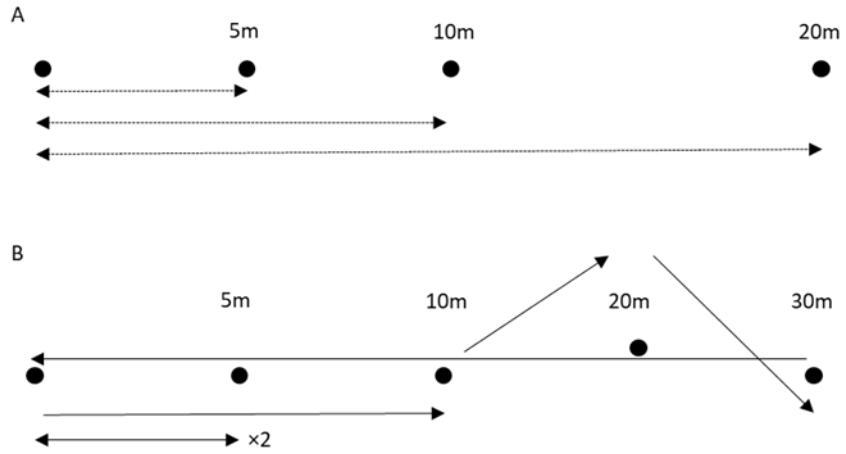

Figure 12. Details the cone placement to conduct the England Anaerobic Endurance test. A = Forwards specific and B = Backs specific.

3.7 Perception of wellbeing

3.7.1 Daily subjective wellness questionnaire

Players were required to complete a wellness questionnaire before any training activity on every specified training day (Appendix E). Each player's data was entered privately and collected daily via an app on an electronic device. Data from all players were then downloaded and added to one database (Microsoft Excel, Microsoft Corporation, Redmond, WA). This type of practice is common in a team sport setting (Taylor *et al.*, 2012). All players were familiar with this practice prior to data collection.

Players provided subjective ratings for subscales of wellness, comprising of physical (upper-body soreness, lower-body soreness, current energy levels), and lifestyle-related domains (mood, sleep duration, sleep quality). Each measure was quantified on a 5-point Likert scale, whereby 1 represented the lowest and 5 the highest possible rating of wellness respectively, the summation of all scores provided an individual's daily total wellness score (6 – 30 AU). Reliability measures were established for the questionnaire, the interclass correlation coefficients (ICC) calculated to indicate test-retest reliability was ICC = 0.77, and the typical error (expressed as CV %) was 6%. Results from Hills and Rogerson (2018) reported similar validity and reliability values.

3.8 Blood sampling and analysis

Blood samples for all studies were obtained from a branch of the basilica vein at the antecubital fossa using the venepuncture technique. For Chapter 5, 5 × 4 ml vacutainers containing di-potassium ethylene diamine tetra-acetic acid (EDTA) and 5 × serum (10 ml) were collected. Only 1 × EDTA (10 ml) vacutainer was required for Chapters 6 and 7. Specific information regarding the timing of these collections is detailed in the respective chapters. All samples were taken at the club's training facility (Figure 13) and transported to the university laboratory for analysis within an hour of collection, allowing sufficient time for serum tubes to clot. The 4 ml EDTA tubes were taken to a local hospital for haematological analysis. The remaining samples from the 10 ml EDTA and serum tubes were centrifuged at 3000 rpm (4˚C) for 10 min, and the resulting plasma and serum supernatant were aspirated into aliquots and stored at −80 ˚C for later analysis.

Figure 13. An example set-up of the morning blood sampling process.

3.8.1 Full blood count

Full blood count analysis was measured in whole blood using an automated haematology system (Sysmex XE-2100, Illinois, US). According to data provided by the laboratory, the CV's for this procedure are typically <10%.

3.8.2 Creatine Kinase

Serum creatine kinase (CK) analysis was performed with an automated system (Cobas 8000 c702, Roche Diagnostics, UK). The CV for CK analysis using this system is typically calculated as < 2 %. Normal reference values for this assay in males is < 190 UL.

3.9 microRNA analysis

3.9.1 RNA isolation

All molecular analysis was conducted at the University of Stirling. Total RNA was extracted from plasma samples using the miRNeasy Serum/Plasma Advanced Kit (miRNeasy Qiagen Ltd., West Sussex, UK) according to the manufacturer's guidelines. During isolation, RNA spike-ins UniSP2, 4 and 5 (Qiagen Ltd., West Sussex, UK) were added for analysis of extraction efficiency. RNA extraction as detailed in Figure 14 was completed as follows.

Prior to extraction, frozen plasma samples were thawed completely at room temperature and inverted to re-constitute any molecules which may have come out of the solution whilst frozen. 200 µl of plasma were then transferred to a reaction vessel. A solution comprising of 59 µl Buffer RPL (lysis of proteins, exosomes and inactivation of RNases) and 1 µl of UniSP2, 4 and 5 spike-in was added to the plasma. The sample was then vortexed for ≥ 5 s and subsequently left at room temperature for 3 min. Twenty µl of Buffer RPP (precipitation of proteins and other contaminants) was added and the samples were mixed vigorously by vortexing ≥ 20 s before being left at room temperature for a further 3 min. The samples were then centrifuged at 12000 x g for 3 min at room temperature to pellet the precipitate. The resulting supernatant (~230 µl) was transferred to a new microcentrifuge tube and 1 volume of isopropanol was added, the samples were again vortexed for ≥ 10 s. Following this the samples were transferred to an RNeasy UCP MinElute Column and centrifuged for 15 s at 8000 x g, any flow-through was discarded. 700 µl of Buffer RWT (a stringent washing buffer) was pipetted into the spin column and then was centrifuged for a further 15 s at 8000 x g, again any flow-through was discarded. 500 µl of Buffer RPE (a mild washing buffer) was also added to the spin column and further centrifuged for 15-seconds at 8000 x g, flow-through was discarded. 500 µl of 80%-ethanol was then added to the spin column before being centrifuged for 2 min at 8000 x g. The flow-through and collection tube were then discarded. Spin columns were placed in a new collection tube and centrifuged with lids open for 5-minutes at maximum speed to dry the spin column membrane. Spin columns were then placed into a new collection tube and eluted using 20ul of RNAse-free water. Samples were incubated for 1 min at room temperature and then centrifuged for 1 min at maximum speed to elute the RNA. RNA samples were then either reverse-transcribed immediately or stored at -20°C until further analysis.

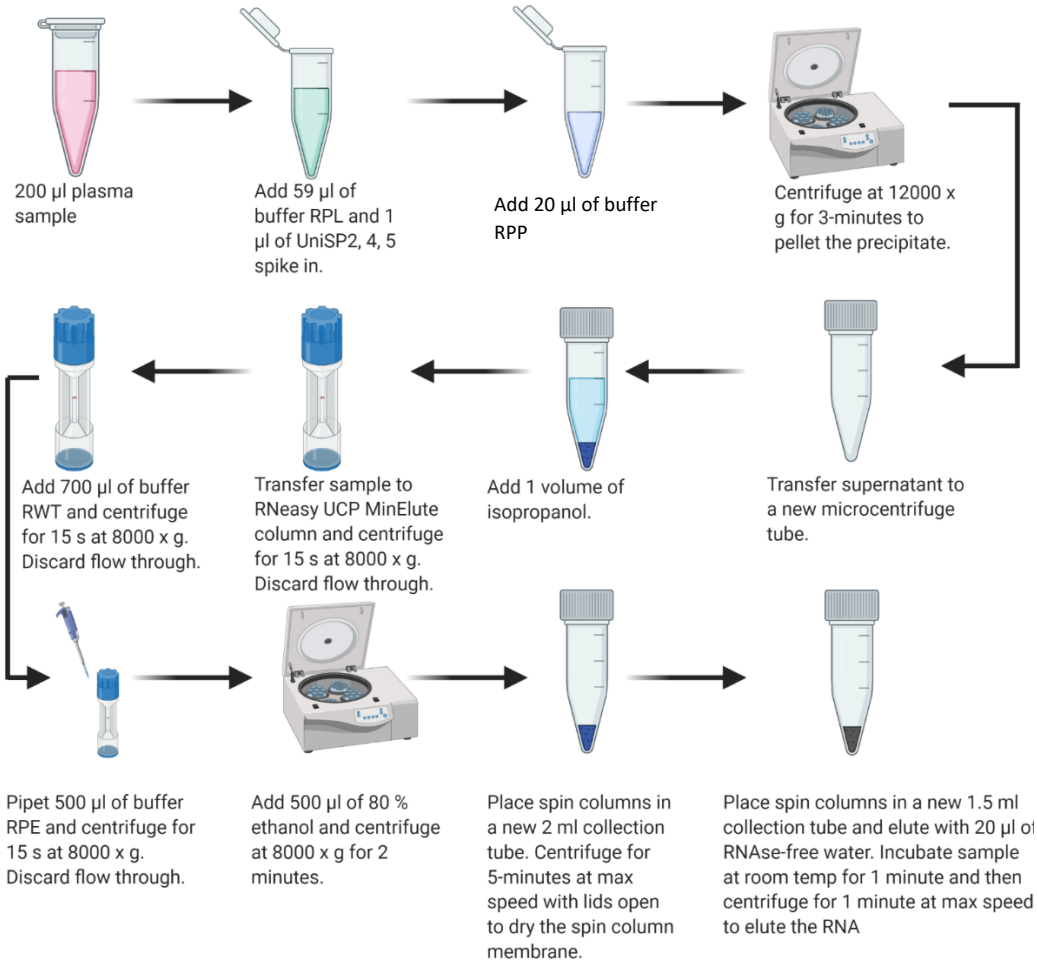

Figure 14. RNA isolation process

3.9.2 Complementary DNA (cDNA) synthesis

From the resulting RNA eluate, RNA was reverse transcribed using miRCURY LNA RT Kit (Qiagen Ltd., West Sussex, UK) following a modified version of the manufacturer's instructions. In addition, a combination of UniSP6 and cel-miR-39 spike-ins were added during cDNA synthesis for analysis of efficiency. Table 9 highlights at each stage of analysis cDNA synthesis reactions were prepared using an optimised RNA input amount according to the end volume required for each stage. The samples were then reverse transcribed using the manufacturer's recommended temperature cycling protocol. Samples were incubated for 60 min at 42°C and then incubated for a further 5 min at 95°C to heat inactivate the reverse transcriptase, following this they were immediately cooled to 4°C. The resulting cDNA samples were used either immediately in qPCR or stored at -20°C until further analysis.

Table 9. cDNA Synthesis reaction setup.

Component	Qiagen Recommended Single Assay	Single Assay	Focus PCR Panel: Plasma	Custom Panel
5x miRCURY RT Reaction Buffer	2 µl	2 µl	4 µl	3 µl
10x miRCURY RT Enzyme Mix	1 µl	1 µl	2 µl	1.5 µl
RNase-free water	4.5 µl	2.5 µl	1 µl	0.75 µl
*cDNA synthesis spike-in **	0.5 µl	0.5 µl	1 µl	0.75 µl
Template RNA	2 µl	4 µl	12 µl	9 µl
Total Reaction Volume	10 µl	7 µl	20 µl	15 µl

Note: * UniSP6 and Cel-miR-39 for plasma panels. UniSP6 alone for custom panels.

3.9.3 Ci-miRNA analysis

Quantitative polymerase chain reaction (RT-qPCR) was conducted using a variety of miRCURY LNA miRNA PCR products; Single Assays, Plasma Focus Panels and Custom Panels (Qiagen Ltd., West Sussex, UK). These were used at different stages to ensure optimised results and appropriate selection of plasma miRNAs for experiments that are specific to an elite rugby cohort. The reaction mix for single assays was optimised through a series of in house experiments, and the focus and custom panel experiment setup was customised to reflect the optimised reaction mix for single assays (Table 10). RT-qPCR were run on a Roche LightCycler 480 (Roche, USA) in 384-well configuration and in accordance with the manufacturer's recommended cycling conditions. UniSP3 was used on both the focus panel and custom panel for analysis of cross-panel qPCR efficiency and subsequent inter-plate calibration.

Table 10. Details of the specific RT-qPCR reaction mixes.

Component	Qiagen Recommended Single Assay	Optimised Single Assay	Serum/Plasma Focus Panel	Custom Panel
2x miRCURY SYBR Green Master Mix	5ul	2.5ul	2.5	2.5ul
PCR Primer Mix	1ul	1ul	-	-
RNase-free Water	2ul	0.5ul	2.4ul	0.5ul
cDNA Template (dilution ratio)	3ul (1:30)	3ul (1:12.5)	0.1ul	4 (1:25)

Total Volume	10ul	7ul	5ul	7ul

Quality control experiments were conducted to assess the efficiency of miRNA extraction and cDNA synthesis in all samples before progressing to focus or custom qPCR panels. Qiagen's miRCURY LNA miRNA PCR assay (hsa-miR-16-3p) was used for the quality control step. All samples were run in duplicate and required both Ct values within 1 cycle and an average Ct value below 35 to progress to either qPCR panel. Qiagen miRCURY LNA miRNA Plasma Focus Panels were used on selected samples (n=12) to identify target miRNAs for analysis. This helped us determine candidate circulating microRNAs (ci-miRNAs) to be used in Chapters 6 and 7. The selected ci-miRNAs that can be seen in Figure 15 were a result of in-house testing and due to their status as myomiRs or a known exercise response within the literature. This resulted in Qiagen miRCURY LNA miRNA Custom Panels being used to analyse 23 miRNAs in all samples (n=72) for the final testing stage (Figure 16). All samples were analysed in technical triplicate on the custom panels.

No.	Type	Origin	Assay
1	Target	Panels	**hsa.miR.421**
2	Target	Panels	**hsa.miR.100.5p**
3	Target	Panels	**hsa.miR.150.5p**
4	Target	Panels	**hsa.miR.92a.3p**
5	Target	Panels	**hsa.miR.885.5p**
6	Target	Panels	**hsa.miR.18b.5p**
7	Target	Panels	**hsa.miR.146b.5p**
8	Target	Panels	**hsa.miR.301a.3p**
9	Target	Panels	**hsa.miR.126.3p**
10	Target	Panels	**hsa.miR.144.3p**
11	Target	Literature	**hsa.miR.1**
12	Target	Literature	**hsa.miR.133a**
13	Target	Literature	**hsa.miR.133b**
14	Target	Literature	**hsa.miR.206**
15	Target	Literature	**hsa.miR.208b**
16	Target	Literature	**hsa.miR.221**
17	Target	Literature	**hsa.miR.149**
18	Control	Endogenous	**hsa.miR.320a**
19	Control	Endogenous	**hsa.miR.30e.5p**
20	Control	Spike-in	**UniSP2**
21	Control	Spike-in	**UniSP4**
22	Control	Spike-in	**Cel-miR-39**
23	Control	PCR panel control	**UniSP3**
24	h20	blank	**Blank**

Figure 15. A list of the circulating miRNA targets was selected for analysis as a result of prior testing and a literature search. The control markers are also provided.

Figure 16. Preparation of 384-well plate Qiagen miRCURY LNA miRNA Custom Panels using a multi-pipette tool.

3.9.4 Ci-miRNA expression quantification

Panels were quantified on a Roche LightCycler LC480 according to the manufacturer's instructions. Late Ct calls (>40 cycles) were manually removed. The absolute median deviation was calculated for the remaining samples to remove outliers. Panels were calibrated using UniSP3 inter-plate calibration spike-in. Delta Ct (ΔCt) values were calculated for the calibrated dataset relative to the geometric mean of the two endogenous controls (hsa.miR.320a and hsa.miR.30e.5p). Delta-delta Ct (ΔΔCt) was calculated relative to the average ΔCt of all samples. Relative expression was calculated as $2^{-\Delta\Delta Ct}$.

Chapter 4

4.0 Quantification of Training and Match-Load across a Season in an Elite Rugby Union Team.

4.1 Introduction

Success in elite-level rugby requires a consistently high level of performance across a season spanning 8 months; matches are played weekly, and players are expected to perform optimally throughout the entire season. This presents a significant challenge for coaches and performance staff, who must plan and action periodisation strategies to ensure effective tactical and physiological athlete needs are met, whilst also trying to reduce injuries (Hägglund *et al.*, 2013).

Training load can be divided into two sub-sections: external and internal. External training load refers to the objective measures of the work performed by the player during training or competition, whilst the internal load is the player's relative physiological and psychological stress as a result of the training or competition (Bourdon *et al.*, 2017). The recent advancement in wearable technology and the development of GPS units have aided the quantification of external training load. GPS devices can quantify distance, and provide speed-derived measures and accelerometer-derived load measures (Malone *et al.*, 2017). This provides coaches and performance staff with an objective measure of training and competitive matches. To date, a large body of literature describes the locomotor and physical requirements of RU match-play (Roberts *et al.*, 2008; Cunniffe *et al.*, 2009; Quarrie *et al.*, 2013; Lacome *et al.*, 2014; Jones *et al.*, 2015). Knowledge of individual players match demands helps coaches plan, prescribe and implement personalised training programmes that takes positional differences into consideration.

Previous research in RU, rugby sevens, and rugby league, has quantified the locomotor demands of preseason (Bradley *et al.*, 2015b; Daniels *et al.*, 2019; Grainger *et al.*, 2020; Tiernan *et al.*, 2020), microcycles (McLean *et al.*, 2010; Moreira *et al.*, 2015b), intensified periods (Bouaziz *et al.*, 2016; Lacome *et al.*, 2018) and full seasons (Cunniffe *et al.*, 2011a; Bradley *et al.*, 2015a; Dubois *et al.*, 2017; Marrier *et al.*, 2019; Dubois *et al.*, 2020b). However, despite the recent advancements in GPS technology, most studies have used the session rating of perceived exertion (S-RPE) method, due to ease of collection. This method of collection provides an internal measure in response to potential changes in the volume and intensity, but it should not be used in isolation (Bourdon *et al.*, 2017) as no context is provided as to specifically how training load is prescribed. Additionally, the majority of the studies have only

reported load at specific time points in the season, with little research examining how training load may be manipulated throughout an entire season.

Of the studies that have previously explored training load throughout an entire season (Table 1), Bradley *et al.* (2015a) only provided the mean weekly training load for forwards and backs and did not include the match loads. These findings do not represent the true 'weekly load', as data is of a training week and not matches, which are a large contributor to the weekly total. Additionally, they only provided a mean of the entire season which does not detail if fluctuations in training load occur throughout a season. Dubois *et al.* (2017) added to these findings by highlighting week-to-week changes, as well as the changes in mesocycle blocks. However, data were only reported for backs. The most recent paper from Dubois *et al.* (2020b) is the most informative to date providing training data from a French Pro 14 team for weeks and mesocycle blocks for both forwards and backs. To date though, no study has reported weekly training loads in a professional RU team competing in the English Premiership.

An English Premiership rugby team has a large squad (~ 40 players), and therefore providing all players sufficient training to maintain fitness and ensure game readiness is a challenge. No research in rugby has yet attempted to observe how the match starting status (starting-15, bench or non-squad) impacts the weekly training loads of these groups of players. This is particularly important to understand, as players not replicating game demands and who have a reduced training exposure, may not be match-ready and might require a different training prescription. This type of information would be advantageous to coaches as it would provide specific methods of loading strategies for these players based on their exposure.

Due to the lack of data currently available, there is a knowledge gap in the practices of elite teams when it comes to the quantification of external training load throughout the entirety of a season. The majority of what is currently known in team sports is unpublished and derives from the coaches personal experience, as many elite teams are unwilling to display data surrounding their practice, with the worry other teams will gain a competitive edge (West *et al.*, 2019; Kelly *et al.*, 2020). Recent surveys in professional senior football (Akenhead and Nassis, 2016) and rugby (West *et al.*, 2019) detailed that the vast majority of clubs use GPS as a measure of external load and yet still so little is understood as to how GPS data is practically utilised within research. Providing an overview of professional practices can stimulate applied research, to understand the practical challenges players may face and how coaches' prescribed training load is associated with traditional periodisation models.

It is essential to understand the complexities and demands of preparing a professional rugby player throughout a season in order to maximise performance. This will allow coaches and practitioners to understand what they are preparing players for so that individualised training measures can be put in place and windows of opportunity can be determined. Therefore, this study aimed to quantify the combination of training and match load (the total external physical load) and examine the distribution across the competition period of an English Premiership Rugby team, for (a) playing position and (b) player status.

4.2 Methodology

4.2.1 Participants

Data were collected from a squad of professional RU players (n = 30, height: 185.8 ± 8.0 cm; mass: 104.0 ± 11.0 kg and game minutes played 1173± 408 min) across a 38-week competition phase of the 2018-2019 English Premiership season. All players had been playing RU professionally for at least 2 years. Where players may have been involved in international competition, training loads were still monitored and collected. To analyse positional demands, players were assigned to one of three positional groups: Front-5 (F5, n = 12), Middle-5 (M5, n = 7) and Back-5 (B5, n = 11). These groupings are common positional training groups within professional RU. Each group consists of specific playing positions, the F5 group involves 'props', 'hookers' and 'locks', the M5 group, 'flankers', 'number 8s', 'scrum-half's' and 'fly-half's' and the B5 group 'centres', 'wingers' and 'full backs'. Positional player characteristics can be seen in table 2. All players provided written informed consent before volunteering for the study. Experimental procedures were approved by the Newcastle University research ethics committee.

4.2.2 Experimental approach

An observational longitudinal study design was employed to collect external load data throughout a 10-month period (end of August - middle of May). All data was collected from the club's training ground and at no point were data collection or training methods altered to accommodate this study. In this study, every 1st team training session, home and away matches were included in the analysis. Training sessions were inclusive of the starting line-up, bench, and non-squad players but senior academy players were not included. Individual sessions outside the specified training schedule, rehabilitation, and any modified sessions

were also excluded from the analysis. Over the 38-week competitive season, 220 training and match days were recorded, totalling 4364 individual sessions.

4.2.3 Study design

The competitive season comprised of 33 competitive games, 1 mandatory rest week and 4 no-game weeks, as detailed in Figure 17. Training load throughout the competitive period was predominantly maintenance based with a focus on recovery at the start of the week and then prioritising game readiness as a forthcoming competitive fixture approaches. Figure 18 displays the manipulation of training volume and intensity as a game week progresses. Top-up conditioning throughout the season was individually prescribed and based on the needs of the players and the training and match load exposure following the previous week's game. External load variables were recorded daily and observed as a weekly total. All training and match-load data were categorised into 3 mesocycle phases to enable a full season's analysis of the training and match load. The season was therefore characterised into 'early', middle and 'end' of season blocks (Figure 17).

The sum of all session external training loads for a given training week represented an individual's weekly training load. The weekly training load was dependent upon a microcycle that was adjusted accordingly to optimise game readiness and recovery. One confounding factor that adjusted an individual's weekly training load was the player status on a given week. Players were grouped as 'starting 15', 'bench' and 'non-squad', as can be seen from Figure 18 their participation in different training sessions throughout the week was a determinant of their match starting status. This enables the analysis of external load exposure and potential high-competition exposure of different team status groups throughout the duration of a competitive season. To enable a full competitive season analysis of the potential exposures, the game weeks (n = 33) were split into 3 competitive blocks (Figure 17).

4.2.4 External load quantification

The player's external load was monitored and quantified using GPS devices (Optimeye X4 devices, Catapult Sports, Melbourne, Australia). The data were collected using the techniques described in section 3.4.1 (Global positioning system). The observed training and match-play variables identified for analysis are detailed in section 3.4 (Training load quantification) and Table 8.

4.2.5 Data analysis

All data are expressed as mean ± standard deviation (SD), with statistical significance set at $P < 0.05$ a priori, mean difference and 95 % confidence intervals are also provided where applicable. All analyses were performed using IBM SPSS Statistics 25 for Windows (Surrey, UK).

To quantify the differences in external training load variables between positional groups (F5 vs M5 vs B5) and player status (starting 15 vs bench vs non-squad) a one-way independent analysis of variance (ANOVA) was used, no-game weeks were removed from this analysis. The dependant variables (TD, TLSR, THSR, HIR, VHIR, PL, AD Efforts, IS Efforts) remained the same for both of these analyses. A one-way ANOVA was also utilised to analyse the differences between stages of the season, determined by the mesocycle blocks. As the analysis focused specifically on the external running loads, TD, TLSR and THSR were analysed.

Diagnostic checks (Shapiro-Wilks test of normality and Levene's homogeneity of variance test) were performed on all dependent variables. Welch's *F* was used if a test failed heterogeneity of variances, and sphericity was assumed if Mauchly's test score returned $P \geq 0.05$, with Greenhouse-Geiser adjustments made where appropriate. In the case of VHIR, the analysis revealed skewed distributions and this data set was subsequently log-transformed before statistical procedures. Cohen's d effect sizes (ES) were also provided where relevant, magnitude of effects were considered trivial (< 0.2), small (0.2 - 0.6), moderate (0.6 - 1.2), large (1.2 - 2.0) and very large (2.0 – 4.0) (Batterham and Hopkins, 2006).

Professional Rugby Season																																					
Month	Aug	Sep					Oct				Nov		Dec						Jan				Feb				Mar				Apr					May	
Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Season Block	Block 1 - Early												Block 2 - Middle											Block 3 - End													
Periods	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	R	CG	CG	CG	CG	CG	CG	NG	CG	NG	CG	CG	NG	CG	CG	NG	CG
Competition Block	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2		2	3	3	3	3	3		3		3	3		3	3		3

Figure 17. Schematic representation of how the season was organised and how the experimental design was employed. The weeks shown are individual weeks within the annual training cycle. The season was split into 3 blocks representing different time periods, 'early', 'mid' and 'end'. The week type was characterised as a competitive game (CG), rest week (R) and no-game (NG). Competitive blocks were only inclusive of game weeks, therefore, did not include rest weeks and no games, these were designated to 3 different time points.

Week Overview							
	Monday (MD + 2)	Tuesday (MD + 3)	Wednesday (MD + 4)	Thursday (MD - 2)	Friday (MD - 1)	Saturday (MD)	Sunday (MD + 1)
AM	Whole-body gym session	Upper body gym session	Off (Recovery)	Power-based gym session	Captains run: Low volume, low intensity for starting 15 and bench players		Off (Recovery)
PM	Rugby focus pitch session & top-ups for players with low exposure from the previous game (bench): Low volume, low intensity	Skills/units session: Low volume, low intensity Rugby tactical/technical pitch session: High volume, moderate-intensity		Skills/units session: Low volume, low intensity Rugby training, match simulated & top-ups applied for non-squad: Low volume, high intensity	Non-squad rugby conditioning session: Moderate volume, high intensity	Game	

Figure 18. The organisation of a typical in-season microcycle of 7 days.

4.3 Results

Demographic characteristics are presented in Table 11. Body mass was significantly different between different positional groups (Welch's $F_{(2, 12.5)} = 25.05$, $P = < .001$), the F5 group is heavier than both the M5 ($P = 0.006$, 16.58 kg, [5.79, 27.37 kg], ES = 2.3) and B5 ($P < .001$, 17.58 kg, [10.32, 24.84 kg], ES = 2.7) group. Additionally, the F5 had a greater sum of skinfolds ($F_{(2, 27)} = 5.26$, $P = 0.012$), when compared to the B5 ($P = 0.012$, 19.74 mm, [3.98, 35.50 mm], ES = 1.3). A difference in height was also observed ($F_{(2, 27)} = 3.76$, $P = 0.036$) however no *post hoc* significance was observed between different positional groups. There were no differences between groups for game minutes ($P > 0.05$).

Table 11. Participant characteristics are categorised on their positional groups within the team. Values are presented as mean ± SD.

	Number (n)	Height (cm)	Body mass (kg)	Sum of skinfolds (mm)	Game Minutes (min)
Total	30	185.8 ±8.0	104.0 ± 11.0	82.7 ± 17.3	1173 ± 408
Front 5	12	190.3 ± 8.6	114.3 ± 4.2	93.6 ± 15.7	1019.7 ± 378.4
Middle 5	7	182.4 ± 7.3	97.7 ± 9.3 *	78.0 ± 13.9	1321.4 ± 444.8
Back 5	11	183.0 ± 5.5	96.7 ± 8.3 *	73.9 ± 15.4 *	1246.1 ± 396.2

* signifies significant difference from the Front 5 positional group ($P < 0.05$).

4.3.1 Season overview

The average weekly TD for all positions over the 38 weeks is 15075 ± 2872 m (Figure 19A) and the weekly average distances for TLSR and THSR were 6574 ± 1081 m and 1310 ± 281 m (Figures 19B and 19C). With respect to the 3 mesocycles that break up the season, average weekly TD, TLSR and THSR demands are presented in Figure 20. A significant change in all variables were detected across the different mesocycles (TD and TLSR: $P < .001$, THSR: $P = .001$). External running loads towards the closing stages of the season appear to be the smallest. The weekly TD volumes in block 1 and 2 were much greater when compared to block 3 ($P < .001$, 3068 m, 95% CI range, [1902 to 4233 m], ES = 1.67 and $P < .001$, 2499 m, [1332 to 3664 m], ES = 1.28 respectively; Figure 20A). TLSR distances was lower in mesocycle 3 compared to mesocycle 1 ($P < .001$, -1296 m, [-1728 to -863 m], ES = 1.94; Figure 20B) and mesocycle 2 ($P < .001$, -1011 m, [-1443 to -579 m], ES = 1.40). The greatest average weekly THSR distances occurred in block 2 (1587 m ± 491; Figure 20C). These distances were much greater than block 3 ($P < .001$, 488 m, [179 to 797 m], ES = 0.99) but, no different to block 1 $P > 0.05$).

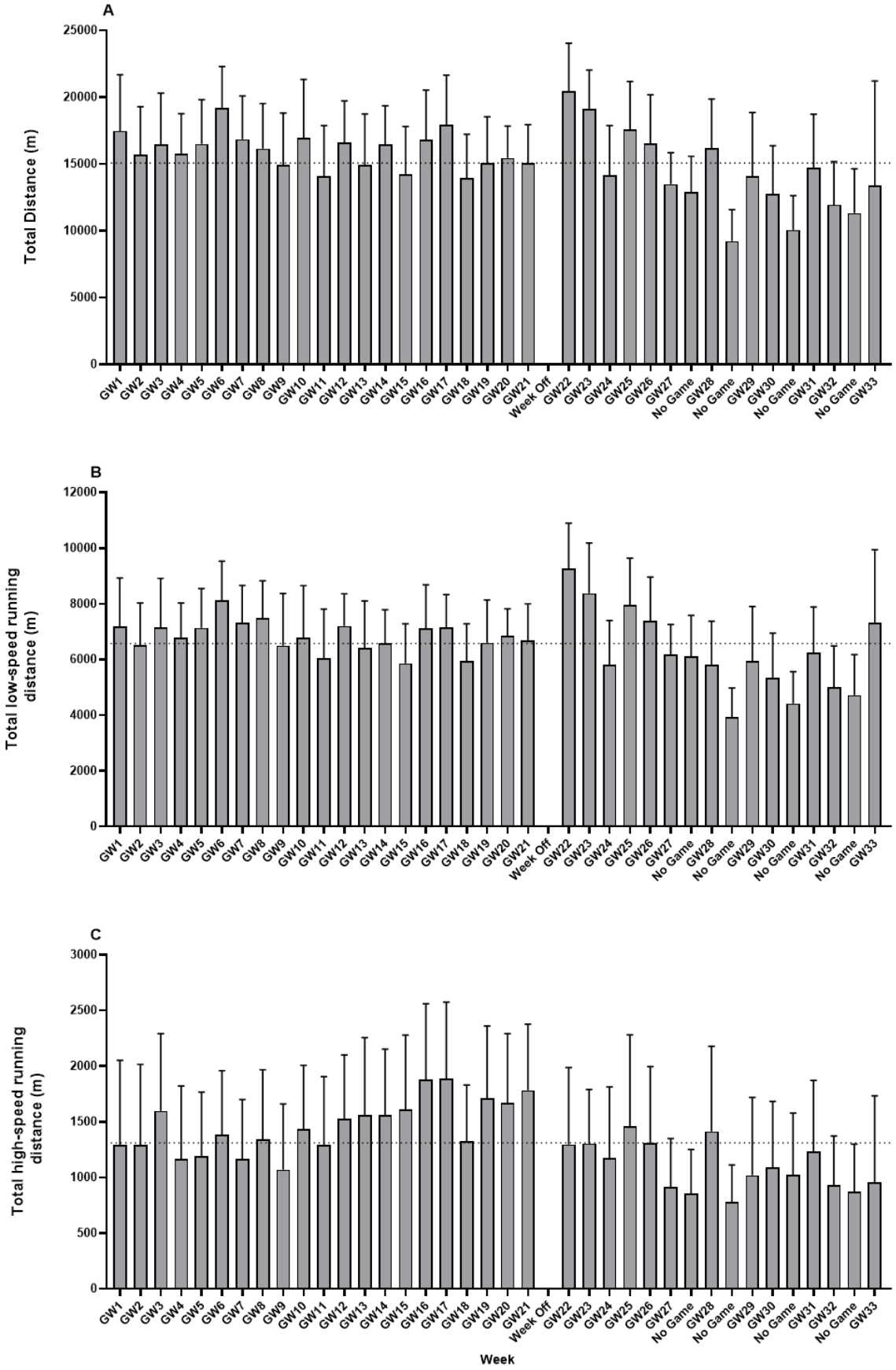

Figure 19. Mean ± SD total distance (A), total low-speed running (B) and total high-speed running (C) for each training week during a competitive season for all players. 'GW' refers to game-week and the dotted line indicates the season average for each specific variable.

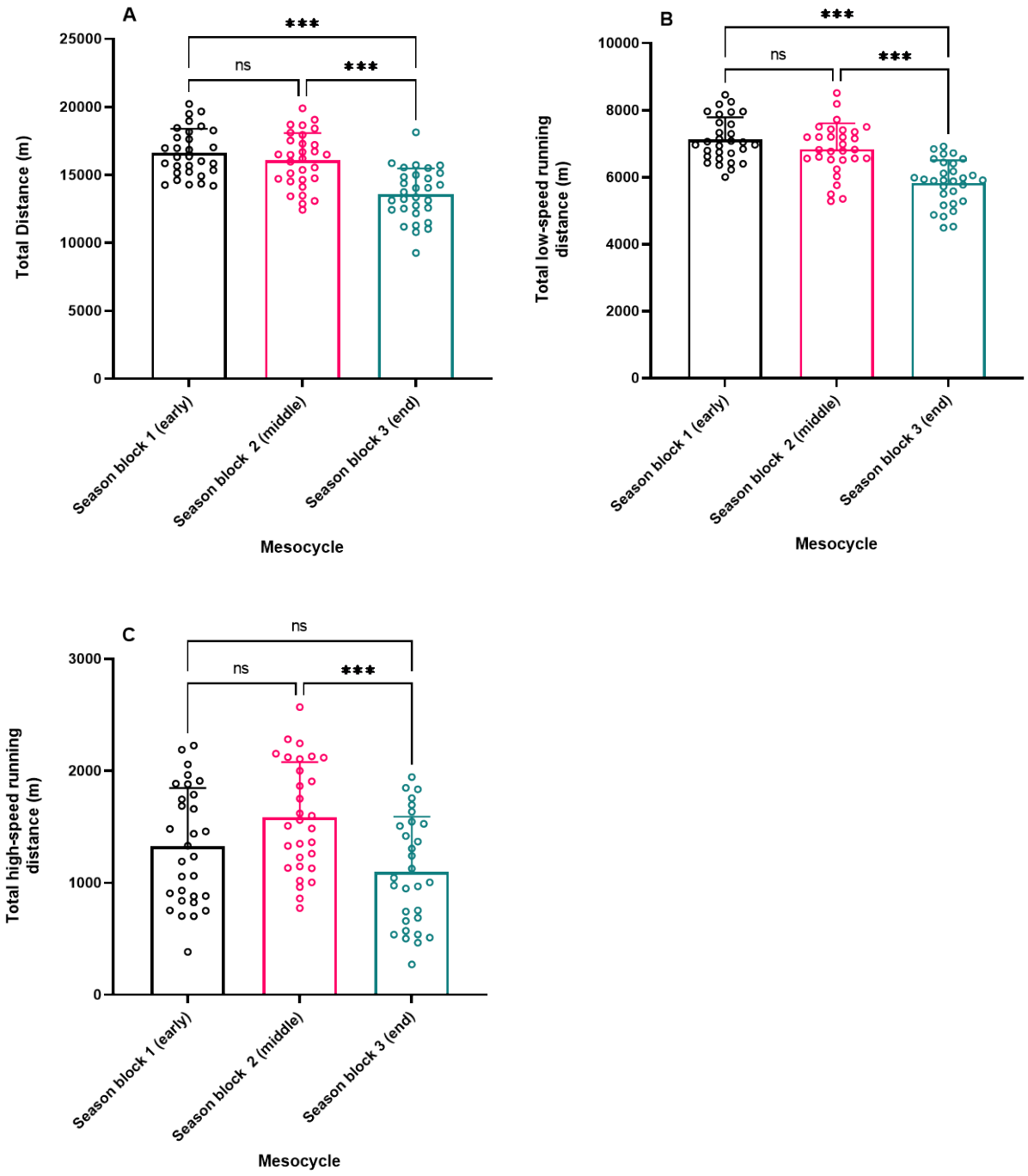

Figure 20. Mean ± SD weekly total distance (A), total low-speed running (B) and total high-speed running (C) values for mesocycle blocks over the duration of the season. ns = Not significant, * denotes significant difference between another mesocycle period (* P = 0.01 – 0.05, ** P = 0.001 – 0.01, *** P < .001).

4.3.2 Differences in positional external training load demands.

There were differences in all variables between the positional groups (TD; $F_{(2, 96)} = 16.91$, $P < .001$, TLSR; $F_{(2, 96)} = 3.48$, $P = 0.035$, THSR; Welch's $F_{(2, 63)} = 102.27$, $P < .001$; Figure 21). The average weekly TD for F5 group is less than the M5 ($P = 0.005$, -1653 m, [-2876 to -429 m], ES = -0.8; Figure 21A) and B5 ($P < .001$, -2982 m, [-4205 to -1759 m], ES = 1.4; Figure 21A). The same pattern is also observed for THSR, as the F5 group cover less distance at these speeds compared to the M5 ($P < .001$, -626 m, [-822 to -430 m], ES = 1.4; Figure 21C) and B5 ($P < .001$, -944 m, [-1102 to -785 m], ES = -3.5; Figure 21C). Players in the B5 group have the greatest average weekly TD (17449 ± 2144 m) and THSR distances (1827 ± 249 m), these were both higher than M5 players ($P = 0.030$, 1329 m, [106 to 2552 m], ES = 0.6 and $P < .001$, 317 m, [129 to 505 m], ES = 1.0, respectively). There was no difference in TLSR distances between M5 and B5 groups ($P > 0.05$), however a small reduction was seen when comparing F5 to B5 players ($P = 0.029$, -628m, [-1294 to -52 m], ES = -0.6; Figure 21B).

Figure 22 provides a breakdown of THSR, displaying both HIR (Figure 22A) and VHIR (Figure 22B) average weekly distances. Players in the B5 group covered higher weekly HIR and VHIR distances than both the F5 ($P < .001$, ES = 3.2 and $P < .001$, ES = 3.4, respectively) and M5 ($P = 0.017$, ES = 0.7 and $P < .001$, ES = 2.1, respectively). The M5 positional group also cover greater HIR and VHIR distance than the F5 group ($P < .001$, ES = 2.0 and $P < .001$, ES = 1.2, respectively). When normalising total training and match meters to the minutes of exposure (m/min) significant differences were observed between the positional groups ($F_{(2, 96)} = 11.62$, $P < .001$). The F5 group (70 m/min) had lower m/min than both the M5 (75 m/min) and B5 (77 m/min) positions ($P = 0.003$ and $P < .001$, respectively). No significant difference were shown between the B5 and M5 ($P = 0.35$) positional groups.

Differences in accelerometer derived measures are displayed in Figure 23. No differences in PL and AD efforts were observed between M5 and B5 ($P > 0.05$). There were differences however between the F5 and M5 positional groups for PL ($P = 0.021$, -158 AU, [-297 to -19 Au], ES = -0.7) and AD efforts ($P < .001$, -59 n, [-88 to -31 n]). Significant differences were also observed between the B5 and F5 groups for both PL ($P = 0.031$, -149 AU, [-288 to -10 AU], IS = -0.7) and AD efforts ($P < .001$, -83 n, [-112 to -55 n]). The M5 group undertook more IS efforts than the F5 ($P < .001$, 7 n, [5 to 9 n], ES = 2.0), but less than the B5 group ($P < .001$, -5 n, [3 to 7 n], ES = 1.2). Additionally, the B5 players also completed more weekly IS efforts than the F5 ($P < .001$, 12 n, [9 to 14 n], ES = 3.4).

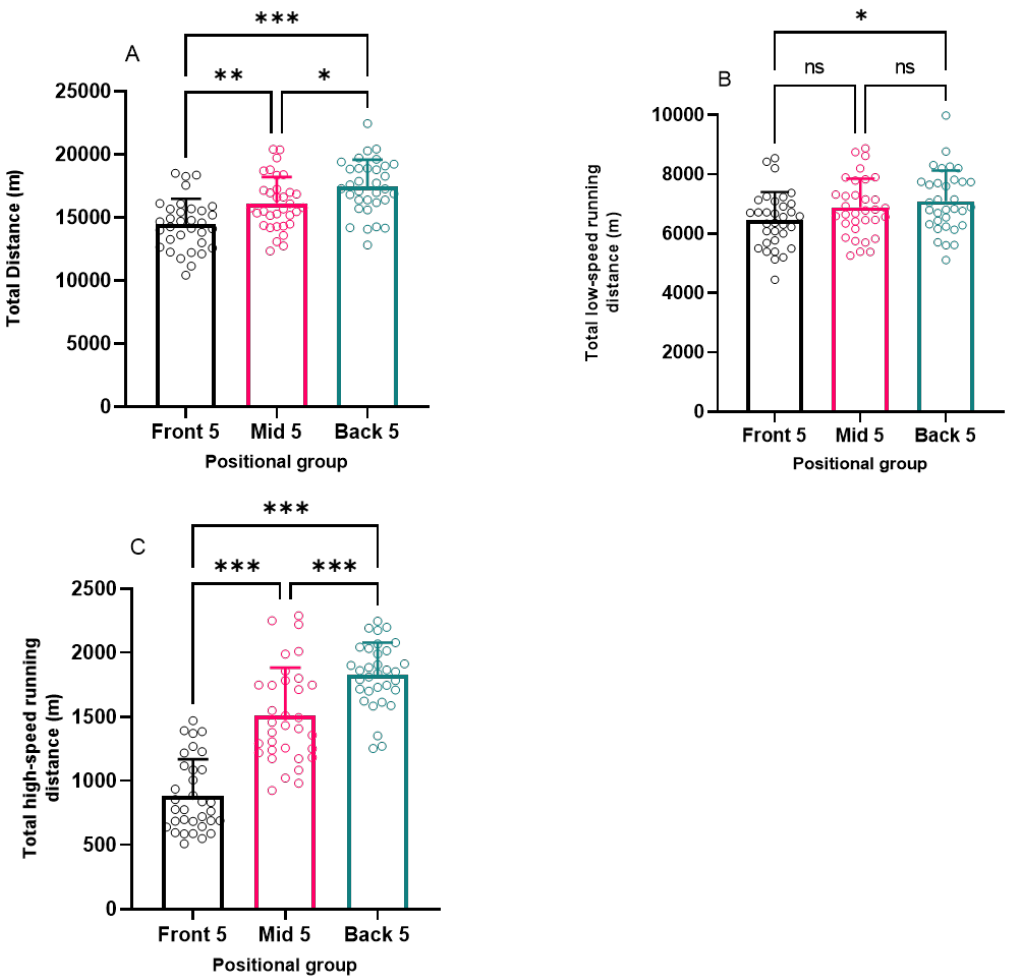

Figure 21. Mean ± SD weekly external running load variables for each positional group; A – total distance, B – total low-speed running distance, C - total high-speed running distance. ns = Not significant, * denotes significant difference between another positional group (* *P* = 0.01 – 0.05, ** *P* = 0.001 – 0.01, *** *P* < .001).

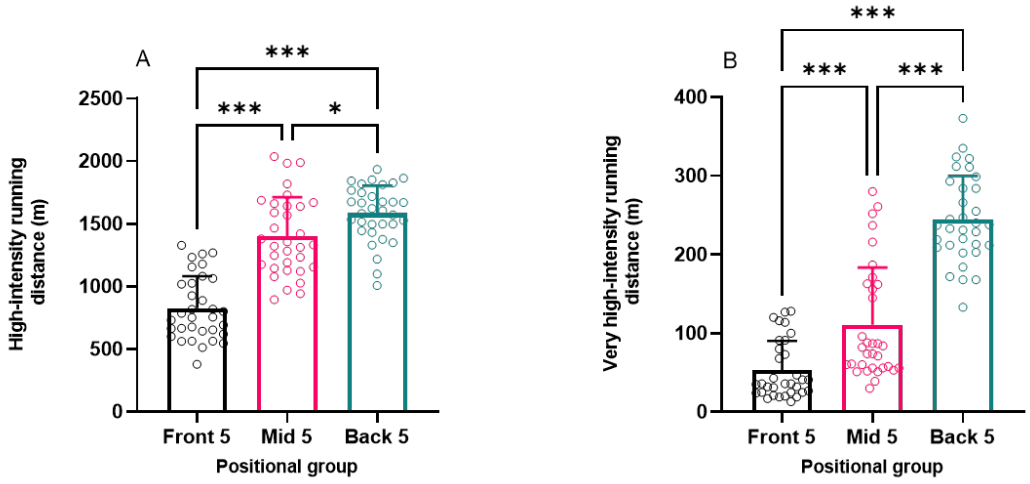

Figure 22. Mean ± SD weekly external high-speed running load variables for each positional group; A – high-intensity running, B – very high-intensity running. ns = Not significant, * denotes significant difference between another positional group (* *P* = 0.01 – 0.05, ** *P* = 0.001 – 0.01, *** *P* < .001).

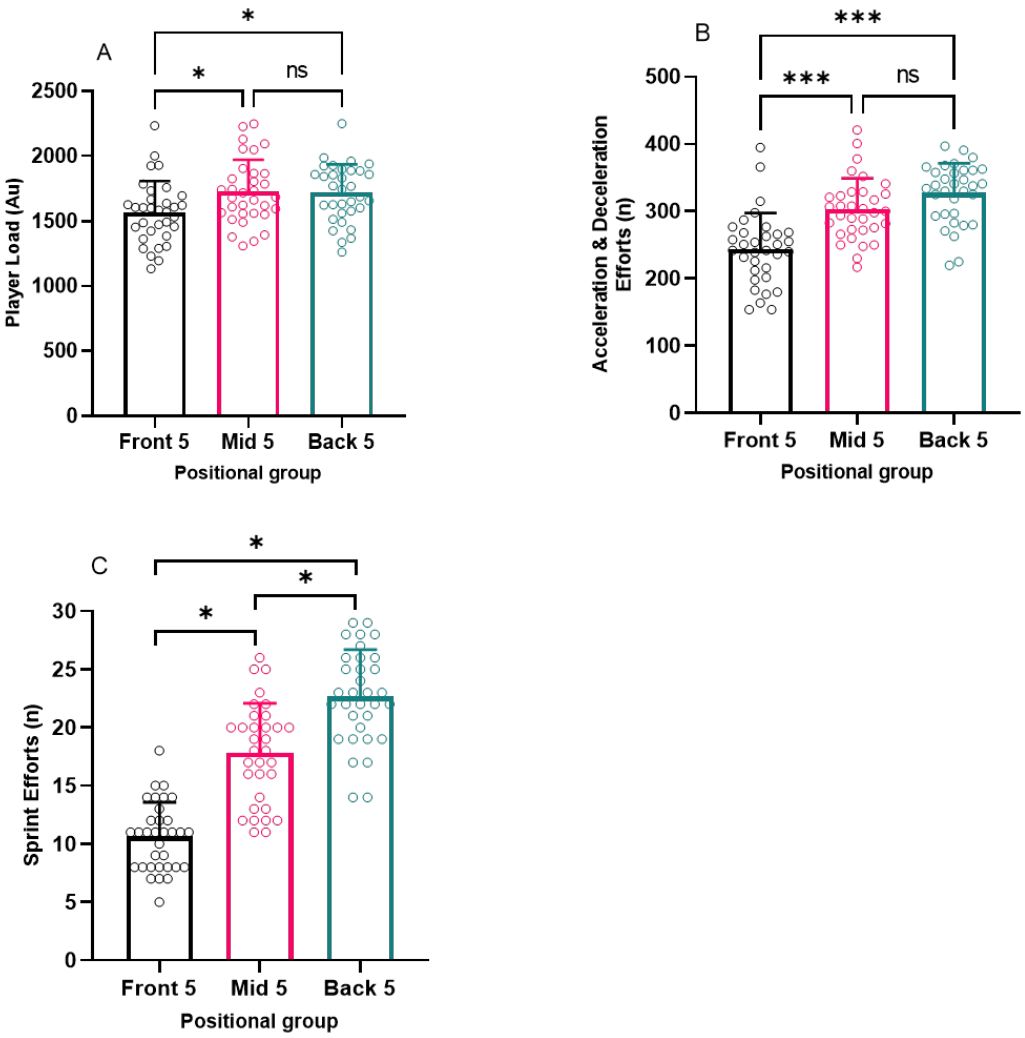

Figure 23. Mean ± SD weekly effort counts and external load variables for each positional group; A – Player Load, B – acceleration and deceleration efforts, C – sprint efforts. ns = Not significant, * denotes significant difference between another positional group (* *P* = 0.01 – 0.05, ** *P* = 0.001 – 0.01, *** *P* < .001).

4.3.3 Player status and external training load demands

Dependant on a players status, differences were observed for TD (Welch's $F_{(2, 61.4)}$ = 52.17, *P* = < .001; Figure 24A), TLSR (Welch's $F_{(2, 63.0)}$ = 32.53, *P* = < .001; Figure 24B), THSR ($F_{(2, 96)}$ = 15.86, *P* = < .001; Figure 24C), HIR ($F_{(2, 96)}$ = 15.86, *P* = < .001; Figure 25A), VHIR ($F_{(2, 24.4)}$ = 60.25, *P* = < .001; Figure 25B), PL (Welch's $F_{(2, 62.7)}$ = 55.57, *P* = < .001; Figure 26A), AD Efforts ($F_{(2, 96)}$ = 16.54, *P* = < .001; Figure 26B) and IS Efforts ($F_{(2, 96)}$ = 40.21, *P* = < .001; Figure 26C).

The starting 15 group covered a greater weekly TD than both the bench group (*P* < .001, 4640 m, [3439 to 5841 m], ES = 2.3) and the non-squad group (*P* < .001, 3887 m, [2555 to 5219 m], ES = 1.7). No difference in TD was observed between non-squad and bench players (*P* < 0.05). The average weekly TLSR distance performed by the starting 15 players is significantly more

than both the bench (*P* < .001, 1804 m, [1219 to 2388 m], ES = 1.8) and non-squad players (*P* < .001, 1609 m, [926 to 2292 m], ES = 1.4). A large increase is observed for THSR when comparing starting 15 players to the bench (*P* < .001, 450 m, [259 to 643 m], ES = 1.5) and a moderate increase when compared to non-squad (*P* = .003, 274 m, [82 to 466 m], ES = 0.8).

No significant differences in HIR distance were evident between the non-squad group and bench (*P* > 0.05) however, starting 15 players covered significantly greater weekly HIR distance than both the bench group (*P* < .001, 361 m, [192 to 532 m], ES = 1.4) and the non-squad group (*P* = .001, 258 m, [88 to 428 m], ES = 0.9). VHIR distance covered typically throughout a week appears similar between the starting 15 and non-squad groups, post hoc analysis revealed no group differences (*P* > 0.05). A large increase in VHIR was evident, however, between the starting 15 and the bench (*P* < .001, ES = 1.6) as well as a moderate increase between the non-squad and bench players (*P* < .001, ES = 1.2).

The similarity of weekly PL values between the bench and non-squad weekly averages is supported by the lack of significance *P* > 0.05. PL values for the starting 15 group were significantly greater than both the bench (*P* < .001, 498 AU, [375 to 622 AU], ES = 2.4) and non-squad groups (*P* < .001, 463 AU, [317 to 609 AU], ES = 1.9). The number of AD Efforts in a game week increased from the bench to non-squad, to starting 15 groups, in that order. Starting 15 AD efforts were significantly greater than both the bench (*P* < .001, 75 n, [44 to 107 n], ES = 1.5) and non-squad groups (*P* = .001, 49 n, [17 to 81 n], ES = 0.9). No differences were observed between the bench and non-squad groups (*P* > 0.05). Starting 15 players performed the highest average IS Efforts in a game week compared to the other groups. Differences were observed between this group, the bench (*P* < .001, 9 n, [6 to 11 n], ES = 2.3) and non-squad (*P* < .001, 6 n, [4 to 11 n], ES = 1.3). The non-squad players (15 ± 5 n) also performed more weekly IS Efforts when compared to the bench players (*P* = .046, 3 n, [0 to 5 n], ES = 0.6).

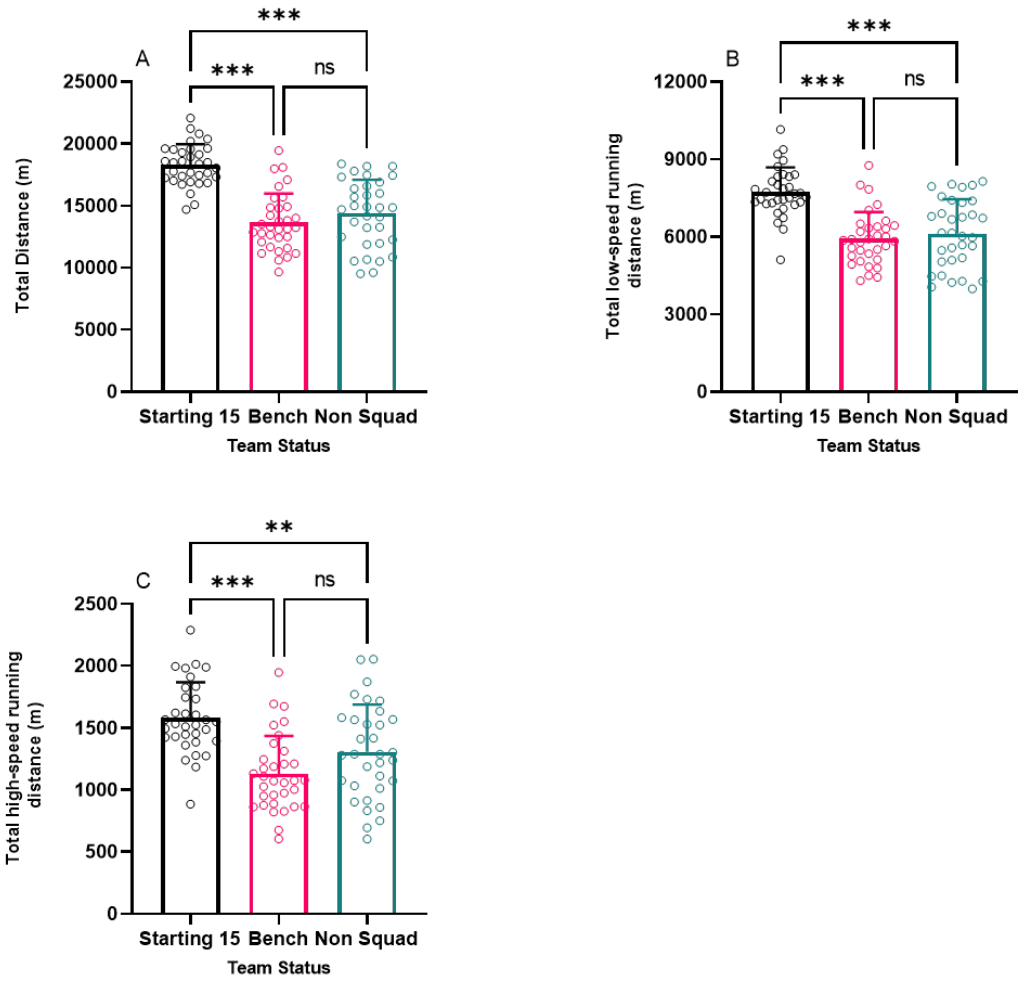

Figure 24. Mean ± SD weekly external running load variables determined by player status; A – total distance, B – total low-speed running distance, C - total high-speed running distance. ns = Not significant, * denotes significant difference between another player status group (* *P* = 0.01 – 0.05, ** *P* = 0.001 – 0.01, *** *P* < 0.001).

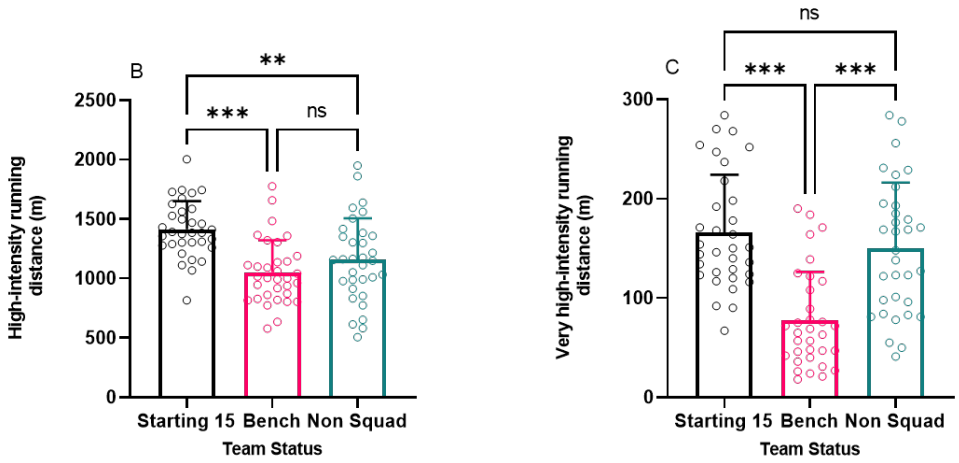

Figure 25. Mean ± SD weekly external high-speed running load variables for each player status group; A – high-intensity running, B – very high-intensity running. ns = Not significant, *

denotes significant difference between another player status group (* P = 0.01 – 0.05, ** P = 0.001 – 0.01, *** P < 0.001).

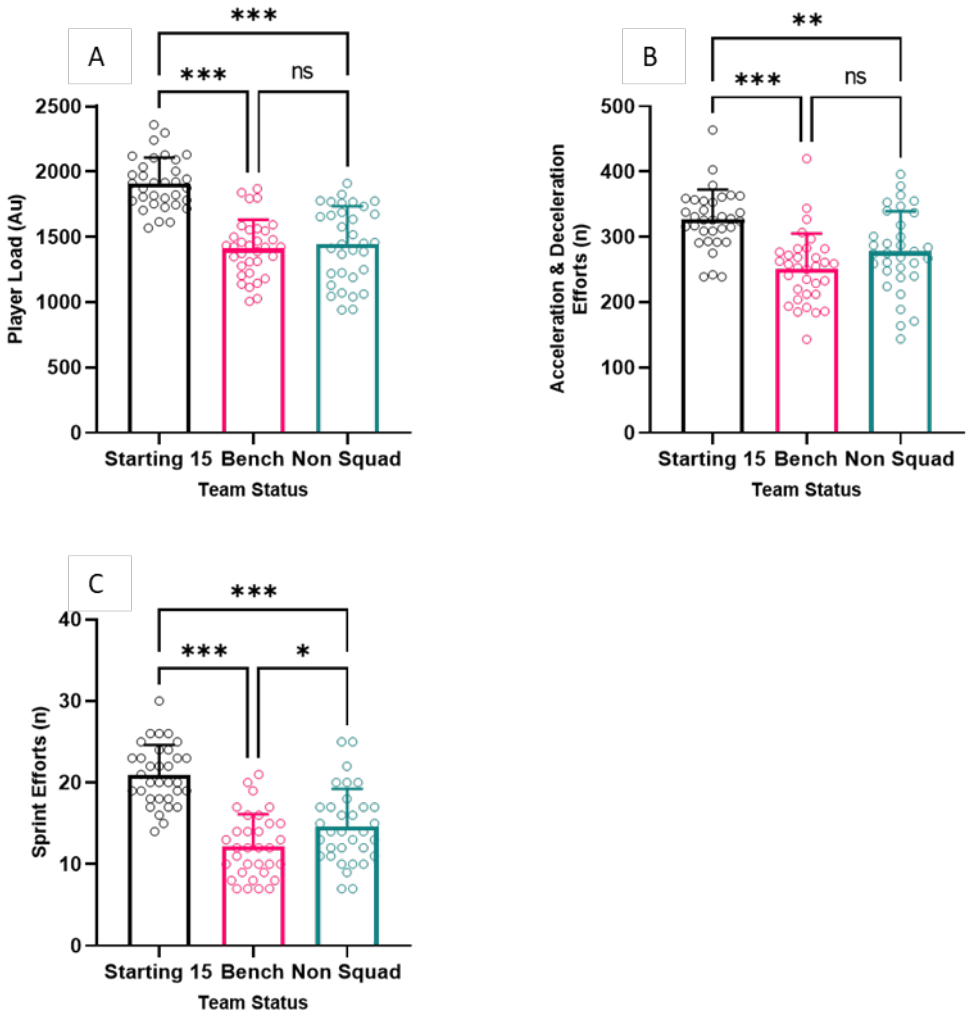

Figure 26. Mean ± SD weekly effort counts and external load variables for each player status group; A – Player Load, B – acceleration and deceleration efforts, C – sprint efforts. ns = Not significant, * denotes significant difference between another player status group (* P = 0.01 – 0.05, ** P = 0.001 – 0.01, *** P < .001).

4.4 Discussion

The current study aimed to examine the external load incurred by elite RU players over the course of a competitive season. Emphasis was placed on understanding the potential effect of playing position and starting status on weekly load demands. The main findings of this study report distinct differences in both running-related and collision-related metrics. The average weekly external workload demands were influenced by both a player's position and starting status within the team. The B5 positional group was characterised by the greatest TD, and they covered greater distances in all the high-speed zones compared to the other groups throughout a training week. They also undertook the most AD and IS efforts. External physical

load were also dependent on starting status, as starting 15 players undertook different external load patterns compared to the bench and non-squad players.

4.4.1 Season workload: overview

Effective in-season training plans need to allow for the recovery from competition, facilitate technical and tactical preparation for the upcoming fixture, whilst also ensuring a 'maintenance' dose is applied to preserve physical fitness qualities developed during the preseason (Bradley *et al.*, 2015a; Moreira *et al.*, 2015a; Ritchie *et al.*, 2016). The results of this study suggest a consistent weekly load is maintained throughout the course of the season, however, a significant reduction is applied as the end of the season approaches (Figure 20). It has previously been shown in rugby (Dubois *et al.*, 2017; Dubois *et al.*, 2020b) and football (Malone *et al.*, 2015) that the running distances in-season are determined by what players become accustomed to in preseason. This study presents the need for consistent external load monitoring in order to achieve this consistency, as coaches need an understanding of the weekly training loads. As seen in Figure 19, week to week variations in load can be observed, this could primarily be due to the different game turnarounds (Moreira *et al.*, 2015b). Appendix D shows the impact of these differing turnarounds and highlights another area that needs to be accounted for when planning training load quantities.

This study reported a significant reduction in the total distance at season block 3 compared to the early and middle phases (Figure 20A) and no changes in high-speed running throughout the season other than a drop between middle and end. This could have been due to a technical or tactical difference associated with the fixture schedule (Figure 20C). Thus far research in rugby season periodisation is very much based on the anecdotal evidence of coaches as little research is available on rugby specific periodisation modelling. Prior evidence in elite sport has suggested a strategy whereby training volume (TD) is reduced towards the end of the season and training intensity is maintained (THSR) (Malone *et al.*, 2015; Ritchie *et al.*, 2016; Dubois *et al.*, 2017; Marrier *et al.*, 2019; Dubois *et al.*, 2020b). This is approach is applied to prevent any further training-induced fatigue as the season progresses, whilst limiting the risk of detraining via the lack of a sufficient stimulus (Marrier *et al.*, 2019). The results of this study support this periodisation model but it is apparent that future research is needed to optimise workload planning to prioritise performance and limit a player's fatigue status as the season draws to a close.

4.4.2 Positional workload differences

This study's findings support previous studies that demonstrate varying movement demands of weekly workloads between positional groups (Bradley *et al.*, 2015a; Dubois *et al.*, 2020a; Dubois *et al.*, 2020b). GPS analysis of the training sessions and games revealed that the average weekly TD, THSR, AD and IS efforts differed significantly between all the positional groups. It is difficult to directly compare the training load derived metrics from this study to the findings of previous studies as the velocity based thresholds and definitions for GPS metrics differ. Additionally, the quantified training load is representative of different professional teams. Typically, studies have represented positions as forwards and backs (Bradley *et al.*, 2015a; Dubois *et al.*, 2020a; Dubois *et al.*, 2020b), but the greater participant numbers in this study allowed the separation of more specific positional groups based on game demands. Previous research has supported the separation of loose forwards from tight forwards, as the game has evolved and now requires them to have a more dynamic and roaming role (Duthie *et al.*, 2005; Eaton and George, 2006).

The B5 group covered the highest weekly distances and a greater proportion of this distance was also in the higher speed zones, they also completed a greater number of AD and IS efforts (Figure 21, 22, 23). In agreement with the present findings, previous studies have reported that backs have the greatest external weekly workloads when compared to forward positions (Bradley *et al.*, 2015a; Dubois *et al.*, 2020a; Dubois *et al.*, 2020b). This may not be surprising as published literature has concluded during a match, distances covered at high speed and collisions vary considerably between these positions (Roberts *et al.*, 2008; Austin *et al.*, 2011; Cahill *et al.*, 2013; Jones *et al.*, 2015).

Additionally, these findings are further explained by the contrasting positional training regimes. Positional groups have specific unit sessions built into their weekly schedule; for forward positions, this requires scrum and lineout training; for the back positions, this requires starter plays and ball-in-hand running. The back row may also be involved in some of the backs unit sessions. As scrums and lineouts require limited movement activity, these sessions will reduce running demands for forward positions and could explain the differences in positional total and high-speed distances. Coaches need to consider the impact of the reduced running load in these sessions for forwards and especially the F5 group with respect to potential undertraining. It should also be noted that GPS measures cannot be the sole determinant of 'external load' as they are unable to measure the impact of these demanding activities.

Therefore, they are unable to reflect the difference in training as there is currently no quantification of the scrums or lineouts. Dubois *et al.* (2020a) observed significant differences in the weekly locomotor activities between positions but no difference in S-RPE or TRIMPS, highlighting the fatiguing nature of non-locomotive demands such as the scrums and lineouts. This suggests the need to incorporate these activities as part of the coaches monitoring systems.

4.4.3 Player status workload differences

To the author's knowledge, this is the first study to report that player starting status as well as playing position are both factors explaining observed differences in external weekly workload parameters in elite RU players in the English Premiership. Additionally, this is the first study to observe 'non-squad' players as well as starters and substitutes. Not only do the findings of this study show starting-15 players complete a greater weekly total distance than the bench and non-squad players, but they also completed higher distances in both high-speed zones; HIR and VHIR (Figure 24, 25, 26).

Considering around 35% of the weekly external load is provided via match activity, this explains the increased activity patterns in the starting-15 players, as they are provided further opportunities to cover distance and engage in higher intensity activities through weekly match-play. It has previously been suggested that participating in the match itself is the most appropriate stimulus for preparing individuals for the physical demands (Anderson *et al.*, 2016). Additionally, the difference in running and activity demands between starting players and those on the bench suggests the after game 'top ups' are insufficient. Top-ups are conducted to make up for the reduction in match demands and avoid 'underloading' (Lacome *et al.*, 2018). This could be because the team has limited time to implement these, especially if the game is away.

Previous studies that have observed the impact of player status on external workload in rugby (Dubois *et al.*, 2020a) and football (Anderson *et al.*, 2016) have reported no apparent difference in total distance, yet do show significant differences in high-speed running demands. It is difficult to directly compare teams but the weekly distances reported by Dubois *et al.* (2020a) are substantially lower than those reported in this study. Lacome *et al.* (2018) reported likely moderate changes in total distance between players with high and low exposure but comparable levels of high-speed activity. They described how they utilised 'top-

ups' to ensure compensatory adjustments to high-speed load for individuals who did not play adequate game time. It is important to understand, however, that this was a 19-day intensified tournament which may have allowed greater control over the prescription of training load, due to less potentially confounding variables such as travel, match-day turnaround and injury replacements over a shorter period of time.

Other than IS efforts and VHIR distance, the other running demands and efforts are no different between bench and non-squad players (Figures 24, 25, 26). This suggests the load provided in the additional non-squad rugby conditioning session (Figure 18) is sufficient to match the loads of bench players. Interestingly however, there was a difference in VHIR distance, which could be due to the bench typically having more forwards than backs. This is relevant as this metric is absolute (> 7.0 $m \cdot s^{-1}$) and has previously been shown to underestimate 'high-speed running' distances for props and second rows (Reardon, Tobin, & Delahunt, 2015). Although the IS efforts are a relative measure and a smaller difference is observed, there is still a significant change between these two groups. The non-squad top-up session also provides another opportunity for repeated sprint training; this addition may explain the difference between the two groups.

The different weekly loading patterns for the various player statuses reported in this study highlight the difficulties for coaching staff to maintain overall squad fitness throughout a season (Anderson *et al.*, 2016; Lacome *et al.*, 2018). Weekly in-season training loads for starting players need to provide appropriate exercise stress whilst also ensuring adequate recovery. The discrepancy in loading patterns for bench and non-squad players is a challenge that needs to be considered for programme design and part of the moderate and long-term processes (Anderson *et al.*, 2016; Dubois *et al.*, 2020a). Players who are regularly on the bench or part of the non-squad may start to de-train if they are not consistently undertaking sufficient stimulus. In particular the requirement of sufficient high-intensity running and actions as it is crucial for game requirements and rugby-specific fitness development (Robineau *et al.*, 2017). It is also important to acknowledge the heightened injury risk to the bench and non-squad players when they are required to start games and complete the loads of the starters. The potential risk exists as they may be unaccustomed to the change in external load (Gabbett, 2004; Anderson *et al.*, 2016; Lacome *et al.*, 2018). Therefore, it is recommended players should undertake 'top up' training to ensure they are exposed to high intensity running, sprinting and acceleration and deceleration efforts.

4.4.4 Limitations

Despite the novel findings presented in this study, the data is not without limitations. The results are based on a single team which may not be representative of the training practices and demands of all teams participating in this or other professional leagues. Additionally, this is only reflective of the locomotive external load of pitch-based training, this does not consider any off-feet top-up sessions. These sessions generally take place in the gym to provide players with a sufficient training stimulus whilst not incurring the on-feet demands. Due to the nature of professional rugby, players are rotated throughout the season to ensure maximal performance. Additionally, players may miss periods of the season due to injury. This results in players changing between sub-groups. To account for injury, players who were out for significant periods of the season were removed as well as any physio and return-to-play sessions. Although the statistical methods used in this study are appropriate, a linear mixed model analysis may be better at handling missing data and changes in groups over a longitudinal period (Krueger and Tian, 2004).

4.4.5 Conclusion

In summary, the present study illustrates the weekly external training and match workload demands of elite RU players and the workload throughout an entire competitive season. The findings from the study displayed a strategical variation in training load prescription across different periods of the season. Future research on optimising rugby specific periodisation would improve the current anecdotal approach. Positional and match status differences in external workload demands were also observed. Importantly for coaches, this study stresses the need to quantify the external load of all player statuses to ensure team fitness and game readiness. Where players are not attaining sufficient stimuli it is suggested that training needs to be manipulated to ensure comparable workloads. This is to guarantee all players irrespective of match-load are ready for competition.

4.4.6 Practical applications

- Practitioners should be aware of both training volume and intensity when planning season-long periodisation techniques. Failure to maintain intensity as volume drops towards the end of the season could result in under-training and negative consequences for fitness and injury risk.
- The data provided has practical implications for training programme design and recommends that both bench and non-squad players should engage with additional

high-intensity training practices to ensure the physical load is representative of starting-15 players.

Chapter 5

5.0 Monitoring biological, physiological and self-reported wellbeing measures in professional Rugby Union players throughout an entire season

5.1 Introduction

The requirements of a modern-day RU player have changed since the sport turned professional in 1995. As well as the full-time training and game demands (detailed in Chapter 4.0), players now also need to account for additional media and sponsorship duties as well as long-distance travel and time away from family (Lindsay *et al.*, 2015b; Quarrie *et al.*, 2017; Hills and Rogerson, 2018). The management of this all-encompassing 'load' from a physical perspective is difficult due to the ever-changing nature of each individual player's schedule. Contextual factors such as the players' position and game status, match location and match turnaround, need to be accounted for when planning and managing weekly training loads (West *et al.*, 2020). All of this necessitates consistent monitoring of player's recovery and readiness, as the demands to play professionally can result in significant strain on physiological systems that can impact performance and health.

The need to optimise the training process is a priority for coaches, as it enables players to achieve a consistently high level of performance, whilst avoiding non-functional adaptations. Therefore, the understanding of the 'dose-response' relationship throughout a full season is critical. The risks of excessive load demands without sufficient recovery could lead to a significant increase in injury and overtraining (Halson and Jeukendrup, 2004; Brooks *et al.*, 2008). Hence, understanding the short, medium, and long term impact of training-and-competition loads on individuals is important for managing players' health and ensuring performance. Thus far, research on the influence of training-and-competition loads is lacking in professional team sports (Akenhead and Nassis, 2016; Bourdon *et al.*, 2017; West *et al.*, 2019; Kelly *et al.*, 2020).

Monitoring markers have proved critical to understanding the dose-response relationship. These tools enable the identification of individuals who may not have had adequate recovery and may be showing early signs of non-functional adaptations (Coutts *et al.*, 2007). To date, no one marker can accurately quantify the response to a bout of training (Bourdon *et al.*, 2017). Therefore, an array of markers is required to provide coaches with sufficient knowledge to make evidence-based decisions regarding how best to optimise an individual's performance. Measures of external training load quantification (Dubois *et al.*, 2017; Dubois *et al.*, 2020b), physiological and neuromuscular function (Coutts *et al.*, 2007; Twist and Highton, 2013), haematology (Banfi *et al.*, 2006; Cunniffe *et al.*, 2011a; Lindsay *et al.*, 2015b), hormone regulation (Tiernan *et al.*, 2020) and self-reported wellness (Hills and Rogerson, 2018) have all

previously shown utility as markers of competition and training tolerability in rugby. These studies have tended to observe these measures in isolation making it difficult to identify how a collective of diagnostic markers may fluctuate throughout a season, providing a more robust understanding.

Prior research has discussed the performance detriments and biochemical disturbances that occur acutely following a rugby game (Takarada, 2003; Gill *et al.*, 2006; Smart *et al.*, 2008; McLean *et al.*, 2010; McLellan *et al.*, 2010; McLellan *et al.*, 2011a; Duffield *et al.*, 2012; Jones *et al.*, 2014; West *et al.*, 2014; Shearer *et al.*, 2015; Oxendale *et al.*, 2016). Collectively, these findings guide the training practices between games and stress the importance of balancing both the recovery and preparation for a game. However, these studies do not provide an understanding of the potential longer-term ramifications of repeated competitive games and the potential effects of accumulated residual fatigue.

The research to date has examined intensified periods, such as preseason (Tiernan *et al.*, 2020) or an international tournament (Cunniffe *et al.*, 2011b; Lacome *et al.*, 2018) and have thus far reported differing associations between markers of recovery and training load. The applicability of these results to in-season training is difficult due to the more challenging demands of these time periods. Additionally, although some prior studies have confirmed associations between training load and measures of performance and perceived wellbeing (Coutts and Reaburn, 2008; Gathercole *et al.*, 2015), the methods required an abnormal increase in load which may not be applicable in an elite sport setting (Hills and Rogerson, 2018). The need for longitudinal observational monitoring in an elite setting is therefore essential, to enable a full understanding of the cumulative effects of rugby over a season.

Prior studies have undertaken a season-long observation of a RU team; however, they are either missing a measure of training load (Banfi *et al.*, 2006), or only include backs players (Dubois *et al.*, 2017). The most complete study to date by Dubois *et al.* (2020b), utilised multi-dimensional monitoring of 14 professional RU players throughout a Top 14 season. They observed changes in training load and physiological, biochemical, and psychological variables. However, they only observed the impact of position and playing status on external workload measures and not on any of the diagnostic measures. The results of the previous chapter (Chapter 4.0), highlighted key differences in the physical load of players with a high versus low game exposure, yet there is currently a scarcity of research investigating the relationship with

diagnostic markers. This information is important to assess the influence of different competition exposures on physical, biochemical, and wellbeing variables.

This study aimed to quantify training load and assess the changes in the physical, biochemical, and self-reported wellness measures throughout a professional RU season. Additionally, a further aim is to understand the influence of both playing position and game exposure on these variables. Finally, to provide greater insight to sports scientists and coaches, this study also sought to determine the association between measures of training load and physical and biochemical diagnostic markers.

5.2 Methodology

5.2.1 Participants

Forty first-team players were recruited to participate in the season-long study. One participant withdrew from the study and twelve participants were removed due to a long term injuries. Strict inclusion criteria were set to ensure a high quality of data collection. Players who were not able to attend a biomarker collection time point were removed. Additionally, if a player was not training due to an injury or was in modified training following an injury in the 2 weeks before a sampling time point, they were also removed from the study. The final sample consisted of fifteen first-team professional RU players (Age: 26 ± 3 years, Height: 186.9 ± 12.3 cm, Body Mass: 102.4 ± 12.3 kg), competing in the 2018-2019 season of the English Premiership. Due to the demanding requirements of this study, it resulted in a smaller number of participants compared to Chapter 4.0, and therefore the positional groupings were labelled as ‘forwards’ and ‘backs’. The forwards' group consists of props, hookers, locks flankers and number 8s, the backs group consists of scrum-half’s, fly-half’s, wingers and full-backs. All players provided written informed consent before volunteering for the study. Experimental procedures were approved by the university’s research ethics committee.

5.2.2 Experimental approach

An observational longitudinal study was undertaken. Players were monitored throughout the preseason and competitive phase, to understand how players were adapting and coping with a full professional RU season and the associated workloads. Figure 27, details how throughout the season, alongside external training load measures for every pitch training session and game, a variety of biochemical, physiological, and wellbeing measures were assessed. This consisted of a variety of blood sampling measures, performance tests, and self-assessment

questionnaires. All data were collected from the club's training ground and at no point was data collection or time points interfered with to accommodate this study.

5.2.3 Training design

The season comprised a 9-week preseason period and an in-season period which consisted of 3 competitive blocks of 12-13 weeks each. The intense 9-week preseason period included progressive training designs, a week off, friendly fixtures, and concluded with a 2-week taper as preparation for the competitive season. The preseason period was structured to include a physical preparation block, to improve physical attributes critical to coping with the running and collision demands of elite level RU. A general preparation period followed this to improve the tactical and technical qualities of the game. Throughout this training, a mix of specific running and conditioning games is progressively increased and orientated toward aerobic and anaerobic conditioning. Figure 28 provides an example of a typical preseason weekly schedule. Training volume is reduced in the taper period towards the latter of preseason; this aims to sustain a weekly load that is lower than the general preparation period but higher than anticipated in-season competition loads. Over the 9-week preseason period, players completed 30 ± 2 training days, with a total of 450 individual pitch based training sessions recorded.

The 38-week competitive period comprised 33 competitive games, 1 mandatory rest week and 4 no-game weeks over a 9-month period. Training load throughout the competitive period is predominantly maintenance based with a focus on recovery at the start of the week and game readiness leading towards the week's fixture. Conditioning throughout the season was individually prescribed and based on the needs of the players and the number of days between games. During the season, a typical 7-day mesocycle consisted of a day's rest following a game (MD + 1), the first training session back was performed at low intensity (MD + 2). Additional top-ups were required in this session for players with a low training load from the previous week and for bench players with low game time. The following day consisted of rugby focused sessions where contact skills and drills were incorporated, typically a day off would follow this day (MD + 3). With two days before the game, this training day was the highest intensity day for drills focusing on higher outputs than a typical match, the total training volume for this day however is reduced (MD – 2). The day prior to a game (MD – 1) is different depending on the player's needs, those who are required for the game will perform a very small low-intensity session. Those who are not required for the game will complete training sessions aimed to

replicate match intensities. Over the 38-week competitive period, players completed 126 ± 12 training days, with a total of 1895 individual pitch-based training sessions recorded.

Professional Rugby Season																								
Month	June	July					August				September					October				November				December
Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Season Block	Preseason									Block 1 - Early												Block 2 - Middle		
Games							F	F	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Wellness		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Performance tests	B						B											IS 1						
Blood Sampling	B								EP									IS 1						

Professional Rugby Season																								
Month					January				February				March				April					May		
Week	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Season Block											Block 3 - End													
Games	X	X	X	X	X	X	R	X	X	X	X	X	X	NG	X	NG	X	X	NG	X	X	NG	X	
Wellness	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Performance tests								IS 2											IS3					ES
Blood Sampling								IS 2																ES

Figure 27. Description of the season layout and a schematic representation of the sampling and testing points.

	Example Preseason Week Overview						
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	Low intensity	*High intensity*	*Low/Recovery*	*Moderate intensity*	*High intensity*		
AM	Speed Session	Upper body gym session	Off feet aerobic training	Whole body weights	Upper body gym session	Off	Off
	Whole body gym session	Skills session		Skills session	Contact conditioning		
			Pool recovery				
PM	Rugby session	High intensity rugby session		Rugby session	High intensity rugby session		

Figure 28. An example of a typical preseason training week.

5.2.4 Procedures

5.2.4.1 External load quantification

External pitch-based training load and matches were recorded throughout the study in accordance with descriptions provided in section 3.4.1. GPS variables are detailed in Table 8.

5.2.4.2 Subjective wellness

Players were required to complete the 6-item wellness questionnaire (detailed in section 3.7.1) every morning before training commenced. No data was provided in week 1 of the preseason.

5.2.4.3 Performance measures

To assess changes in physical performance, players completed countermovement jumps (CMJ) (section 3.5.1), weighted squat jumps (section 3.5.2) and drop jumps (section 3.5.3). All measures apart from RSI were taken on the very first day back to training (baseline) following the off-season. Due to the higher risk and degree of skill associated with the drop jump, baseline RSI measures were taken on week 7 of preseason. All measures were subsequently taken a further 4 times within the competitive season (Figure 27). Testing was scheduled to take place at specific time points throughout the competitive season; In-season 1, 2 & 3 as well as at the end of the season. These specific time points are detailed in Table 12. Testing was performed between 08:00 and 10:00 h during the morning gym sessions.

Table 12. Timeline of the season collection points.

Time point	Training week	Description of sampling point
Baseline (B)	Week 1	Start of preseason: following 6 weeks of rest away from the clubs training base
End of preseason (EP)	Week 9	End of preseason: following 9 weeks of high load general and specific rugby training plus 3 friendly preseason games
In-season 1 (IS1)	Week 18	Intense competition block: following 8 × Gallagher Premiership, 2 × European cup games
In-season 2 (IS2)	Week 32	Fixture break: following a mandatory winter fixture break. Prior to this 6 × Gallagher Premiership, 4 × European cup and 3 × Premiership cup
In-season 3 (IS3)	Week 43	No game week: following 2 × Premiership cup, 6 × Gallagher Premiership, 2 × no game weeks
End of season (ES)	Week 48	Less competitive period and end of the season: following 3 × Gallagher Premiership, 1 × no game weeks

5.2.4.4 Blood sampling and biochemical analysis

Full blood count (section 3.8.1) and creatine kinase (section 3.8.2) analysis was undertaken following the collection of blood samples detailed in section 3.8. The sampling points throughout the season were chosen to incorporate periods of high and low training loads and intense and lighter competition periods. These pre-determined collection times as described in Table 12, were not altered during the study period and training programs were not altered to accommodate this experimental study. The collection of blood samples took place between 07:00 and 09:00 am on each occasion to avoid circadian variations, players arrived at their specific time slots and these were consistent throughout. Samples were taken on a Monday at the start of each week, where a game was played on a Sunday the sample would be moved to ensure at least 48 hours following competition to avoid the influence of acute fatigue.

5.2.5 Data analysis

All data are expressed as mean ± standard deviation (SD), with statistical significance set at $P < 0.05$ a priori, the mean difference and 95 % confidence intervals are provided where applicable. Cohen's d effect sizes (ES) were also provided where relevant, magnitude of effects were considered trivial (< 0.2), small (0.2 - 0.6), moderate (0.6 - 1.2), large (1.2 - 2.0) and very large (2.0 – 4.0) (Batterham and Hopkins, 2006). Between player group factors were determined based on their position within the team and labelled as either forwards or backs. Players were also grouped based on high or low game exposure. To calculate this, each player's game minutes for the season were totalled, the median was then calculated and players were split based on being higher or lower than the median.

Independent samples t-tests were used to analyse differences between participant group characteristics. Statistically significant differences between the means of performance and blood biomarker measures were determined by one-way repeated measures analysis of variance (ANOVA) with Tukey post hoc analysis.

GPS (total distance: TD, total low-speed running: TLSR and total high-speed running: THSR) and wellness were measured using a mixed model ANOVA; 2 group levels (forwards vs backs & high vs low game exposure) by 4-time levels (preseason, competitive block 1, competitive block 2 and competitive block 3). For between player group (forwards vs backs) comparisons of all performance measures and blood indices (after being corrected for % change from

baseline), a two-way mixed [group x time] ANOVA, with 'time' (Performance measures: baseline, in-season 1, in-season 2, in-season 3 and end of season; Blood measures: baseline, end of preseason, in-season 1, in-season 2 and end of season) as a repeated factor and player 'group' as the between factor, was used. Separate 2-way mixed ANOVAs were used to observe the exposure group (high exposure vs low exposure) of all performance measures and blood indices. Follow up analyses on the main or interaction effects were performed using Bonferroni's post hoc test.

The strength and direction of a linear relationship between external workload parameters and blood biomarker responses were performed using Pearson's Correlation Coefficient (r) by considering the acute (7- day) and chronic (28 - day) total values. The aim was to observe if any relationships existed between the acute and chronic GPS variable values and the % change in baseline measures of blood marker and performance indices. To calculate the various acute and chronic GPS variable values (total distance, total-low speed running, total high-speed running, high-intensity running, very high-intensity running, Player load, acceleration and deceleration efforts and individualised sprint efforts; Table 8), the sum of each GPS variable from the previous 7 (acute) or 28 (chronic) days before the collection of a blood sample was recorded. These values were then compared to the relevant time point of each blood measure value (after being corrected for % change from baseline). Baseline samples for all blood biomarker measures were therefore removed from this analysis. The magnitude of each effect was analysed as follows: small $r = 0.1–0.3$, moderate 0.3–0.5, large 0.5–0.7, very large 0.7–0.9, nearly perfect 0.9–0.99, and perfect $r = 1$ (Hopkins, 2000; Hopkins *et al.*, 2009).

Diagnostic checks (Shapiro-Wilks test of normality and Levene's homogeneity of variance test) were performed on all dependent variables. Welchs's *F* was used if a test failed heterogeneity of variances and sphericity was assumed if Mauchly's test score returned $P \geq 0.05$, with Greenhouse-Geiser adjustments made where appropriate. In the case of creatine kinase, the analysis revealed skewed distributions and this data set was subsequently log-transformed prior to statistical procedures.

IBM SPSS Statistics (version 24, IBM, Armonk NY) software package was used to analyse the data.

5.3 Results

5.3.1 Participant descriptors

Participant physical characteristics are displayed in Table 13. The forwards were taller (P = 0.006, 9.5 cm, [3.3 to 15.7 cm], ES = 1.7), heavier (P < 0.001, 22.7 kg, [18.7 to 26.8 kg], ES = 6.3) and had a greater sum of skinfolds (P = 0.014, 17.9 mm, [4.3 to 31.5 mm], ES = 1.5) compared to backs. No differences in age or total game minutes were found (P > 0.05).

Table 13. Participant characteristics are categorised on their positional groups within the team.

	Number (n)	Age	Height (cm)	Body mass (kg)	Sum of skinfolds (mm)	Total game Minutes (min)
Group	15	26.3 ± 3.4	186.9 ± 7.3	102.4 ± 12.3	79.7 ± 15.1	1255.3 ± 394.3
Forwards	8	25.0 ± 2.1	191.4 ± 5.2 *	113.0 ± 4.0 *	88.0 ± 9.1 *	1268.6 ± 458.8
Backs	7	27.9 ± 4.1	181.9 ± 6.0	90.2 ± 3.2	70.1 ± 14.7	1240.0 ± 341.7

Note: Values are presented as mean ± SD. * signifies a significant difference from the forwards positional group (P < 0.05).

5.3.2 External training load assessment

Mean TD, TLSR and THSR distances for each position and game exposure group for the preseason and competitive periods are presented in Table 14. No interaction effect or main group effects were observed for either position or game exposure and mesocycles block for TLSR distances (P > 0.05). However, main time effects were observed, showing mean distances in preseason, block 1 and block 2 were higher than block 3 (P < 0.05).

No interaction or group effects were observed for THSR for players with high or low game exposure (P > 0.05), however, like position, a significant time effect was observed (P < .001). The average weekly THSR distance covered in competitive block 3 were lower than preseason (P < 0.05), block 1 (P < .001) and block 2 (P < .001). The greatest THSR distances were reported in block 2 which were significantly higher than in block 1 (P = 0.001) and block 3 (P < .001). A main group effect was reported for position as, backs covered greater distances at high speed than forwards (610 m, [339 to 881 m], P < .001).

No interaction effect was observed for positional weekly total distances ($F_{(3, 39)}$ = 2.02; P = 0.166) however, there was a main time ($F_{(3, 39)}$ = 16.91; P < 0.001) and group ($F_{(1, 13)}$ = 6.62; P

= 0.023) effect. The lowest average weekly total distance occurred in block 3, this is lower than preseason (*P* < .001, -3103 m, [-4559 to -1467 m], ES = 1.7), block 1 (*P* < .001, -2987 m, [-3710 to -2263 m], ES = 1.8) and block 2 (*P* < .001, -3137 m, [-3718 to -2556 m], ES = 1.6). Additionally, group effects highlighted that backs players covered higher weekly total distances when compared to forwards (*P* = 0.023, 1339 m, [215 to 2462 m]).

An interaction effect was observed for the total distance measures based on exposure ($F_{(1.9, 24.2)}$ = 4.55; *P* = 0.023). Simple main group effects highlighted a difference between game exposure groups at block 2 (*P* = 0.019, 2158 m, [408 to 3906 m], ES = 1.4) and block 3 (*P* = 0.025, 2150 m, [317 to 3984 m], ES = 1.3). Simple time effects showed that for the high exposure groups, the total distances in block 1 (*P* = 0.02, 2595 m, [1132 to 4059 m], ES = 2.5) and 2 (*P* < .001, 3142 m, [1962 to 4322 m], ES = 2.6) were greater than block 3. For the low exposure group differences were observed between preseason (*P* = 0.028, 4841 m, [584 to 9098 m], ES = 2.4), block 1 (*P* = 0.012, 3552 m, [925 to 6178 m], ES = 1.8) and block 2 (*P* = 0.002, 3135 m, [1432 to 4839 m], ES = 1.6) with block 3.

Table 14. Mean ± SD, weekly training and match loads during each phase of the season for total distance, total low-speed running distance and total high-speed running distance.

	Preseason (W1 - W9)	Competitive Block 1 (Early)	Competitive Block 2 (Middle)	Competitive Block 3 (End)
Total Distance (m)				
Total	**16541 ± 1693 ¥**	**16411 ± 1511 ¥**	**16509 ± 1875 ¥**	**13369 ± 1932**
Forwards	16434 ± 590	16119 ± 1449	15471 ± 1314	12308 ± 1848
Backs †	16663 ± 2500	16745 ± 1622	17695 ± 1762	14583 ± 1225
ES	*-0.13*	*-0.41*	*-1.45*	*-1.43*
High Exposure	16084 ± 1416	16968 ± 980 ¥	17516 ± 1341 ¥, β	14373 ± 1093 β
Low Exposure	17063 ± 1938 ¥	15574 ± 1822 ¥	15358 ± 1791 ¥	12223 ± 2106
ES	*-0.58*	*0.83*	*1.38*	*1.31*
Total low-speed running distance (m)				
Total	**6906 ± 1088 ¥**	**7098 ± 601 ¥**	**7084 ± 838, ¥**	**5844 ± 786**
Forwards	6723 ± 530	7122 ± 711	6772 ± 928	5332 ± 681
Backs	7117 ± 1528	7098 ± 601	7440 ± 595	6430 ± 389
ES	*-0.36*	*0.08*	*-0.84*	*-1.94*
High Exposure	6630 ± 633	7228 ± 503	7408 ± 873	6120 ± 553
Low Exposure	7222 ± 1440	6949 ± 706	6714 ± 670	5529 ± 932
ES	*-0.55*	*0.46*	*0.88*	*0.79*
Total high-speed running distance (m)				
Total	**1445 ± 336 ¥**	**1295 ± 438 ¥, ‡**	**1597 ± 471 ¥**	**1072 ± 461 ‡**
Forwards	1278 ± 220	973 ± 268	1284 ± 305	736 ± 236
Backs †	1635 ± 357	1663 ± 260	1956 ± 359	1456 ± 329
ES	*-1.23*	*-2.61*	*-2.03*	*-2.55*
High Exposure	1498 ± 300	1374 ± 397	1725 ± 455	1188 ± 454
Low Exposure	1383 ± 388	1205 ± 496	1452 ± 480	940 ± 467
ES	*0.34*	*0.38*	*0.58*	*0.54*

Note: Data is presented for both positional and status groups. Significant effects highlighted by ¥ > Block 3 (End); ‡ < Block 2 (Middle); † > Forwards; β > Low exposure($P < 0.05$) . ES = effect size.

5.3.3 Self-reported wellbeing

A longitudinal display of average wellbeing totals dependent on position or game exposure is presented in Figure 29. Self-reported wellbeing was not significantly altered by position or game exposure, showing no time, group, or interaction effects ($P > 0.05$).

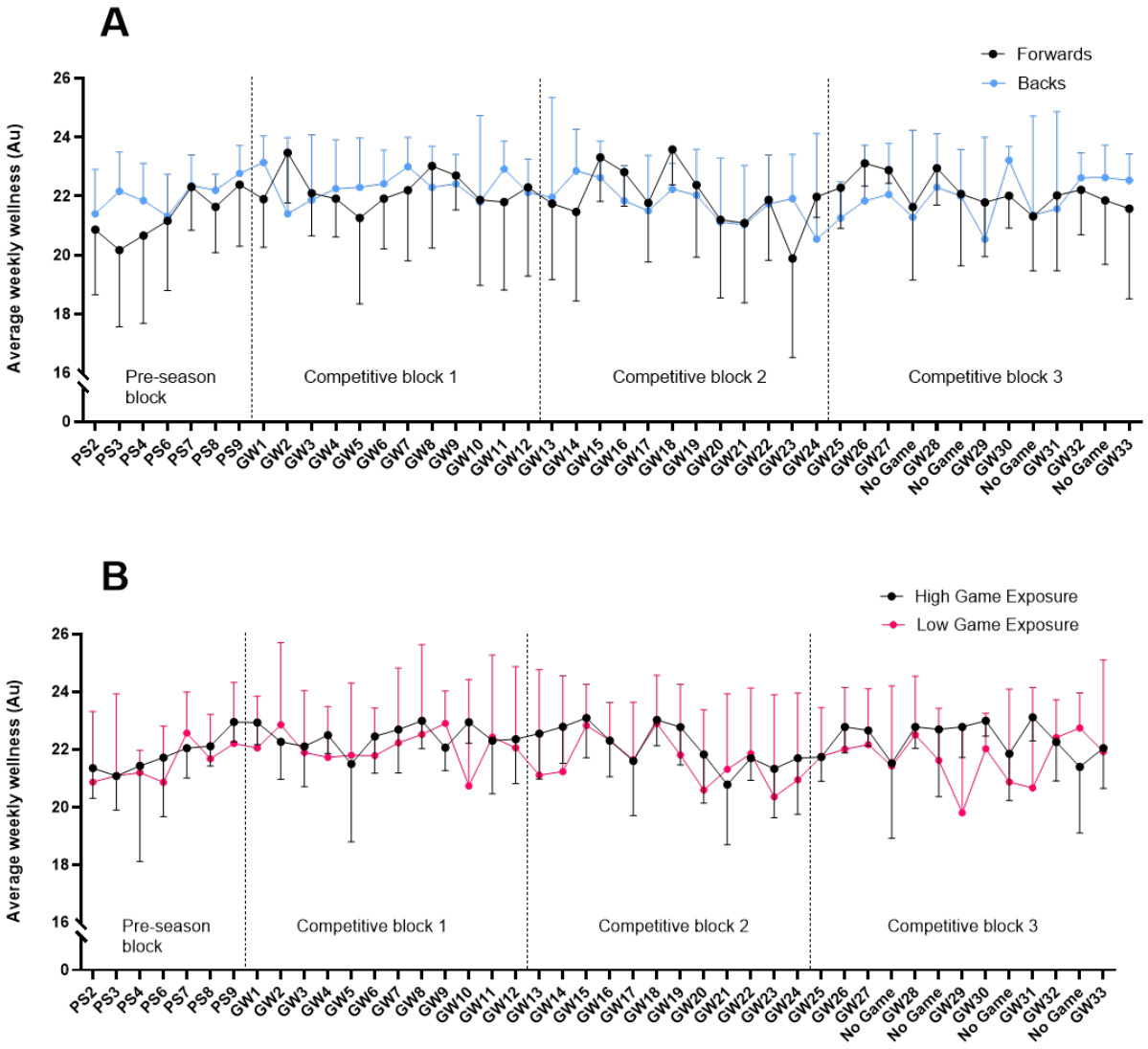

Figure 29. A: Mean wellness ± SD for forwards and backs across the season. B: Mean wellness ± SD for players with high vs low game exposure across the season.

5.3.4 Changes in performance

Group measures for jump height, RSI, mean velocity and peak velocity are presented in Figure 30. CMJ ($F_{(4, 56)} = 6.16$; $P = 0.002$) and mean velocity ($F_{(4, 52)} = 3.85$; $P = 0.008$) showed main time effects. Differences were observed between baseline and end of season time points for CMJ ($P = 0.013$, 3.9 cm, [0.7 to 7.2 cm], ES = 0.6; Figure 30A), and between in-season 2 and in-season 3 for mean velocity measures ($P = 0.006$, -0.11 m·s, [-0.19 to -0.02 m·s], ES = 0.7; Figure 30C). No time, group or interaction effects were present for RSI or peak velocity measures ($P > 0.05$).

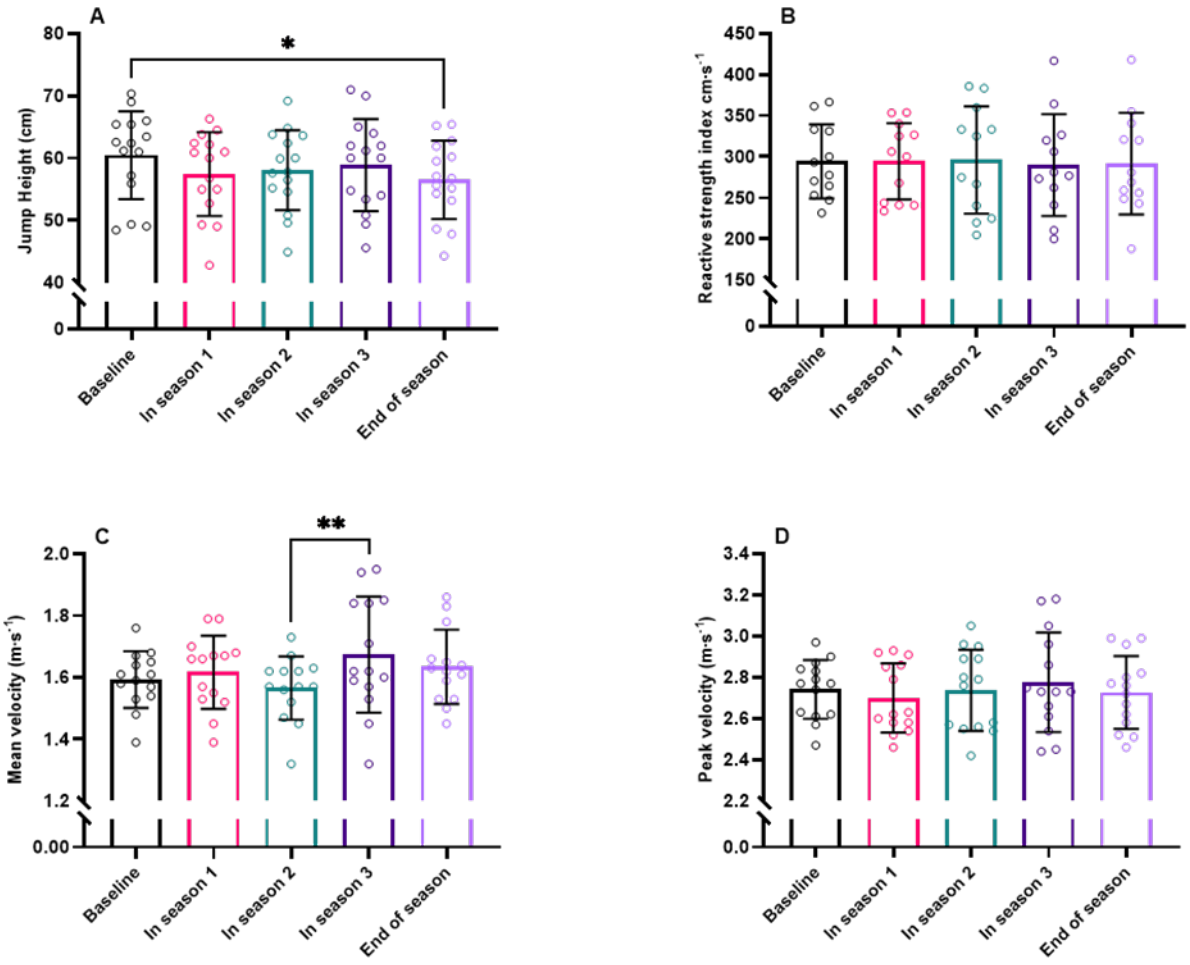

Figure 30. Changes in performance variables across the different periods of the season. * *P* = 0.01 – 0.05, ** *P* = 0.001 – 0.01, *** *P* < 0.001.

No time, group or interaction effects were present for RSI or peak velocity measures for either positional variable (*P* > 0.05). Based on position both CMJ and mean velocity displayed significant time effects (*P* < .001 and *P* = 0.036, respectively), but no group or interaction (*P* > 0.05). Post Hoc analysis revealed no significant pairwise comparisons for mean velocity however, differences were observed between baseline CMJ measures and at the end of the season (*P* = 0.011, -6.4 %, [-11.5 to -1.2 %], ES = 1.5, Figure 31A).

When observing the influences of a high or low game exposure no time, group or interaction effects were observed for peak velocity measures (*P* > 0.05). There was a significant time effect for mean velocity (*P* = 0.011) but no significant differences were found with post hoc pairwise comparisons. Interaction effects were evident for both CMJ and RSI performance measures (*P* < .001 and *P* = 0.045, respectively). Simple main group effects for CMJ highlighted a larger decrease in baseline performance when comparing the low game exposure group with the

high, at time points in-season 1 (*P* = 0.022, -6.6 %, [-12.3 to -1.2 %], ES = 1.3, Figure 32A) and end of season (*P* = 0.019, -6.8 %, [-12.2 to -1.3 %], ES = 1.4). Simple main time effects for CMJ highlighted a difference between baseline and end of season (*P* = 0.029, 9.8 %, [1.1 to 18.6 %], ES = 2.6). No simple main time effects for RSI were observed, but main group effects were apparent at time points (Figure 32B), in-season 1 (*P* = 0.032, -13.5 %, [-25.5 to -1.5 %], ES = 1.4) and in-season 3 (*P* = 0.001, -20.9 %, [-30.2 to -11.6 %], ES = 2.9).

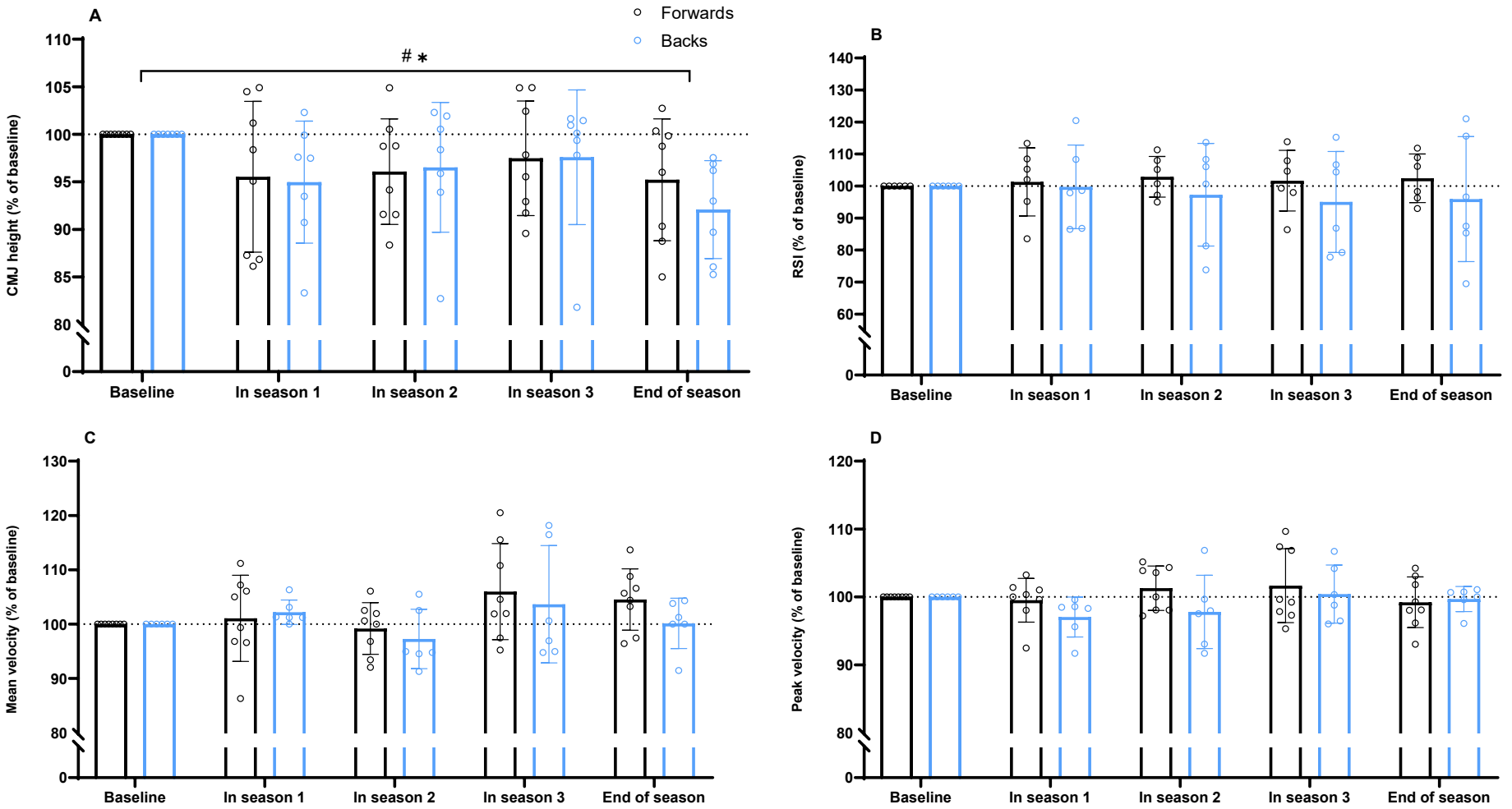

Figure 31. Positional changes in the % difference of performance measures throughout the season. # represents a significant time difference. * $P < 0.05$.

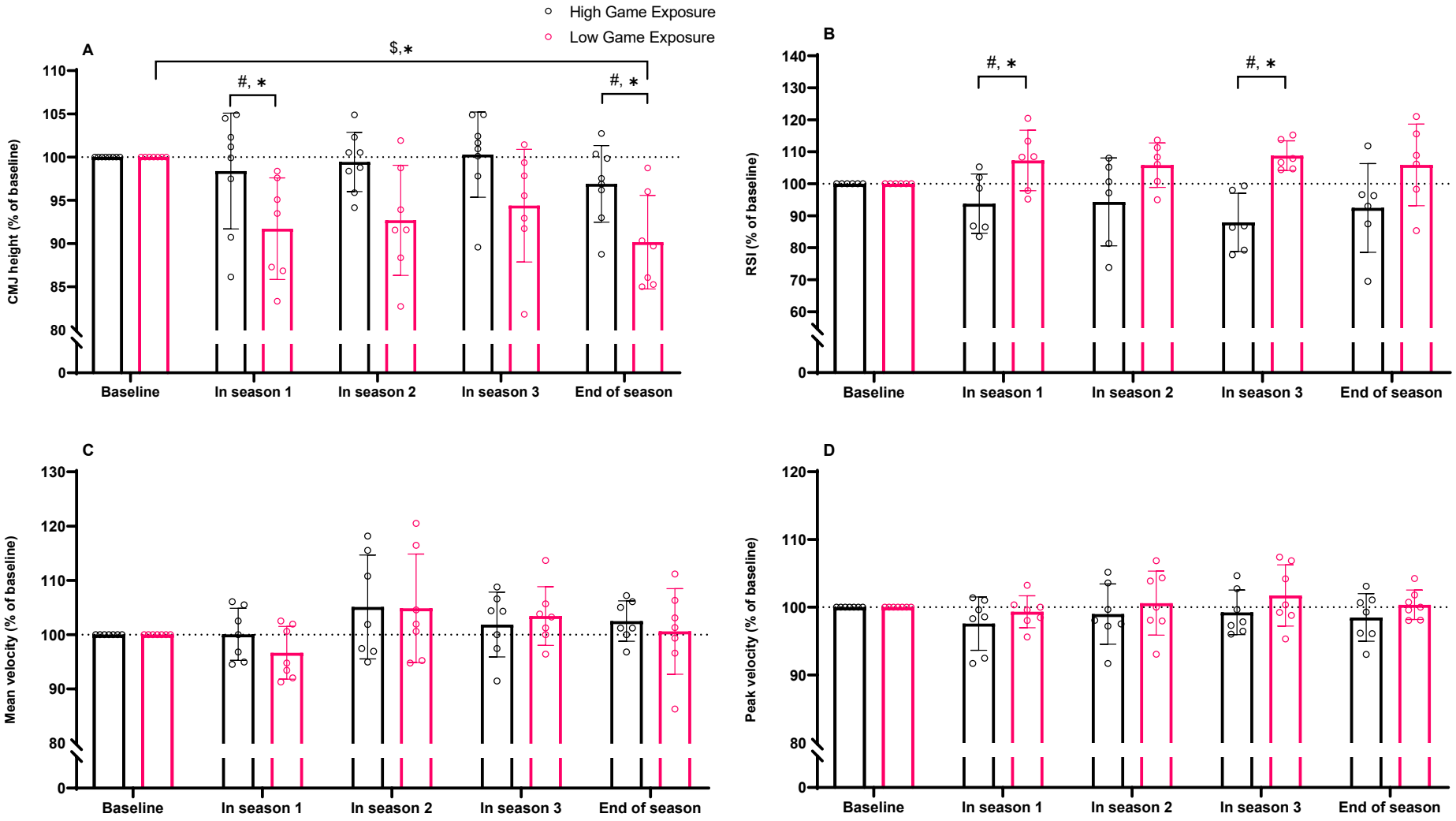

Figure 32. Game exposure changes in the % difference of performance measures throughout the season. $ represents a significant time difference and # a significant group difference. * $P < 0.05$

5.3.5 Changes in blood biomarker measures

Table 15 displays the mean absolute values of full blood count and CK values over the season at specified time points. Significant main time effects were observed for log transformed CK ($F_{(2.6, 36.3)} = 10.38$, $P < .001$), HB ($F_{(4, 56)} = 4.932$, $P = 0.002$), RBC ($F_{(4, 56)} = 6.93$, $P < 0.001$), MCV ($F_{(4, 56)} = 15.04$, $P < 0.001$) and MCH ($F_{(4, 56)} = 3.92$, $P = 0.007$). No significant time effect was evident for HCT, platelets or WBC ($P > 0.05$). Differences in CK were observed between end of preseason and baseline ($P = 0.001$, ES = 1.4), in-season 1 ($P = 0.043$, ES = 1.1) and in-season 2 ($P = 0.002$, ES = 1.6). Additionally, CK results at the end of the season were greater than in-season 2 ($P = 0.008$, ES = 1.3). RBC concentrations at baseline and in-season 2 were higher compared to the end of season sample ($P = 0.001$, ES = 1.3 and, ES = 0.9, respectively). MCV results were higher at the end of season time point compared to all other time points (Baseline: $P < 0.001$, ES = 1.0; end of preseason: $P = 0.001$, ES = 0.9; in-season 1: $P < 0.001$, ES = 1.0; in-season 2: $P = 0.002$, ES = 0.8). At in-season 2 MCV values were lower than end of preseason ($P = 0.020$, ES = 0.5) and in-season1 ($P = 0.032$, ES = 0.5).

Table 15. Mean values and standard deviations of the haematological parameters across different periods of the season.

Blood parameters (units)	Baseline - (W1)	End of preseason - (W9)	In-season 1 - (W18)	In-season 2 - (W32)	End of season -(W48)
CK ($U \cdot L^{-1}$)	227.5 ± 58.3 †	413.7 ± 168.6	250.9 ± 136.7 †	208.6 ± 94.0 †, ‡	401.7 ± 198.9
HB (g / L)	158.5 ± 5.1	155.3 ± 6.2	155.6 ± 7.7	155.3 ± 7.4	150.4 ± 7.0 *
HCT (%)	46.3 ± 1.6	45.2 ± 2.0	45.2 ±1.9	46.1 ± 2.0	45.2 ± 1.6
RBC (10^{*12} / L)	5.19 ± 0.19 ‡	5.05 ± 0.26	5.07 ± 0.24	5.14 ± 0.24 ‡	4.91 ± 0.24
MCV (fl)	89.2 ± 3.2 ‡	89.6 ± 2.6 ‡	89.2 ± 3.0 ‡	89.8 ± 2.5 ‡	92.1 ± 2.9
MCH (pg)	30.5 ± 1.0	30.8 ± 1.2 ¥	30.7 ± 0.9 ¥	30.2 ± 1.1	30.6 ± 0.9
Plts (10^{*9} / L)	220.5 ± 35.0	223.9 ± 39.2	219.7 ± 38.3	223.1 ± 40.3	222.7 ± 42.7
WBC (10^{*9} / L)	5.52 ± 1.09	5.38 ± 1.17	5.77 ± 1.23	5.97 ± 1.18	5.56 ± 1.07

Note: Values are expressed as mean ± SD. * < Baseline; † < End of preseason; ¥ > In-season 2. ‡ < End of season ($P < 0.05$). CK = Creatine kinase; HB = Haemoglobin; HCT = Haematocrit; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; Plts = Platelets; WBC = White blood cells.

Figure 33 displays the significant differences between forwards and backs over the course of the season for blood biomarker measures and Figure 34 shows the differences between players with high and low game exposure. No time, group or interaction effects were observed for Platelets or WBC when accounting for position or the level of exposure ($P > 0.05$). Relative to baseline values, significant time effects are seen for both differences in position and game exposure for CK (Position; $F_{(4, 52)} = 8.13$, $P = 0.003$ & Exposure; $F_{(4, 52)} = 8.13$, $P = 0.002$), HB

(Position; $F_{(4, 52)} = 4.99$ $P = 0.002$ & Exposure; $F_{(4, 52)} = 4.91$, $P = 0.002$), RBC (Position; $F_{(4, 52)} = 7.19$, $P < 0.001$ & Exposure; $F_{(4, 52)} = 7.32$, $P < 0.001$) and MCV (Position; $F_{(4, 52)} = 15.52$, $P < 0.001$ & Exposure; $F_{(4, 52)} = 15.07$, $P < 0.001$).

MCV was greater in backs than forwards ($P = 0.023$, 1.27 %, [0.2 to 2.3 %]) whilst no group effect was observed between high and low exposure ($P = 0.486$). HCT in the high exposure group was significantly lower compared to the players with low exposure ($P = 0.041$, -2.64 %, [-0.13 to -5.15 %]).

An interaction effect was observed between the MCH values of players with different exposure levels over the course of the season ($F_{(4, 52)} = 3.32$, $P = 0.017$). Simple main group effects revealed in-season 2 values of MCH for players with low exposure were significantly less than at end of preseason ($P = 0.009$, -3.4 %, [-0.95 to -5.77 %], ES = 1.2) and at in-season 1 ($P = 0.008$, -2.59 %, [-0.78 to -4.40 %], ES = 1.1). No simple main time effects were evident ($P > 0.05$).

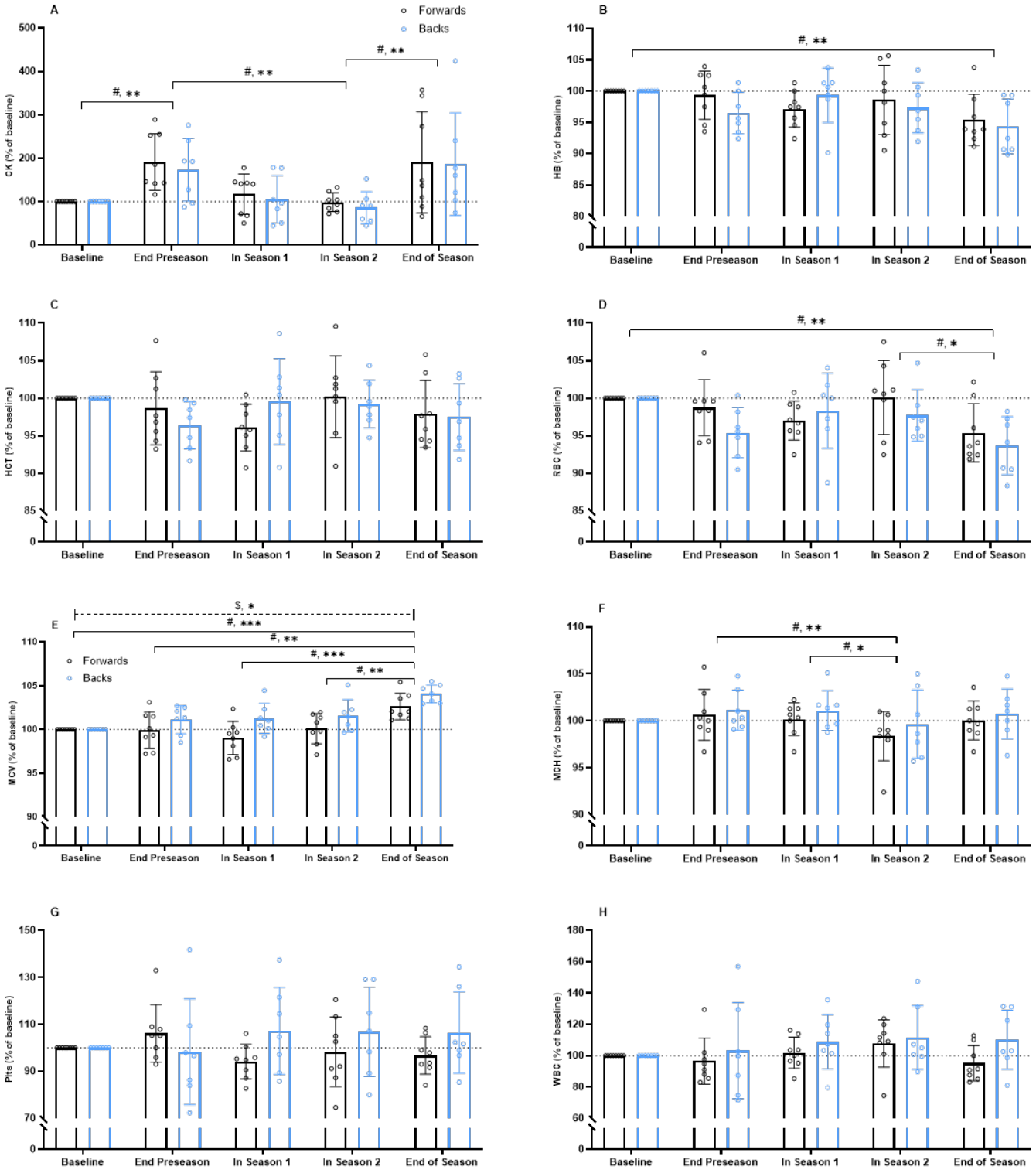

Figure 33. Positional changes in the % difference of haematological parameters throughout the season. # represents a significant time difference and $ a significant group difference. * P = 0.01 – 0.05, ** P = 0.001 – 0.01, *** P < 0.001. CK = Creatine kinase; HB = Haemoglobin; HCT = Haematocrit; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; Plts = Platelets; WBC = White blood cells.

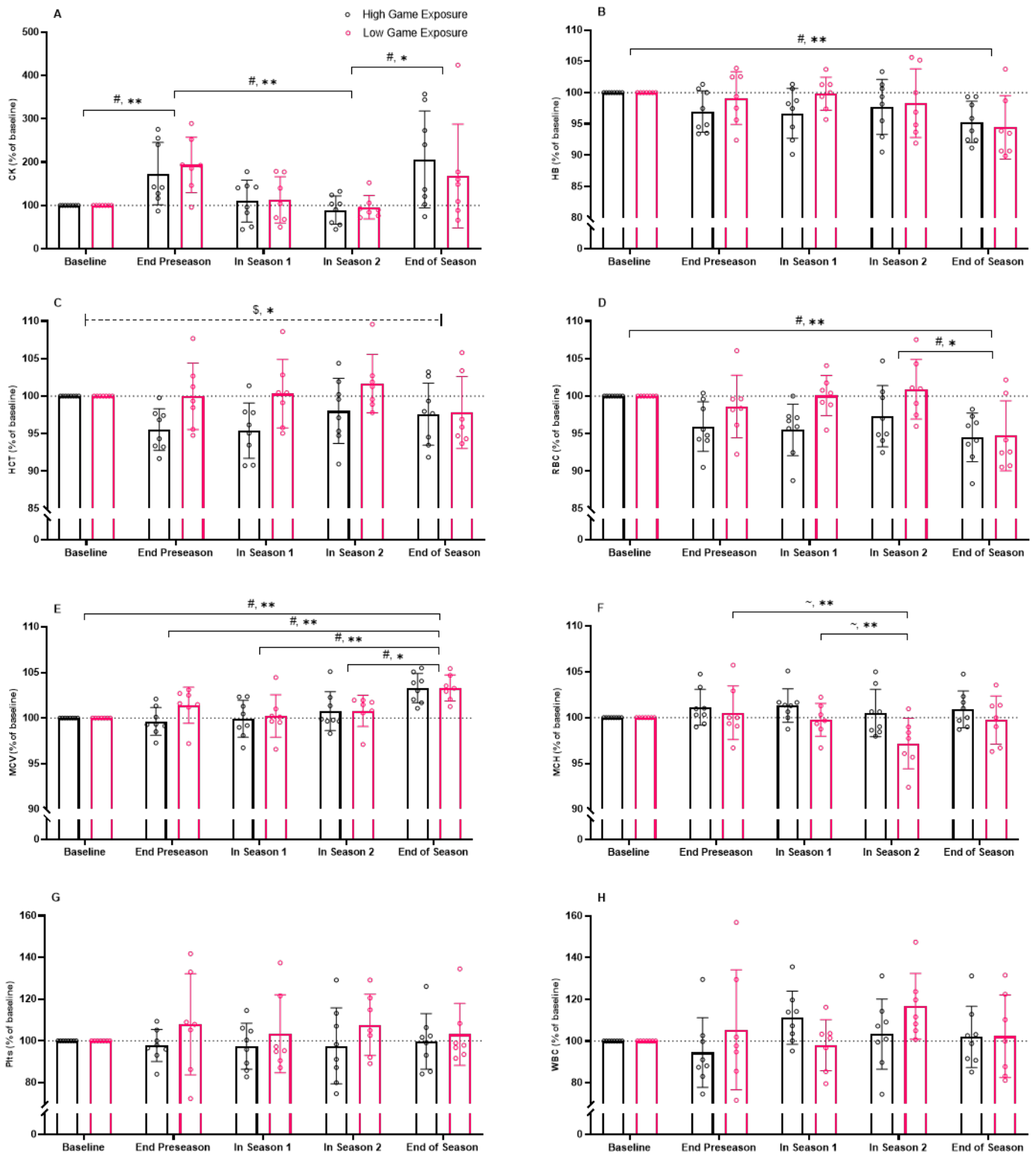

Figure 34. Game exposure changes in the % difference of haematological parameters throughout the season. # represents a significant time difference, ~ represents a significant time difference for the low exposure group and $ a significant group difference. * $P = 0.01 – 0.05$, ** $P = 0.001 – 0.01$, *** $P < 0.001$. CK = Creatine kinase; HB = Haemoglobin; HCT = Haematocrit; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; Plts = Platelets; WBC = White blood cells.

5.3.6 Influence of external workload parameters on blood and performance measures

Medium to large correlations between the relative change from baseline of performance measures and biomarkers and the acute and chronic GPS variables can be seen in Table 16.

Moderate negative correlations were observed between the percentage changes in mean velocity and the chronic THSR distances (P = 0.024) and the chronic number of sprints (P = 0.022). No further correlations were observed between the various performance changes and the GPS variables (P > 0.05).

Acute total distance values were correlated with CK (P < 0.001) and MCH (P = 0.04) in a positive manner and negatively with HCT (P = 0.03). Significantly small negative correlations were seen when comparing RBC changes to the acute Player load (P = 0.04), the acute number of acceleration and deceleration efforts (P = 0.05) and the acute number of sprints (P = 0.03). Player load and acceleration and deceleration efforts were the only chronic variables to display correlations with blood biomarker changes. Negative correlations were apparent when chronic Player load were compared to MCV (P = 0.01) and when chronic acceleration and deceleration efforts are compared to MCV (P = 0.05). Furthermore, CK change was significantly correlated to acute Player load (P < 0.001) and the acute number of accelerations and decelerations (P = 0.01). Correlations were also observed between the relative change in HCT levels, the acute number of accelerations and decelerations efforts (P = 0.02), the acute Player load values (P = 0.03) and the acute number of sprint efforts (P = 0.03). A small positive correlation was also seen between MCH and acute acceleration and deceleration efforts (P = 0.04).

Table 16. Pearson correlations between GPS variables and relative change of performance measures and haematological parameters.

	Total Distance (m) - A	**Total Distance (m) - B**	**High-speed running distance (m) - A**	**High-speed running distance (m) - B**	**Player load sum (AU) - A**	**Player load sum (AU) - B**	**Acceleration & deceleration efforts (n) - A**	**Acceleration & deceleration efforts (n) - B**	**Sprint efforts (n) - A**	**Sprint efforts (n) – B**
Performance measures										
CMJ (cm)	*r* = -0.06	*r* = 0.03	*r* = 0.00	*r* = -0.13	*r* = -0.06	*r* = 0.00	*r* = -0.07	*r* = 0.20	*r* = -0.11	*r* = 0.06
RSI ($cm \cdot s^{-1}$)	*r* = -0.11	*r* = -0.06	*r* = -0.14	*r* = -0.11	*r* = -0.13	*r* = -0.03	*r* = -0.09	*r* = -0.01	*r* = -0.15	*r* = 0.04
Mean velocity ($m \cdot s^{-1}$)	*r* = -0.07	*r* = -0.14	*r* = -0.11	***r* = -0.30 ***	*r* = -0.02	*r* = -0.08	*r* = -0.05	*r* = -0.17	*r* = -0.14	***r* = -0.31 ***
Peak velocity ($m \cdot s^{-1}$)	*r* = -0.18	*r* = -0.05	*r* = 0.02	*r* = -0.04	*r* = -0.16	*r* = 0.00	*r* = -0.08	*r* = 0.14	*r* = -0.09	*r* = -0.14
Blood parameters										
CK ($U \cdot L^{-1}$)	**r = 0.38 ****	r = 0.16	r =-0.01	*r = -0.19*	**r = 0.50 ****	**r = 0.29 ***	**r = 0.33 ***	r = 0.06	r = 0.08	r = 0.04
HB (g / L)	*r* = -0.07	*r* = -0.02	*r* = -0.12	*r* = -0.13	*r* = -0.14	*r* = -0.01	*r* = -0.12	*r* = -0.05	*r* = -0.17	*r* = -0.10
HCT (%)	***r* = -0.28 ***	*r* = -0.20	*r* = -0.20	*r* = -0.20	***r* = -0.27 ***	*r* = -0.20	***r* = -0.30 ***	*r* = -0.22	***r* = -0.28 ***	*r* = -0.22
RBC (10^{*12} / L)	*r* = -0.22	*r* = -0.09	*r* = -0.21	*r* = -0.18	***r* = -0.26 ***	*r* = -0.05	***r* = -0.26 ***	*r* = -0.11	***r* = -0.27 ***	*r* = -0.21
MCV (fl)	*r* = -0.13	*r* = -0.25	*r* = -0.02	*r* = -0.10	*r* = -0.05	***r* = -0.32 ***	*r* = -0.10	***r* = -0.26 ***	*r* = -0.01	*r* = -0.08
MCH (pg)	***r* = 0.26 ***	*r* = 0.13	*r* = 0.18	*r* = 0.12	*r* = 0.22	*r* = 0.07	***r* = 0.26 ***	*r* = 0.13	*r* = 0.20	*r* = 0.20
Plts (10^{*9} / L)	*r* = -0.06	*r* = -0.14	*r* = -0.14	*r* = -0.04	*r* = -0.04	*r* = -0.15	*r* = -0.07	*r* = -0.14	*r* = -0.04	*r* = 0.07
WBC (10^{*9} / L)	*r* = -0.24	*r* = -0.20	*r* = -0.09	*r* = 0.03	*r* = -0.23	*r* = -0.17	*r* = -0.16	*r* = -0.04	*r* = -0.14	*r* = -0.09

Note: CMJ = Counter movement jump; RSI = Reactive strength index; CK = Creatine kinase; HB = Haemoglobin; HCT = Haematocrit; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; Plts = Platelets; WBC = White blood cells. A = 7 day (acute), B = 28 day (chronic). * = statistically significant at $P < 0.05$ level and ** = statistically significant at $P < 0.01$ level.

5.4 Discussion

The aim of this study was to identify if and when changes in diagnostic markers (performance, self-reported wellbeing, and blood biomarker variables) occurred and additionally any associations with external training load. The results of this study demonstrated key time points within the season that is crucial to monitor and optimise, to ensure the repeated performance of players throughout a season. Additionally, the need for an individualised approach to monitoring is apparent, due to the differences observed as a result of position and playing status.

5.4.1 Season long observation of competition and training load.

This is the first study to include preseason in the distribution and content of training load of professional RU players in the English Premiership. Preseason plays a critical role in the preparation of players for the upcoming competitive season (Argus *et al.*, 2010; McLaren *et al.*, 2018). The results of this study report no difference in TD or TLSR between preseason and competitive blocks 1 and 2 (Table 14). This suggests that preseason loads were progressed to a level whereby they could be sustained throughout the competition period. Additionally, distances of THSR in preseason were amongst the highest of the season, this implies a training load prescription method that ensures players can tolerate the training and competition loads that they will be subjected to in-season.

Few studies have reported preseason training loads as part of the season-long observations into professional RU. Dubois *et al.* (2020b) and Dubois *et al.* (2017) both included preseason blocks to display the load changes throughout a season and are in agreement with the findings of this study, that the highest workload demands are observed in this period. Research in both football and Australian football reaffirms the importance of a high workload during the preseason, as it prepares players for the season ahead, reduces the likelihood of injury and increases participation in training and match availability (Murray *et al.*, 2017; Ekstrand *et al.*, 2020).

In addition to highlighting the importance of the preseason period, the results of this study have confirmed that the training load varies throughout the season. All of the measured variables (TD, TLSR and THSR) report the lowest average distances in the last competitive block (Table 14). TD and TLSR were significantly lower in mesocycle block 3 compared to all other time points. THSR is a good indicator of intensity, and distances recorded in mesocycle block

2 were significantly greater than both mesocycle block 1 and 3, but not preseason. As well as the differences in the blocks throughout the season, clear differences were also reported between position and a player's game exposure. Backs cover a greater weekly TD and THSR distance compared to forwards. These findings are supported by numerous other studies, observing the training and competition load (Bradley *et al.*, 2015b; Dubois *et al.*, 2020a; Dubois *et al.*, 2020b). Interestingly no differences are reported between exposure groups at any of the time points suggesting that player top-ups are sufficient. However, as the season progresses it appears the high exposure group maintains a higher TD than the low exposure group. This could have implications for the management of players from an injury-risk perspective, as individuals may not be accustomed to the increased training loads of starting players towards the end of the season.

5.4.2 Self-reported wellbeing

Prior research has suggested that psychometric and wellbeing questionnaires in team sports can help identify early signs of high levels of physical strain and stress (Coutts and Reaburn, 2008; Saw *et al.*, 2016). The findings from this study, however, showed no difference in reported wellbeing between the periods of the season or the influence of position and game exposure. The use of The Recovery-Stress Questionnaire for Athletes (RESTQ-S) is a popular tool within team sports to measure the recovery-stress balance to training stressors. Previous findings in football using this tool reported similar findings to this study: no difference in total stress or total recovery between players with a high versus low match exposure (Meister *et al.*, 2013). Additionally, findings from professional handball found no significant changes in total stress or total recovery scores throughout the season (Bresciani *et al.*, 2010). These findings appear to confirm that the season-long training design does not result in significant changes in reported wellbeing, implying players were able to cope with the prescribed loads.

A caveat could be that this measure or method of collection was not sensitive enough to detect minor perturbations in subjective wellbeing. Similarly to this study, Hills and Rogerson (2018) used a custom-made short-form questionnaire due to its ease of application within a practical team sport setting. Unlike this study's results, they did report significant fluctuations in players' responses and supported its role as a tool within player monitoring. However, they observed weekly changes over 12 weeks of the competitive season rather than blocks. It could be suggested that the lack of findings reported in the study could be attributed to a loss in sensitivity by observing the block averages in wellbeing as opposed to weekly changes.

Although not included in this study, previous findings have shown the acute impacts of training load on reported wellbeing responses in rugby (Hills and Rogerson, 2018; Dubois *et al.*, 2020b). This suggests that self-reported wellbeing may have a place in identifying 'at-risk' players within a training week and should be utilised to optimise player training loads and avoid the risk of under-recovery.

5.4.3 Performance changes

The literature on the ability of performance tests to assess a player's training tolerability is equivocal and highly situation dependant (Meister *et al.*, 2013; Hills and Rogerson, 2018; Dubois *et al.*, 2020b). Prior studies have reported no significant changes in seasonal performance testing (Meister *et al.*, 2013), whilst also others have reported on their ability to notify when a player may have had inappropriate training with inadequate recovery (Hills and Rogerson, 2018). The findings in the present study show a significant reduction in CMJ at the end of the season and an improvement in mean velocity during the week off at the in-season 3 time point (Figure 30).

The crux of these diagnostic tools is to indicate when players may be at risk. The ability to suggest if a player needs additional rest or squad rotation is key to optimising a team's performance. Both CMJ and RSI reported in-season game exposure group differences (Figure 32). Study findings showed CMJ performance was maintained throughout the season in the high exposure group. Although this may sound contrary to what is expected, as this measure was usually taken on the morning of the captain's run, this could suggest those starting players were ready for performance and not showing signs of fatigue. However, the RSI in the high exposure group did not return to baseline following baseline tests, suggesting this performance test could be a more adequate measure of residual fatigue.

In addition to the variety of in-season performance changes, negative associations were observed between weighted jump squat mean velocity and chronic external workload parameters (THSR distance and IS efforts). This confirms the neuromuscular fatigue that results from high loads of high-speed running demands (Marrier *et al.*, 2017) and that measures of CMJ incorporating a measure of velocity are more sensitive to detecting measures of fatigue (Johnston *et al.*, 2013). Interestingly, the results of the present study not only support this but offer coaches an indicator to measure as part of their player monitoring

system. It should also be highlighted that mean velocity is found to be a better indicator than peak velocity for the weighted jump squat.

5.4.4 Blood-based biomarker changes

CK is a commonly used marker for muscle damage (Brancaccio *et al.*, 2007) and has previously been used as a longitudinal diagnostic marker in team sports athletes (Meyer and Meister, 2011; Heisterberg *et al.*, 2013; Dubois *et al.*, 2017; Dubois *et al.*, 2020b). High measures are representative of intense training periods and the resulting exercise-induced muscle damage (Mougios, 2007). Hence, it is no such surprise that a significant rise in CK is observed in the preseason due to the intense running and resistance training associated with this time point. However, it is interesting to observe that although it appears players were able to recover from the training loads at in-season 1 and the week off at in-season 2, significant increases were then observed at the end of the season.

The association of CK activity and high-intensity actions such as sprints and acceleration and decelerations has previously been shown in Australian Rules football (Young *et al.*, 2012) and football (Meister *et al.*, 2013; Varley *et al.*, 2017). Additionally, RU associations were also observed for heavy contact impacts (Dubois *et al.*, 2020b), high-speed running distance and sprint efforts and distance (for backs) (Jones *et al.*, 2014). This study observed associations between CK and acute AD efforts, supporting the hypothesis that high-intensity mechanical actions contribute to muscle damage and result in an increase in CK activity. Interestingly, PL provided the strongest association with CK. This measure utilises the accelerometer to provide an all-encompassing measure of external load which is said to take into account contact as well as running and mechanical demands. These findings present coaches with non-invasive indicators of muscle damage induced from match play and training. This presents the prospect of tailoring individual recovery strategies.

Associations were observed between CK and acute TD, PL and AD efforts. This highlights the importance of monitoring actions within training as well as games due to the resulting impact on muscle damage. This poses important considerations for in-season weekly training design and player readiness. Comparable to findings observed in footballers (Meister *et al.*, 2013), no time point differences in CK were reported between players with a high vs low game exposure. This has raised questions on the utility of monitoring CK levels longitudinally as CK values tend to return to baseline 48-hours following match play. This suggests this measure is not

appropriate to determine the long term evaluation of muscle recovery and accurately diagnose overtraining syndrome (Urhausen and Kindermann, 2002). This study, however, observed a positive correlation between chronic player load and CK, suggesting that it could be a useful marker.

It is important to understand how regular rugby training coupled with an extensive amount of professional games impact haematological variables throughout the season. The stability of haematological status could provide key insights into maintaining optimal player performance (Banfi *et al.*, 2006; Owen *et al.*, 2018). Decreased HCT and HB values are suggested to be directly related to periods of high-intensity training or competition in sport (Heisterberg *et al.*, 2013). Additionally, it has been shown in well-trained athletes that these concentrations decrease at the end of a competitive season (Banfi *et al.*, 2006; Meyer and Meister, 2011; Heisterberg *et al.*, 2013; Anđelković *et al.*, 2015; Dubois *et al.*, 2020b). The findings of this study reported no change in HCT but a significant decrease in HB at the end of the season (Table 15). Whilst this could be suggestive as an indicator of intense training at the end of the season, it is more likely that the tolerability to training is diminished within this period, as measures of training load reduced towards the end of the season. Although, no differences in HCT were found between the in-season time points a significant group effect was observed between the high and low game exposure groups. Players with high game exposure reported the greatest decrease in HCT, signalling the heavy effort and intensity of competitive games (Banfi *et al.*, 2006). This raises an important consideration for the rotation of players to maintain player readiness and increase the ability to perform optimally.

A significant increase in MCV at the end of the season compared to all other time points was detected and a moderate correlation with chronic PL was reported. Owen *et al.* (2018) observed a strong correlation between total competitive minutes played and MCV values in football players. MCV, therefore, deserves more attention in future studies to identify the impact of chronic training loads. Negative associations were also observed between RBC and acute measures of PL, AD and ID efforts. Previous findings have reported a negative correlation between RBC and the number of acute heavy impacts (Dubois *et al.*, 2020b). These findings support the increased RBC destruction due to exercise known as march haemoglobinuria (Mairbäurl, 2013). Measures of RBC were reduced during training and game periods compared to baseline and in-season 2. As this measure appeared to recover during in-season 2 testing which was taken following a week off, this highlights the acute nature of the

damage. The longitudinal findings also support previous literature which observed decreases in RBC during the competition period (Banfi *et al.*, 2006).

5.4.5 Limitations

The findings of this study are based on one professional RU team during a single season. This makes generalising the findings difficult as training and match demands amongst other contextual factors contribute to a high level of variability.

This study has a small sample size but ensuring large subject participation within elite-level sports research is very difficult. The longitudinal nature of this study requires participants to consistently meet the inclusion criteria, which is difficult due to the numerous external influences such as injury or illness (Owen *et al.*, 2018).

It is difficult to completely isolate the chronic effects of training and match demands from acute influences (Meyer and Meister, 2011), and to specifically identify time points in the season that are a risk. Correlations between GPS variables and haematological variables provide evidence that some of these measures represent the acute effects. Methodological parameters such as ensuring samples were obtained at least 48 hours following competition were used to limit this impact.

Although numerous factors may impact a blood sample, care was taken to control these as much as possible. All blood samples were obtained at the same time for each player and before any training. Nutrition and hydration status influences a blood sample, in this study participants were told to arrive having abstained from caffeine which is the only attempt to control for this. The study may have benefitted from players completing food diaries and following this before all visits.

5.4.6 Conclusion

This study reported not only the external training demands that are experienced by elite RU players but, also provides new information on the utility of frequently used training load and performance readiness markers to capture how players tolerate load throughout a competitive season. The findings show, that during the preseason and at the end of the season, many of the markers deviate significantly from baseline, suggesting these periods should be monitored closely to optimise performance and manage training loads. The study findings also suggest that player rotation may be necessary, as there were differences in both performance and haematological parameters between players with high and low game

exposure. Finally, the observed correlations between GPS variables and diagnostic markers of performance and biomarkers provide, a useful and non-invasive indicator of a player's response to training load. This will help to refine the number of player monitoring tools that could be used.

5.4.7 Practical applications

- This study provides baseline elite level data that can be compared to future studies and highlights crucial time points within a season to optimise.
- The need for rotational policies and periodisation is supported by the biomarker findings, however, they will not replace the regular measures of training load management due to the currently limited testing frequency.
- Correlations between GPS measures and changes in biomarkers can help inform recovery strategies following training and games and inform a personalised approach to training prescription.
- If practitioners want to support their training load monitoring programme with biomarker testing, it is essential that a database for players is established and key in-season time points for testing are identified.

Chapter 6

6.0 Circulating miRNA levels in elite male Rugby Union athletes and their associations with anthropometric and performance phenotypes

6.1 Introduction

Rugby Union is a unique sport that requires individual clusters of positions to possess specific technical qualities and varied physiological and anthropometric characteristics (Duthie *et al.*, 2003; Heffernan *et al.*, 2015). These positional specific phenotypes are discussed in a review by Brazier et al. (2018), who highlighted key differences in anthropometric and physiological characteristics in elite rugby players. The differences between elite positional groups are also reviewed in more depth in the literature review (section 2.3); however, Figure 35 describes some of the key characteristics and differences between forwards and backs players. Nonetheless, what determines which level and position a rugby athlete achieves are still to be fully understood.

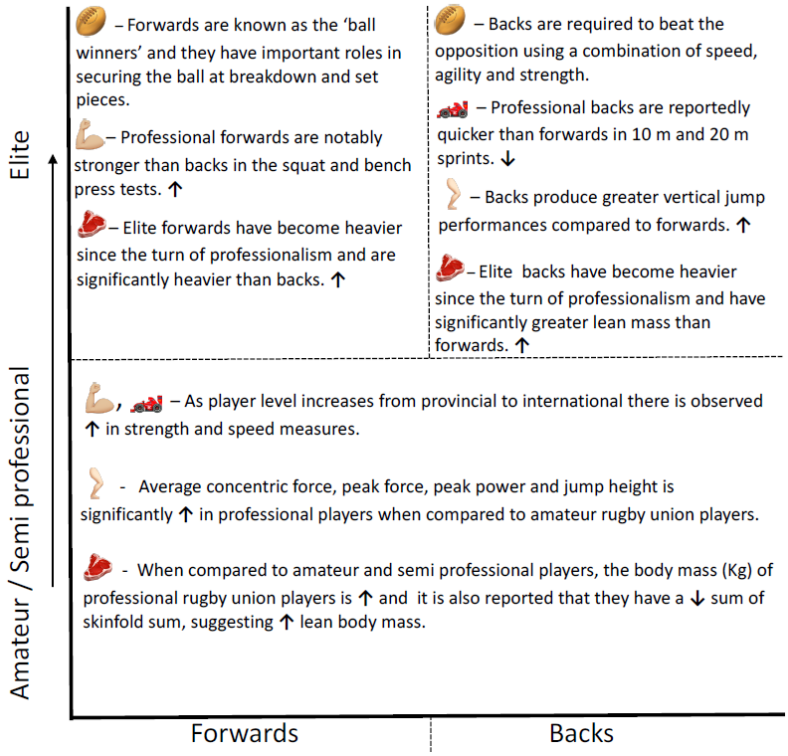

Figure 35. Differences between forwards and backs rugby union players and playing level. Figure created from research detailed in section 2.3.

Such physiological differences are in part derived from differences in training and diet (Bradley *et al.*, 2015b; Black *et al.*, 2019). However, there is known to be a large heritable component to sporting performance. Heritable factors are often as, or more, important than environmental factors explaining upwards of 50% of the variance between individuals (De Moor *et al.*, 2007; Livshits *et al.*, 2016; Zempo *et al.*, 2017). Subsequently, a number of specific

genetic variants, for example *FTO* rs9939609, have been shown to differ by position in elite rugby athletes (Heffernan *et al.*, 2017). Other genes such as *ACTN3* and *HIF1A* have been shown to influence physiological characteristics important for rugby performance, such as strength or speed, in elite athletes from other sports (Yang *et al.*, 2003; Gabbasov *et al.*, 2013). However, only a small number of specific variants have been identified and much of the biological variation underpinning these important characteristics remains elusive. Greater knowledge of how genetics govern exercise-induced adaptations could be exploited to enhance performance (Coffey and Hawley, 2007). Chapter 4 detailed the specific positional external training requirements of elite RU players; however, this is only one part of a larger training response model, as can be seen in Figure 36.

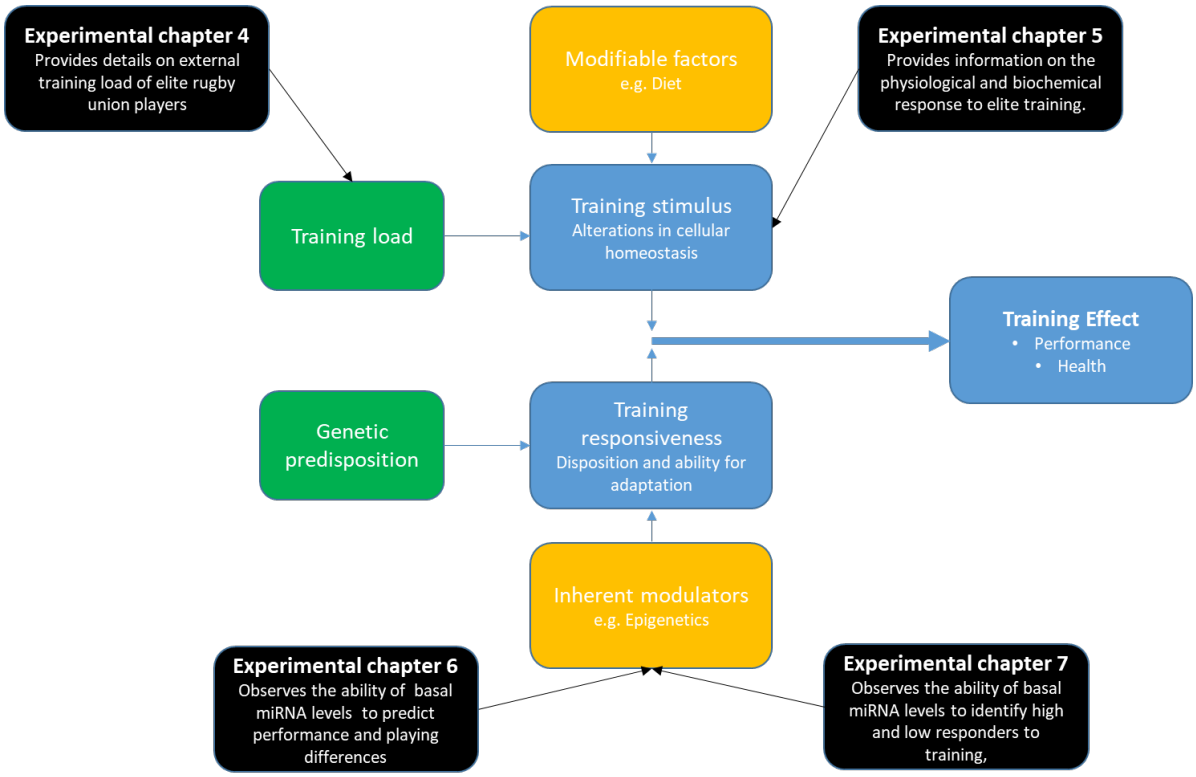

Figure 36. Concepts of training response adapted from (Hecksteden *et al.*, 2015). Schematic representation of the multiple interactions between nature and nurture elements. Black boxes display the experimental chapters within this thesis and where they add information to this process.

Epigenetic variations, such as microRNA (miRNA) expression levels, are also known to associate with exercise (Davidsen *et al.*, 2011). Almost 2,000 human miRNAs have been identified in a variety of tissues and fluids (Griffiths-Jones, 2004; Griffiths-Jones_Lab, 2018). It is estimated that miRNAs target up to a third of all genes and that each miRNA may act on up to 200 mRNAs (Lewis *et al.*, 2005). Hence, miRNAs are formidable regulators of physiological processes. The signature of tissue miRNAs has been shown to differ between elite power-lifters and controls (D'Souza *et al.*, 2017a) and to relate to resistance exercise responsiveness in untrained individuals (Davidsen *et al.*, 2011). In non-athletes, they have also been shown to play important roles in mediating a wide range of physiological processes important for sports performance including muscle hypertrophy (Diniz and Wang, 2016) and angiogenesis (Kir *et al.*, 2018). However, to date, no study has investigated tissue miRNA involvement in the performance of elite RU players.

Additionally, approximately 10% of human miRNAs are found in plasma. Here they are known as ci-miRNAs. These offer an advantage when working with elite athletes as they can be obtained from routinely collected blood samples without the need for more invasive techniques such as muscle biopsy. In elite athletes, ci-miRNAs have been shown to differ by mode of exercise (strength versus endurance) (Wardle *et al.*, 2015). In non-elite athletes, they have been shown to both respond to exercise and associate with training status (Baggish *et al.*, 2011). They have additionally been associated with several important performance phenotypes in non-athletes, such as aerobic fitness (Bye *et al.*, 2013) and power (Cui *et al.*, 2015). Whilst a few studies have investigated ci-miRNAs in team-sport athletes, where a broad range of physiological qualities are required (Domańska-Senderowska *et al.*, 2017; Li *et al.*, 2018), none have examined them in elite RU players.

The aim of this study sought to gain a better understanding of ci-miRNAs role in the physiological adaptation of elite team sport athletes. First, it was examined whether ci-miRNA profiles differed between elite RU players from different positional groups. Second, the association of ci-miRNAs with anthropometric and performance variables was investigated.

6.2 Methodology

6.2.1 Participants

Nineteen elite athletes from a professional Rugby Union club, playing at the highest level of English and European competition were recruited. Participants were drawn from two

positional groups: forwards (n = 12) and backs (n = 7). Participant characteristics are described in Table 17. All players provided written informed consent before volunteering for the study. Experimental procedures were approved by the university's research ethics committee.

Table 17. Participant characteristics by group.

	All (n = 19)	Forwards (n = 12)	Backs (n = 7)
Age (years)	26 ± 4	26 ± 4	27 ± 4
Height (cm)	187.7 ± 5.4	188.9 ± 5.9	185.6 ± 3.7
Body mass (kg)	105.8 ± 11.2	**112.3 ± 6.8****	94.8 ± 8.3
Sum of 8 skinfolds (mm)	90.4 ± 20.4	98.6 ± 15.7	76.5 ± 21.0
EAET (s)	234.5 ± 12.4	241.6 ± 10.1	223.9 ± 6.3
Bronco (s)	294.9 ± 15.7	299.3 ± 17.3	288.7 ± 11.6
1RM squat strength (kg)	173.1 ± 23.3	179.2 ± 26.4	160.8 ± 6.6
1RM bench strength (kg)	133.2 ± 13.8	135.8 ± 15.1	127.0 ± 8.4
10 m sprint (s)	1.80 ± 0.07	**1.82 ± 0.05***	1.75 ± 0.07
Countermovement jump height (cm)	57.1 ± 6.9	55.9 ± 7.6	59.1 ± 5.7
Squat jump height (cm)	53.3 ± 7.0	52.7 ± 8.1	54.3 ± 5.5
40 kg squat jump velocity ($m \cdot s^{-1}$)	2.72 ± 0.20	2.74 ± 0.18	2.70 ± 0.25
Reactive strength index ($cm \cdot s^{-1}$)	284.5 ± 40.8	278.1 ± 51.0	291.9 ± 27.5

Note: All values are presented as mean ± SD. 1RM = 1 repetition maximum; EAET = England anaerobic endurance test; * Significant difference between positions ($P < 0.05$); ** Significant difference between positions ($P < 0.001$).

6.2.2 Blood sampling

Blood samples were collected on the first day of preseason training following a 6-week period of no training (June 2018). Two weeks before returning, participants were provided with an off-season minimum training program to reduce injury risk on return to full training. Participants arrived at the training ground between 07:30 and 09:00 h following an overnight fast having abstained from strenuous exercise for 48 hours. Venous blood samples were collected into EDTA vacutainers by venepuncture (section 3.8). Samples were immediately

transported to a laboratory where the plasma was separated by spinning at 3,000 rpm (4 °C) for 10 minutes, aliquoted and stored at −80 °C for later analysis.

6.2.3 Skinfold measurements

Skinfold measurements were collected within the first week of preseason training by a certified individual following the recommendations of the International Society for the Advancement of Kinanthropometry (ISAK). Eight skinfold sites were assessed (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, anterior thigh, and medial calf) using Harpenden skinfold callipers (Baty International, West Sussex, UK). Each site was measured in duplicate, a third was collected if the technical error of measurement (TEM) was breached. Results are provided as the sum of all eight sites.

6.2.4 Performance assessments

On their return to preseason training, participants completed a battery of performance measures to assess aerobic fitness, strength, speed and power (section 3.5). These were standard tests used by the club and were familiar to the athletes from previous preseasons.

6.2.4.1 Fitness assessments

Two different tests were performed to determine players' fitness levels. The England Anaerobic Endurance Test as described in section 3.6.1 and the Bronco fitness test. The Bronco test aims to measure an athlete's aerobic fitness and is widely used in the rugby environment (Richard W. Deuchrass *et al.*, 2019). As seen in Figure 37, the test requires players to run in a shuttle-type manner between 0, 20, 40 and 60 m lines, completing five repetitions as quickly as possible to a total of 1200m. All participants completed the test at the same time having completed the same standardised warm-up. The Bronco test was filmed and players were given a precise time on reviewing the footage. The time taken to complete the test is their Bronco score (s).

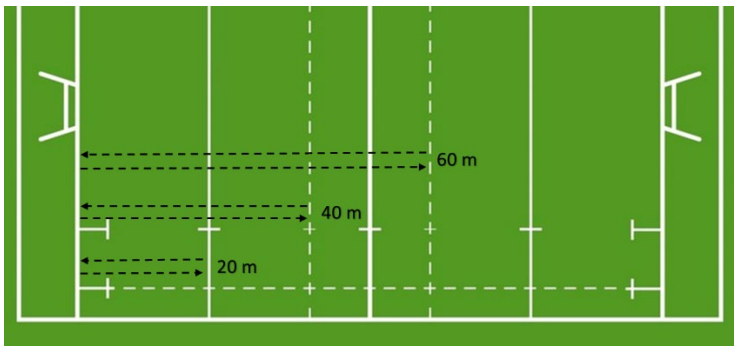

Figure 37. A schematic of the Bronco test completed on a 4G artificial rugby pitch. This shows one repetition performed in a shuttle like manner, the test is completed when five repetitions have been performed.

6.2.4.2 Strength assessments

Both the squat and bench press 1 repetition maximum (1 RM) tests, were undertaken as detailed in the general methods chapter 3.5.4.

6.2.4.3 Speed and power assessments

A variety of sprint and jump tests were used to assess speed and power. Counter-movement jump, weighted jump squats and the drop jump are previously described in sections 3.5.1, 3.5.2 and 3.5.3 respectively.

Similar to the CMJ, squat jumps (SJ) were measured using a timing mat (Just Jump System, Probotics, Huntsville, Alabama, USA). Each participant was instructed to perform three jumps with maximal effort. To perform a valid jump, players squatted to a self-selected depth with hands placed on their hips. When they reached their desired depth, they remained in this position for a three-second count, following which they jumped as high as possible with no knee or hip flexion during the flight phase. The best of three squat jump heights (cm) were recorded.

A Players' acceleration was defined as the minimum time achieved on a maximal 10 m sprint test. The players firstly performed a standardised warm-up followed by three maximal 10 m sprint efforts. To ensure maximal and reliable sprint efforts a minimum of 60 s recovery was provided between each sprint. Sprints were performed on an outdoor artificial 4G rugby pitch and timed using a photocell system (Brower Timing Systems, Utah, USA). The quickest time recorded over 10 m was used for this study.

6.2.5 RNA isolation and complementary (cDNA) synthesis

Please refer to general methods chapter 3.9.1 and 3.9.2 for methodological details.

6.2.6 Quantification of circulating miRNA levels

A list of candidate ci-miRNAs was constructed using background literature and preliminary experiments with Qiagen miRCURY LNA miRNA Plasma Focus Panels. Focus panels contain assays for 84 ci-miRNAs. Twelve samples with the most extreme physiological differences were selected for the Focus panels. The 10 ci-miRNAs with the largest differences between groups were selected for further analysis (hsa.miR.18b.5p, hsa.miR.92a.3p, hsa.miR.100.5p, hsa.miR.126.3p, hsa.miR.144.3p, hsa.miR.146b.5p, hsa.miR.150.5p, hsa.miR.301a.3p, hsa.miR.421 and hsa.miR.885.5p). The most stable ci-miRNAs were identified with NormFinder and selected as controls (hsa.miR.320a and hsa.miR.30e.5p) (Andersen *et al.*, 2004). A further 7 ci-miRNA were added from the literature due to their status as myomiRs (hsa.miR.1, hsa.miR.133a, hsa.miR.133b, hsa.miR.206 and hsa.miR.208b) or known response to exercise (hsa.miR.149 and hsa.miR.221). These 19 ci-miRNAs plus recommended control assays (UniSP2, UniSP4, Cel-miR-39 and UniSP3) were included in Qiagen miRCURY LNA miRNA Custom Panels for final testing on all 19 participants (Figure 15). All samples were analysed in technical triplicate. Please refer to general methods chapter 3.9.3 and 3.9.4 for further details on circulating microRNA analysis and quantification.

6.2.7 Data analysis

Statistical analysis was performed using IBM SPSS Statistics 25 for Windows (Surrey, UK) and Minitab software (version 18; Minitab, State College, PA). Descriptive data are expressed as mean ± standard deviation (SD). All analysis was performed on normally distributed ci-miRNA ΔCt data with outliers removed. Normal distribution was confirmed using the Ryan-Joiner test and outliers were identified via the Grubbs test. Independent samples t-tests were used to compare group differences. Linear regression analysis was undertaken to test associations between ci-miRNA expression levels and quantitative physiological phenotypes. EAET was corrected for position to account for differences in the fitness test. Multiple linear regression was performed if more than one significant association was made with a specific performance variable. Statistical significance was accepted at $P < 0.05$ a priori. In acknowledgement of the small sample size and unequal groups, Cohen's d_s effect sizes (ES) were calculated with the magnitude of effects considered as either trivial (<0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2.0) and very large (2.0–4.0).

6.3 Results

6.3.1 Group ci-miRNA Analysis

Ci-miR-149-5p was expressed at a significantly higher level in forwards compared to backs (Figure 38). Plasma levels of all other measured ci-miRNAs were not significantly different between positional groups ($P > 0.05$; Table 18).

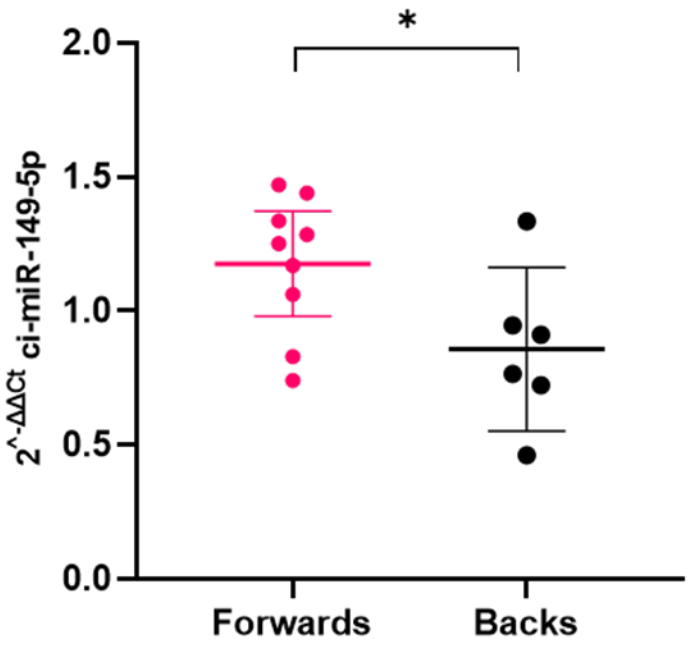

Figure 38. Relative expression levels of ci-miR-149-5p in forwards (n = 9) and backs (n = 6). Horizontal lines show the mean and 95% CI. Circles show individual fold change values. * Significant difference between groups ($P < 0.05$).

Table 18. Mean and 95%CI of all candidate ci-miRNAs in each positional group relative to the overall mean.

ci-miRNA	Forward *Mean [95%CI] (n)*	Back *Mean [95%CI] (n)*	***P* Value**	**Cohens *d***	
ci-miR-1-3p	1.56 [0.82 – 2.30] (12)	0.99 [0.40 – 1.58] (7)	0.282	-0.6	*small*
ci-miR-18b-5p	1.09 [0.84 – 1.34] (12)	1.01 [0.70 – 1.32] (7)	0.712	-0.2	*trivial*
ci-miR-92a-3p	1.06 [0.85– 1.27] (12)	1.04 [0.66 – 1.42] (6)	0.378	0.0	*trivial*
ci-miR-100-5p	1.07 [0.56 – 1.54] (12)	1.87 [0.08 – 3.66] (7)	0.261	0.6	*small*
ci-miR-126-3p	1.06 [0.83 – 1.28] (12)	1.07 [0.74 – 1.40] (6)	0.650	0.1	*trivial*
ci-miR-133a-3p	1.60 [0.81 – 2.38] (12)	0.85 [0.38 – 1.32] (7)	0.147	-0.7	*moderate*
ci-miR-133b	1.58 [0.77 – 2.38] (12)	0.97 [0.36 – 1.57] (7)	0.279	-0.6	*small*
ci-miR-144-3p	1.10 [0.70 – 1.49] (12)	1.41 [0.08 – 2.74] (6)	0.295	0.3	*small*
ci-miR-146b-5p	1.00 [0.86 – 1.15] (11)	1.05 [0.85 – 1.25] (7)	0.983	0.2	*trivial*
ci-miR-149-5p	1.17 [0.98 – 1.37] (9)	0.86 [0.55 – 1.16] (6)	**0.043***	-1.2	*Large*
ci-miR-150-5p	1.05 [0.77 – 1.32] (12)	1.10 [0.73 – 1.47] (6)	0.239	0.1	*trivial*
ci-miR-206	1.39 [0.80 – 1.97] (12)	0.94 [0.55 – 1.33] (7)	0.434	-0.6	*small*
ci-miR-208b-3p	1.78 [0.06 – 3.49] (9)	1.95 [-0.41 – 4.31] (7)	0.568	0.1	*trivial*
ci-miR-221-3p	1.00 [0.87 – 1.13] (12)	1.05 [0.88 – 1.23] (6)	0.533	0.3	*small*
ci-miR-301a-3p	1.10 [0.84 – 1.36] (12)	1.08 [0.58 – 1.57] (7)	0.842	0.0	*trivial*
ci-miR-421	1.15 [0.89 – 1.41] (12)	0.93 [0.58 – 1.28] (7)	0.243	-0.6	*small*
ci-miR-885-5p	1.05 [0.93 – 1.18] (10)	1.04 [0.51 – 1.57] (6)	0.567	0.0	*trivial*

Note: * Significant difference between forwards and backs. n = participant numbers after removal of outliers ($P < 0.05$).

6.3.2 Association of ci-miRNA with anthropometric measures

Three ci-miRNAs correlated with anthropometric measures ($P < 0.05$). Ci-miR-221-3p was negatively correlated with height (Figure 39A). Ci-miR-149-5p was positively correlated with both body mass and the sum of eight skinfolds (Figures 39B and 39C respectively). Ci-miR-150-5p was also positively correlated with the sum of eight skinfolds (Figure 39D). No significant associations were observed for any other measures (Appendix H).

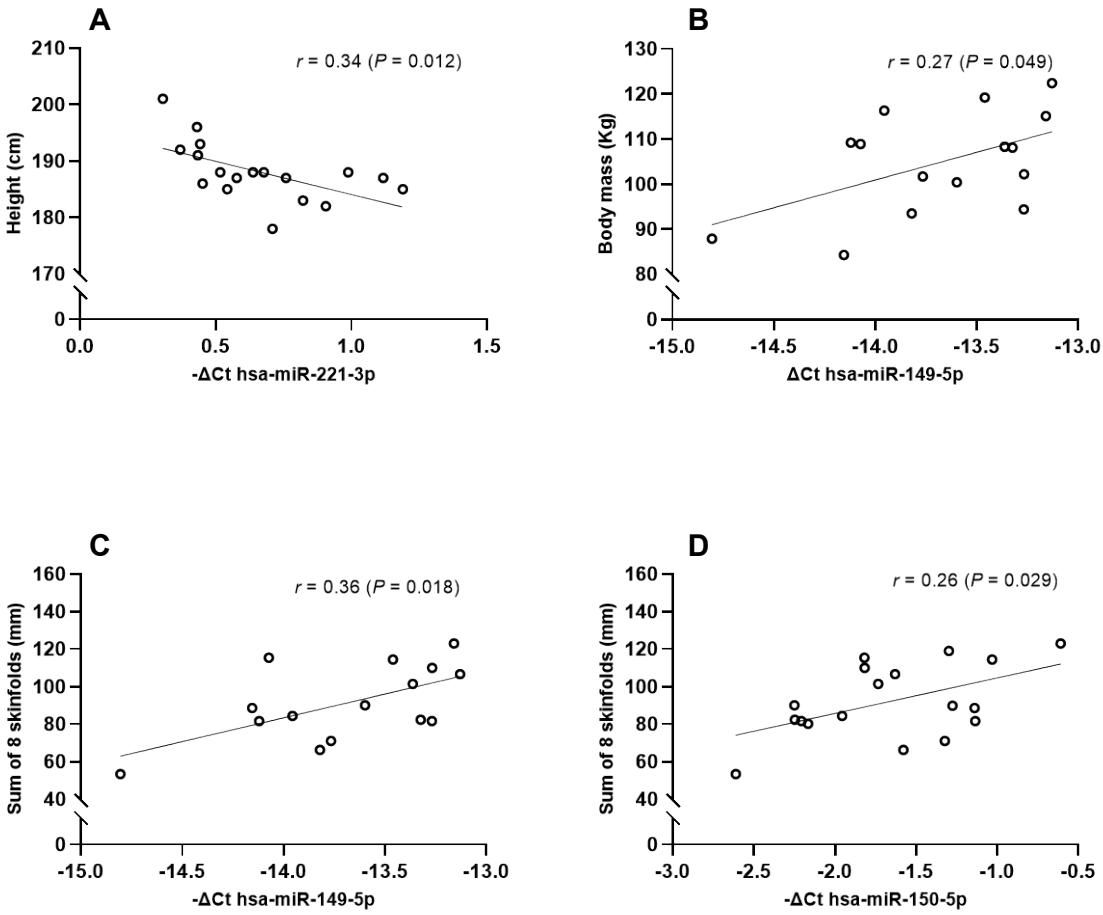

Figure 39. Relationships between anthropometric measurements and ci-miRNA expression: (A) height and ci-miR-221-3p (n = 18); (B) body mass and ci-miR-149-5p (n = 15); (C) sum of skinfolds and ci-miR-149-5p (n = 15); and (D) sum of skinfolds and ci-miR-150-5p (n = 18). Note that higher levels of miRNA expression are to the right of each graph.

6.3.3 Association of ci-miRNA with performance-related phenotypes

Nine ci-miRNAs correlated with aspects of performance assessments ($P < 0.05$). Ci-miR-92a-3p and ci-miR-126-3p correlated negatively with the EAET (Figures 40A and 40B respectively). Ci-miR-133a-3p and ci-miR-206 were positively correlated with a 1RM squat (Figures 40C and 40D respectively). Ci-miR-146b-5p levels were negatively correlated with slower sprint times; whilst ci-miR-149-5p was positively correlated with slower sprint times (Figures 40E and 40F respectively). Ci-miR-100-5p correlated negatively with 40 kg jump velocity (Figure 40G). Whilst ci-miR-146b-5p also correlated positively with 40 kg jump velocity (Figure 40H). Ci-miR-421 correlated negatively with squat jump performance (Figure 40I). A positive correlation was observed between ci-miR-208b and RSI scores (Figure 40J). However, no other significant

associations were observed between ci-miRNA levels and performance-related phenotype (Appendix F).

6.3.4 Combining ci-miRNA to explain anthropometric and performance-related phenotypes

Where two ci-miRNAs correlated with a given anthropometric or performance-related phenotype, multiple regression modelling was used to investigate whether each ci-miRNA explained a significant portion of the variance independently. Whilst the model was significant for each phenotype investigated, the addition of a second ci-miRNA was only independently significant in explaining 40 kg squat jump velocity (Table 19).

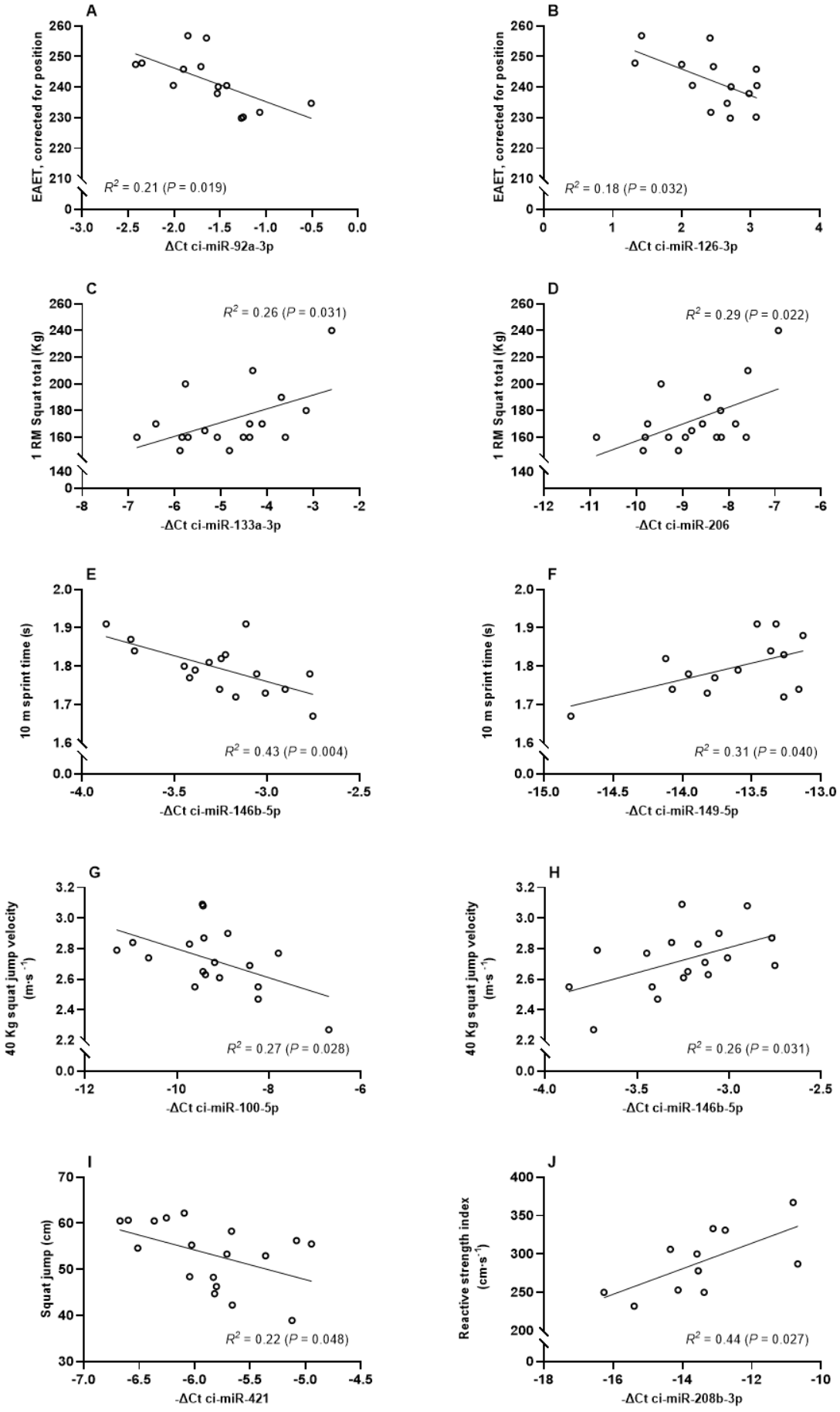
A
EAET, corrected for position
260
250
240
230
220
210
0
R^2 = 0.21 (P = 0.019)
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
ΔCt ci-miR-92a-3p
B
EAET, corrected for position
260
250
240
230
220
210
0
R^2 = 0.18 (P = 0.032)
0
1
2
3
4
-ΔCt ci-miR-126-3p
C
1 RM Squat total (Kg)
260
240
220
200
180
160
140
0
R^2 = 0.26 (P = 0.031)
-8
-7
-6
-5
-4
-3
-2
-ΔCt ci-miR-133a-3p
D
1 RM Squat total (Kg)
260
240
220
200
180
160
140
R^2 = 0.29 (P = 0.022)
-12
-11
-10
-9
-8
-7
-6
-ΔCt ci-miR-206
E
10 m sprint time (s)
2.0
1.9
1.8
1.7
1.6
0.0
R^2 = 0.43 (P = 0.004)
-4.0
-3.5
-3.0
-2.5
-ΔCt ci-miR-146b-5p
F
10 m sprint time (s)
2.0
1.9
1.8
1.7
1.6
0.0
R^2 = 0.31 (P = 0.040)
-15.0
-14.5
-14.0
-13.5
-13.0
-ΔCt ci-miR-149-5p
G
40 Kg squat jump velocity (m·s^{-1})
3.2
3.0
2.8
2.6
2.4
2.2
0.0
R^2 = 0.27 (P = 0.028)
-12
-10
-8
-6
-ΔCt ci-miR-100-5p
H
40 Kg squat jump velocity (m·s^{-1})
3.2
3.0
2.8
2.6
2.4
2.2
0.0
R^2 = 0.26 (P = 0.031)
-4.0
-3.5
-3.0
-2.5
-ΔCt ci-miR-146b-5p
I
Squat jump (cm)
70
60
50
40
30
R^2 = 0.22 (P = 0.048)
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
-ΔCt ci-miR-421
J
Reactive strength index (cm·s^{-1})
400
350
300
250
200
0
R^2 = 0.44 (P = 0.027)
-18
-16
-14
-12
-10
-ΔCt ci-miR-208b-3p

Figure 40. Relationships between performance measurements and ci-miRNA expression: (A) EAET and ci-miR-92a-3p (n = 14); (B) EAET and ci-miR-126-3p (n = 14); (C) 1RM squat and ci-miR-133a-3p (n = 18); (D) 1RM squat and ci-miR-206 (n = 18); (E) 10 m sprint and miR-146b-5p (n = 17); (F) 10 m sprint and miR-149-5p (n = 14); (G) 40 kg jump squat velocity and miR-100-5p (n = 18); (H) 40 kg jump squat velocity and miR-146b-5p (n = 18); (I) Squat jump and miR-421 (n = 18); and (J) reactive strength index and miR-208b-3p (n = 11). Note that higher levels of miRNA expression are to the right of each graph.

Table 19. The multiple regression model included the circulating microRNAs that correlated with phenotypes.

		β Value	***P* Value**	***R*²**
EAET	Model		**0.004***	73%
	miR-92a-3p	-7.71	0.155	
	miR-126-3p	5.07	0.280	
Sum of 8 skinfolds	Model		**0.041***	32%
	miR-149-5p	-0.476	0.083	
	miR-150-5p	-0.259	0.323	
1RM squat	Model		**0.049***	33%
	miR-133a-3p	-0.273	0.345	
	miR-206	-0.358	0.220	
10 m sprint	Model		**0.046***	46%
	miR-149-5p	-0.305	0.255	
	miR-146b-5p	0.499	0.076	
40 kg squat jump velocity	Model		**0.008***	48%
	miR-146b-5p	-0.459	**0.027***	
	miR-100-5p	0.471	**0.024***	

Note: β = standardised coefficient. * Significant $P < 0.05$.

6.4 Discussion

This is the first study to investigate the role of ci-miRNAs in elite RU rugby players. A significant difference in ci-miR-149-5p expression levels between forwards and backs was observed. Additionally, 11 ci-miRNAs correlated with aspects of anthropometry and performance

important for rugby. This makes them potentially useful biomarkers in elite rugby athletes. Combining ci-miRNAs improved their association with 40 kg jump velocity.

Of the 17 candidate ci-miRNAs, only miR-149-5p was differentially expressed between forwards and backs (Figure 38). It was higher in the forwards. Additionally, higher levels of this ci-miR-149-5p were associated with higher body mass, a higher sum of eight skinfolds and slower sprint times (Figures 39B, 39C and 40F). This is consistent with forwards having significantly higher body mass and slower sprint times in this study and greater body mass and the sum of skinfolds in previous research (Brazier *et al.*, 2020). Collectively, this suggests that ci-miR-149-5p may influence sprinting speed through body mass and fat mass rather than alterations in fibre type. One previous study investigated ci-miR-149 in severely obese subjects who had undergone surgery-induced weight loss (Nunez Lopez *et al.*, 2017). The authors found an increase in ci-miR-149 in subjects who additionally took part in a 6-month exercise programme versus those who did not. However, they did not correlate ci-miRNA levels with anthropometric measurements. In the skeletal muscle of mice, miR-149 is believed to promote mitochondrial biogenesis (Mohamed *et al.*, 2014). In bovine cells, it appears to be involved in adipocyte proliferation (Khan *et al.*, 2020). However, others have shown no correlation between changes in ci-miR-149 and skeletal muscle in humans in response to acute exercise (D'Souza *et al.*, 2017b). Future studies should focus on the physiological mechanism by which increased levels of ci-miR-149-5p may link to an increased fat mass in humans.

Levels of ci-miR-221-3p, selected as an exercise responsive ci-miRNA (Andersen *et al.*, 2004) and known to differ between strength and endurance athletes (Wardle *et al.*, 2015), correlated negatively with height (Figure 39A). Previous studies have either not investigated or not reported relationships with height. However, distance runners are typically smaller in stature (Weyand and Davis, 2005), suggesting the negative relationship with height found here may be consistent with previous results in elite athletes (Wardle *et al.*, 2015).

The literature on ci-miR-150-5p is equivocal. In one study it was significantly lower in obese middle-aged adults compared to normal weight (Hijmans *et al.*, 2018). Whilst in another it was increased in women with gestational obesity (Carreras-Badosa *et al.*, 2015). Included in this study via the Focus panels, a positive association with the sum of eight skinfolds was observed (Figure 39D); although, not with other aspects of body mass or stature. However, the participant groups were quite different making direct comparisons difficult.

Other ci-miRNAs identified through the Focus panels displayed associations primarily with measurements of strength and power. Higher levels of both ci-miR-92a-3p and ci-miR-126-3p were indicative of a quicker performance in the EAET (Figures 40A and 40B). However, both have equivocal results in the literature. Ci-miR-92a increased following a dietary and exercise intervention in obese older adults (n=33) with impaired glucose regulation (Fachim *et al.*, 2020). Whilst it decreased following 20 weeks of endurance exercise training in a subcohort (n=20) from the HERITAGE study (Barber *et al.*, 2019). Contrastingly, ci-miR-126 was found to increase in the HERITAGE study (Barber *et al.*, 2019), and with 4 weeks of high-intensity interval training (Schmitz *et al.*, 2019). Although, other studies have found no influence of training (Schmitz *et al.*, 2017). MiRNA-92a is known to be involved in vascular growth and angiogenesis (Li *et al.*, 2014). MiRNA-126 is known to be enriched in vascular endothelial where it enhances the pro-angiogenic actions of VEGF (Wang *et al.*, 2008). This makes both good candidates for involvement in mediating the effects of exercise training. Future studies should investigate its relationship with capillary density, exercise, and fitness.

MiR-146b is believed to be an inflammatory miRNA (Specjalski and Jassem, 2019). Only one study (n=10) has investigated ci-miR-146b and exercise. The authors found a reduction in ci-miR-146b following 12 weeks of progressive resistance training (Liu *et al.*, 2020). This is in contrast with the ci-miR-146b results in this study where increased levels of ci-miR-146b are associated with quicker sprint times and a greater 40 kg jump velocity (Figures 40E and 40H). The exploratory study by Liu *et al.* (2020) observed the resistance training effects in older adults with no prior resistance training. The differences in the study populations could explain the contrasting ci-miR-146b associations. A study of women in early pregnancy (n=92) found a positive association between leisure time physical activity and ci-miR-146b (Badon *et al.*, 2018). A higher leisure time physical activity is known to associate with greater lower body strength (Loprinzi *et al.*, 2017). Future larger studies should continue to consider ci-miR-146b as a potential exercise training responsive miRNA particularly in relation to speed and power-related phenotypes.

MiR-100 is a negative regulator of mTOR in cancer cells (Ye *et al.*, 2020) and adipocytes (Pek *et al.*, 2016). mTOR signalling in skeletal muscle promotes muscle growth (Schiaffino *et al.*, 2013). Only one study has assessed ci-miR-100 and exercise (Sansoni *et al.*, 2018). The authors found a decrease in ci-miR-100 following eight weeks of repeated sprint training. This study reported lower ci-miR-100-5p associated with greater 40 kg squat jump velocity (Figure 40G).

Thus, lower levels of ci-miR-100-5p may improve 40 kg jump performance by allowing a greater expression of mTOR and therefore skeletal muscle growth. Future studies should investigate this potential mechanism of action for ci-miR-100-5p in exercise. When combined with ci-miR-146b-5p, ci-miR-100-5p explains 48% of the variance of the 40 kg jump velocity which is consistent with their mechanisms being through different pathways.

Similarly, ci-miR-421 had a negative association with a power phenotype, squat jump (Figure 40I). No studies have investigated the relationship between exercise training and ci-miR-421. However, like miR-100, miR-421 is also believed to be a negative regulator of mTOR.(Wang *et al.*, 2020) Again, it could be predicted that lower levels of ci-miR-421 would allow greater expression of mTOR and therefore skeletal muscle growth. Future studies should investigate this potential mechanism of action for ci-miR-421 in exercise.

The remaining associated ci-miRNAs were myomiRs. These are skeletal muscle enriched miRNAs crucially involved in muscle proliferation, differentiation and regeneration (Horak *et al.*, 2016). Their involvement in cellular processes is perhaps better understood than many other miRNAs. Two of the investigated myomiRs, ci-miR-133a and ci-miR-206, had positive associations with lower body strength in the 1RM squat (Figures 40C and 40D). Although, circulating levels do not necessarily reflect cellular levels, cellular miR-133a and miR-206 are known to positively promote muscle development making these results consistent with the understanding of their biology: more muscle should lead to better squat performance. Some exercise studies have reported very low levels of circulating ci-miR-133a and ci-miR-206, making measurement difficult (Baggish *et al.*, 2011; Wardle *et al.*, 2015). Others have successfully detected ci-miR-133a and miR-206; although, with equivocal results. One study consistent with this study's findings showed increases in their expression with resistance training in older adults (Zhang *et al.*, 2015). Another showed no association with muscle strength in middle-aged men (D'Souza *et al.*, 2019). Future studies should consider that differences in the strength of associations may come from the different participant groups used.

A third myomiR, ci-miR-208b, had a positive relationship with the reactive strength index (Figure 40J). This is not a measure of strength *per se* but a measure of the body's ability to change from eccentric contraction to concentric contraction and is important for dynamic jumping activities requiring explosive force. Cellular miR-208b is believed to promote muscle growth and cause a shift towards a slow myogenic programme (Horak *et al.*, 2016). This makes

its relationship to explosive force unclear: it could be expected that more muscle and a fast myogenic programme to be important for the reactive strength index. However, no other studies have investigated ci-mi-208b and such explosive phenotypes. Future studies should investigate ci-miR-208b and muscle fibre type alongside explosive contraction measurements.

6.4.1 Limitations

Elite athletes provide a unique opportunity to investigate the biological underpinning of physiological and performance phenotypes due to their high levels of training and skill in completing performance tasks. However, they are rare and difficult to recruit in large numbers and the results of this study must be interpreted with caution due to the small sample size. A number of the target ci-miRNAs showed no association with phenotype. Additionally, there was a lower than expected number of differences in ci-miRNA expression between forwards and backs. This may be a result of the increasing similarity between the positional groups since the advent of professionalism in August 1995 (Quarrie and Hopkins, 2007; Smart *et al.*, 2013). However, both could also be affected by the small number of participants. Additionally, this study may have benefited from a control group, which would have allowed us to draw definitive conclusions about how circulating miRNAs are involved in different phenotypes. Only circulating miRNAs were measured which resulted in the study not being able to identify cause and effect relationships with targeted gene and protein expression levels. The use of a candidate miRNA chosen approach would have resulted in a reduction in new miRNA associations being discovered. However, rigorous methods were designed to find potential new miRNAs as well as test common research derived miRNAs.

6.4.2 Conclusion

This study supports the value of ci-miRNAs as biomarkers of anthropometric and performance variables. The observed associations were consistent with the current understanding of the likely biological mechanisms. Future research should investigate the role of these ci-miRNAs in the proposed mechanisms of adaptive response to exercise.

Chapter 7

7.0 Can Baseline Plasma miRNA Levels Identify Individual Responsiveness to Preseason Training in Elite Male Rugby Union Players?

7.1 Introduction

The preseason period prepares RU players with the necessary levels of skill and fitness qualities required for elite competition performance. Existing research recognises the critical role this period plays; there are several studies on the responses of an elite RU preseason training period spanning four (Argus *et al.*, 2010), eight (McLaren *et al.*, 2018), ten (Bradley *et al.*, 2015b) and eleven weeks (Grainger *et al.*, 2020). Gannon *et al.* (2016) tracked lower body strength and power characteristics of 22 professional English Premiership rugby players over 45 weeks. They identified the greatest opportunity for physical development over an entire playing season as the dedicated preseason block. The targeted enhancement of these physiological qualities is the consequence of functional adaptations underpinned by a multitude of signalling and molecular mechanisms (Coffey and Hawley, 2007). However, the quality of adaptation is prone to significant inter-individual variability, as discussed in section 2.2.2.

Chapter 6.0 supported previous research on the role ci-miRNAs play in the regulation of phenotypes. However, whether ci-miRNAs in elite RU players also contribute to the magnitude of change in response to a period of preseason remains unknown. Initial studies on intramuscular miRNAs indicated that changes in expression levels pre and post a training intervention were associated with the variation of strength responses in individuals with no prior resistance training experience (Davidsen *et al.*, 2011; Ogasawara *et al.*, 2016) and older adults (Zhang *et al.*, 2015). This research is not currently applicable to elite athletes as they are either untrained or older. Additionally, the ability to collect muscle samples from elite players is very limited. Hagstrom and Denham (2018) observed the fold change in serum miRNA levels and found positive associations with greater improvements in upper and lower body strength changes in breast cancer patients. These previous studies have primarily reported associations between fold changes and percent differences in specific miRNAs. This method requires multiple samples (before and after an intervention), which is not often possible in elite sport.

Emerging research suggests that a baseline miRNA sample might be able to predict the variability in response to training, which would be more feasible and appropriate in elite sport. Ogasawara *et al.* (2016) reported 17 miRNAs obtained from skeletal muscle at baseline were differentially expressed between 10 individuals, the top 5 high and top 5 non- hypertrophic responders. The potential diagnostic ability of miRNAs to predict a magnitude of training

response has also been shown by Zhang *et al.* (2015) who reported baseline plasma expression levels of miR-499 showed a significant correlation with the percentage change in knee extensor strength. Additionally, Horak *et al.* (2018) reported that baseline plasma levels of ci-miR-93 were an independent predictor of high and low responders based on improvements in isometric leg extension.

The majority of published research has reported associations with resistance training, so typically the outcome of strength change is predicted. The ability to measure a preseason rugby period allows an opportunity to observe whether ci-miRNAs can be biomarkers for predicting individual responsiveness to other fitness qualities. Previously, Parr *et al.* (2016) manipulated the diet and aerobic exercise of individuals and reported that ci-miRNA-935 and -140 were differentially expressed between high and low responders before and after a weight loss intervention. Additionally, Zhang *et al.* (2017) observed the percentage change in plasma ci-miR-92a-3p as a potential biomarker to predict gait speed responses to aerobic exercise training in obese older adults.

Currently, very few studies have explored the relationship between basal ci-miRNA expression and trainability in elite athletes and, no reliable physical adaptation biomarkers assessing molecular events currently exist. Thus, this study focuses on identifying potential ci-miRNA biomarkers which could predict the magnitude of physiological exercise adaptation in response to a rugby specific preseason program. The ability to understand whether ci-miRNAs are capable of characterising the potential magnitude of physical adaptation to different exercise stimuli would be very beneficial in elite sport. This would allow a movement towards an individualised athlete-centred approach to exercise prescription provided by an understanding of the adaptive potential.

Prior research has reported superior training improvements when undertaking an individualised training approach (Dorrell *et al.*, 2020; Javaloyes *et al.*, 2020; Simpson *et al.*, 2020). Jones *et al.* (2016) tested the genotypes of 28 athletes from different sports and 39 football athletes. Individuals were placed into a power-based or endurance-based resistance group, which either matched or mismatched their individual genotype. They demonstrated amongst the low responders from the differing sports and football only cohort, 82% of athletes were from the mismatched group, additionally greater performance changes were observed in individuals with the matched genotype training.

Only a few studies have analysed the effects basal ci-miRNA levels contribute to the magnitude of a physiological fitness change and none of these has tested elite athletes. This study aims to bridge the gap toward evidence-led interventions by providing observational and association based information on specific ci-miRNAs that have the potential as predictive biomarkers. Biomarkers able to characterise a robust physiological training response will be very useful for an individualised training design. Thus, the purpose of this study was to: (A) Examine the individual fitness responses to preseason training in elite RU players; (B) Determine the association between basal expression levels of candidate ci-miRNAs and the magnitude of change in performance variables associated with rugby performance.

7.2 Methodology

7.2.1 Participants

Twenty-seven participants were recruited from an elite English Rugby Union first-team squad, playing at the highest level of English and European competition. Participation in the study required a baseline blood sample, preseason completion and maximal pre and post-testing. Due to the strict inclusion criteria and the nature of professional sport, the final study sample consisted of nineteen players The nineteen participants (age: 26 ± 4 years, stature: 188 ± 5 cm, body mass: 104 ± 11 kg) included four who had represented internationally. One participant was removed due to an extensive injury that resulted in missing the majority of the preseason training period. The remaining participants completed the whole training period however, some may have missed an element of the testing or re-testing due to injury risk or involvement in a separate competition. Overall pre and post-tests were performed by eleven players for the EAET, sixteen players for the 1 RM squat and fifteen players who performed the 1 RM bench press. All players were familiar with the testing procedures and provided written informed consent before volunteering for the study. Experimental procedures were approved by the university's research ethics committee.

7.2.2 Preseason training period

Players returned to preseason training following a 6-week off-season period; this allows the players time to rest and recover. Two weeks before the return to training, players were provided with an off-season maintenance program which provides a minimum training dose to sustain fitness and avoid injuries on return to training. Preseason was a total of 9-weeks and included both general and specific player preparation periods. As can be seen from Figure

41, the training load is programmed to increase linearly before a taper period is applied two weeks prior to the competitive season commencing. An active recovery week was located in week 5. Figure 28 provides information on the structure of a typical preseason training week which includes a mixture of field-based rugby training sessions, speed sessions and gym work. The main goals of preseason are to improve players' skills, aerobic capacity, repeat sprint ability, maximum velocity, strength and power, these variables are required for successful competitive performance.

7.2.3 Procedures

All testing procedures were selected and planned in cooperation with the clubs' high-performance coaching staff. These tests were completed as part of the club's normal preseason testing regime, therefore, the timing of when the tests were undertaken was rigid in design. A schematic detailing when each testing procedure took place is provided in Figure 41.

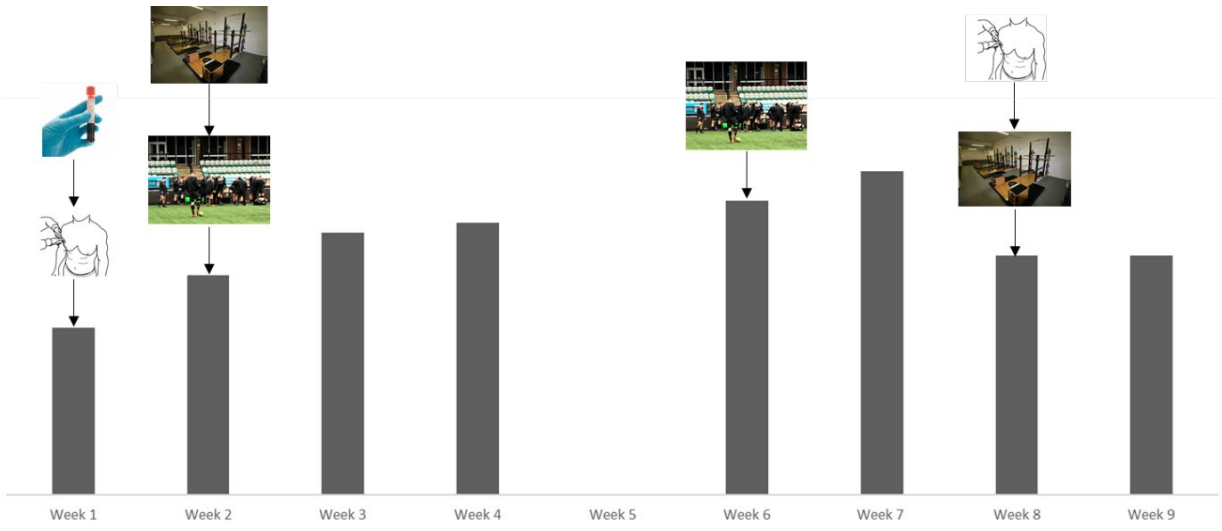

Figure 41. Bars provide an example of load progression over the 9-week preseason period. Blood samples were obtained on the first day of preseason in week 1, anthropometry assessments took place in weeks 1 and 8, England anaerobic endurance test was undertaken in week 2 and week 6 and strength testing took place in week 2 and week 8.

7.2.3.1 Blood sampling

Players arrived for a baseline venous blood sample between 07:30 and 09:00 h in a fasted state, having abstained from caffeine prior to their visit and any strenuous exercise for 48 hours. This sample was obtained on the first day back to preseason training following a 6-week period of no competitive rugby or training during the off-season. Venous blood

samples were taken at the club's training ground and collected from all participants into EDTA vacutainers (section 3.8). Following collection, they were transported to a laboratory for plasma separation and subsequently aliquoted and stored at −80 ˚C for later analysis.

7.2.3.2 Anthropometric testing

Skinfolds and lean mass index calculations were performed to observe any changes in body composition that may occur due to the preseason training period. Baseline tests were performed in week 1 and were retaken in week 8 to monitor the changes over the preseason (Figure 41). Skinfold measures were obtained as detailed in section 6.2.3.

Lean-mass index (LMI) was calculated using the formula developed by Slater *et al.* (2006) as a measure of fat-free mass in trained athletes. It has been shown as a valid method of predicting lean mass in both RU (Slater *et al.*, 2006) and rugby league players (Delaney *et al.*, 2016). Following each individual's skinfold assessment, LMI was calculated as LMI = (m/S^x), where m = body mass, S = sum of seven skinfold thicknesses and x = the LMI exponent. The seven skinfold sites are defined as triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, anterior thigh, and medial calf skinfolds. The exponent is defined as 0.13 for forwards and 0.14 for backs.

7.2.3.3 Assessment of fitness changes

Baseline England anaerobic endurance testing as a measure of anaerobic endurance took place in week 2 before being repeated in week 6 (Figure 41). The England anaerobic endurance test is described in section 3.6.1.

7.2.3.4 Assessment of strength changes

One repetition maximum (1 RM) squat and bench press testing took place at the club's training facility in week 2 and was repeated at the end of preseason (week 8). Both the squat and bench press 1 RM tests, were undertaken as detailed in section 3.5.4.

7.2.4 RNA isolation and complementary (cDNA) synthesis

Please refer to general methods chapter 3.9.1 and 3.9.2 for methodological details.

7.2.5 Quantification of circulating miRNA levels

Please refer to general methods chapter 3.9.3 and 3.9.4 for further details on circulating microRNA analysis and quantification.

7.2.6 Data analysis

All data are expressed as mean ± standard deviation (SD), with statistical significance set at $P < 0.05$ a priori. An α of 0.05 was deemed appropriate for each of the below statistical tests as prior evidence has shown associations between the performance-related phenotypes and the expression levels of candidate ci-miRNA.

Statistical analysis was performed using IBM SPSS Statistics 25 for Windows (Surrey, UK) and Minitab software (version 18; Minitab, State College, PA). All analysis was performed on normally distributed ci-miRNA ΔCt data and any significant outliers were removed. Normal distribution was confirmed using the Ryan-Joiner test and significant outliers were identified via the Grubbs test.

Preseason anthropometric, fitness and strength changes were analysed using a paired samples t-test. Percentage change ((post-measure – pre-measure)/pre-measure × 100) was calculated for the outcomes of all anthropometric, fitness and strength measures. Linear regression analysis was carried out to test the linearity of any associations between ci-miRNA expression levels and the percent change of anthropometric, fitness and strength measures. In acknowledgement of the small sample size, Cohen's *d* effect sizes (ES) were calculated with the magnitude of effects considered as either trivial (< 0.2), small (0.2 - 0.6), moderate (0.6 - 1.2), large (1.2 - 2.0) and very large (2.0 – 4.0) (Batterham and Hopkins, 2006).

7.3 Results

7.3.1 Training-induced changes

7.3.1.1 Anthropometrical changes over the preseason period

Changes in anthropometry following preseason training are shown in Figure 42. As can be seen from Figure 42A participants at the start of preseason (88.6 ± 19.4 mm) had a greater sum of skinfold value compared to the end (83.8 ± 20.1 mm). This was a statistically significant decrease of -4.8 mm ($P = 0.001$, [-7.4, -2.2], $t_{(17)} = -3.9$, ES = -0.2). Following preseason LMI (Figure 42B) showed a small significant increase of 1.2 kg/mm^x ($P < 0.001$, [-0.7, 1.6], $t_{(17)} = 6.0$, ES = 0.2).

7.3.1.2 Performance changes over the preseason period

There was a significant improvement in fitness as seen by the change in the England anaerobic endurance fitness test (Figure 43A) following 4 weeks of conditioning over the preseason

training. A 2 % decrease in England anaerobic endurance fitness test scores was observed (Pre; 229.4 ± 9.1 s, Post; 225.1 ± 10.4 s), paired samples t-test highlighted a small significant reduction -4.3 s (P = 0.026, [-8.0, -0.6], $t_{(10)}$ = -2.6, ES = -0.4).

Changes in strength over the preseason can be seen in Figures 43B – 43E. Squat and relative squat performance increased from 172.8 ± 24.2 kg and 1.6 ± 0.2 kg·kg^{-1} to 195.3 ± 27.9 kg·kg^{-1} and 1.8 ± 0.2 showing an improvement of 13% respectively. These improvements were of significant moderate (22.5 kg, P < 0.001, [16.7, 28.3], $t_{(15)}$ = 8.3, ES = 0.9) and large (0.21 kg·kg^{-1}, P < 0.001, [0.15, 0.26], $t_{(15)}$ = 8.3, ES = 1.0) magnitude increases in performance. An average increase of 8% was observed for bench press (Pre; 132.3 ± 14.5 kg, Post; 142.7 ± 15.1 kg) and relative bench press (Pre; 1.2 ± 0.1 kg·kg^{-1}, Post; 1.3 ± 0.1 kg·kg^{-1}) performance. Paired samples t-test highlighted a significant improvement in bench press 10.3 kg, P < 0.001, [6.8, 13.9], $t_{(14)}$ = 6.3, ES = 0.7 and relative bench press 0.10 kg·kg^{-1}, P < 0.001, [0.07, 0.13], $t_{(14)}$ = 6.6, ES = 0.8.

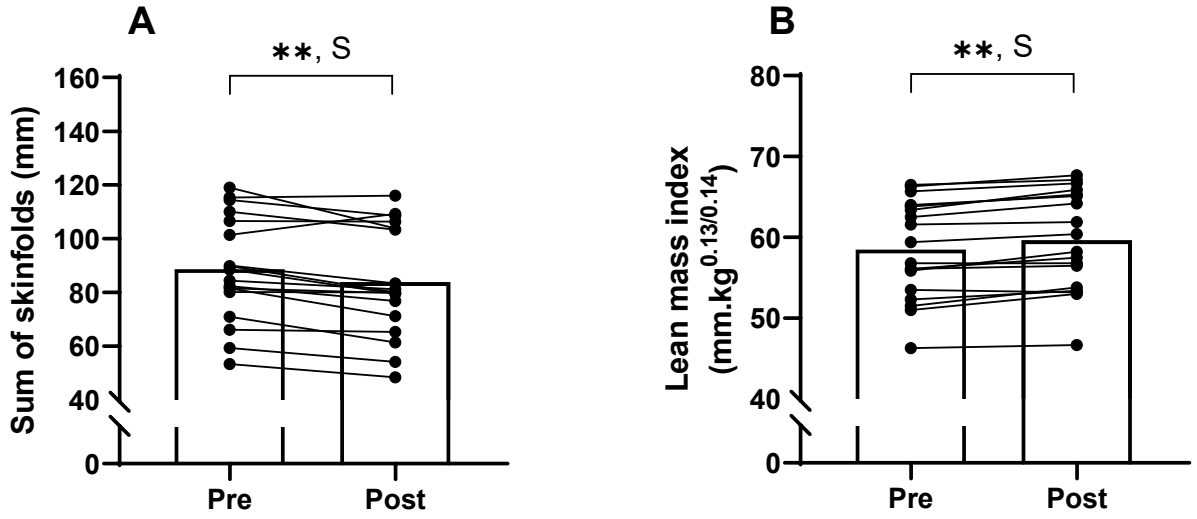

Figure 42. Anthropometrical changes following a preseason period. (A) Changes in skinfold; (B) changes in lean mass index. * signifies P < 0.05, ** signifies P < 0.01, subscripts denote small (S), moderate (M), large (L) and very large (VL) Cohens d descriptor.

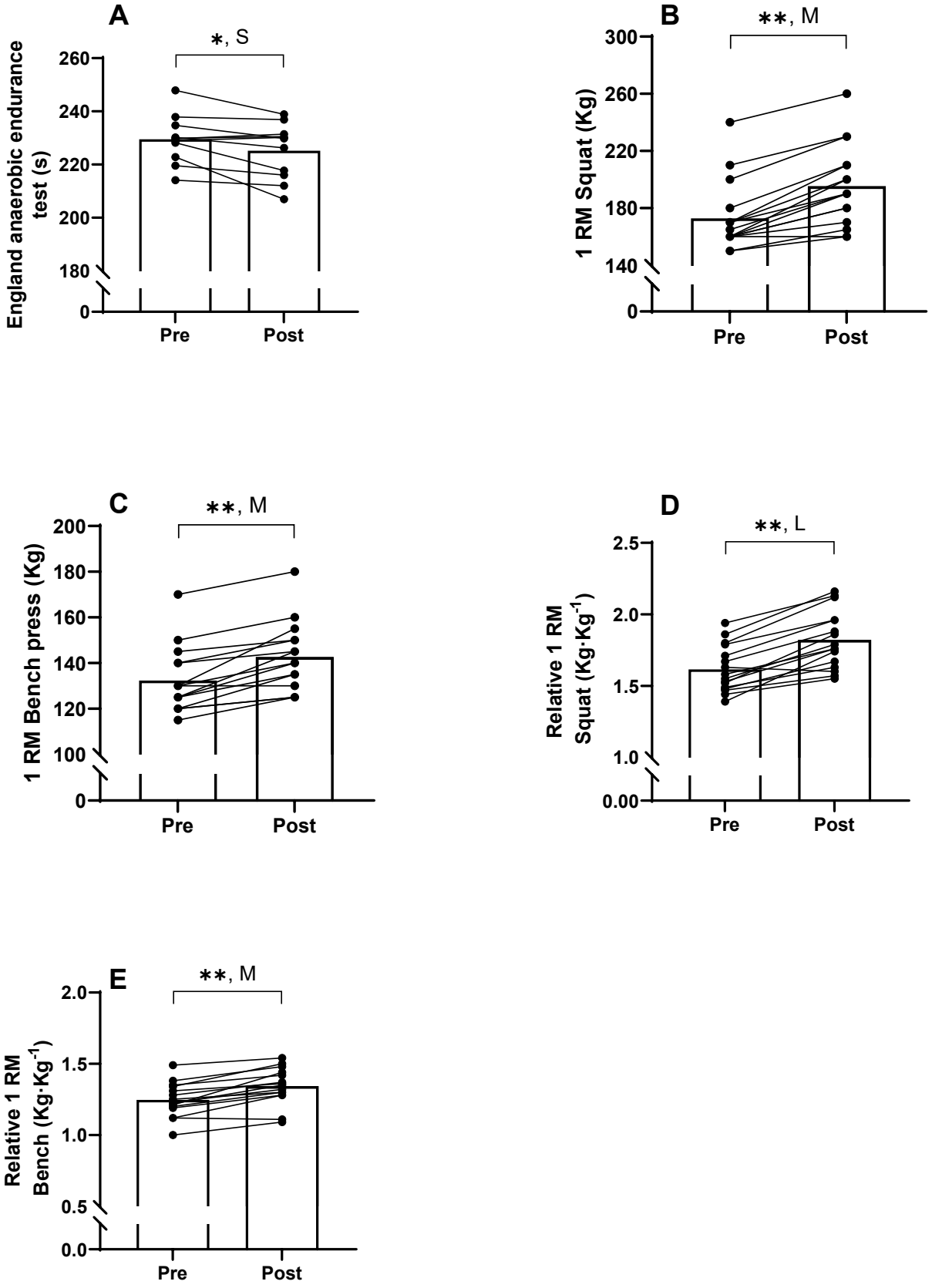

Figure 43. Individual performance responses following a period of preseason training. 1 RM = 1 repetition maximum.* signifies P < 0.05, ** signifies *P* < 0.01, subscripts denote small (S), moderate (M), large (L) and very large (VL) Cohens d descriptor.

7.3.2 Relationships between changes in anthropometry measures and circulating miRNAs at baseline

There were significant negative associations between the percent change in the sum of skinfolds and baseline level of ci-miR-100-5p and 92a-3p. A significant association between the sum of skinfolds and miR-100-5p can be seen in Figure 44A (R^2 = 0.52, P < 0.001), suggesting a higher baseline expression level is predictive of a greater decrease in skinfold percentage change. The same pattern is also observed for miR-92a-3p (R^2 = 0.57, P < 0.001, Figure 44B). No other significant correlations were observed.

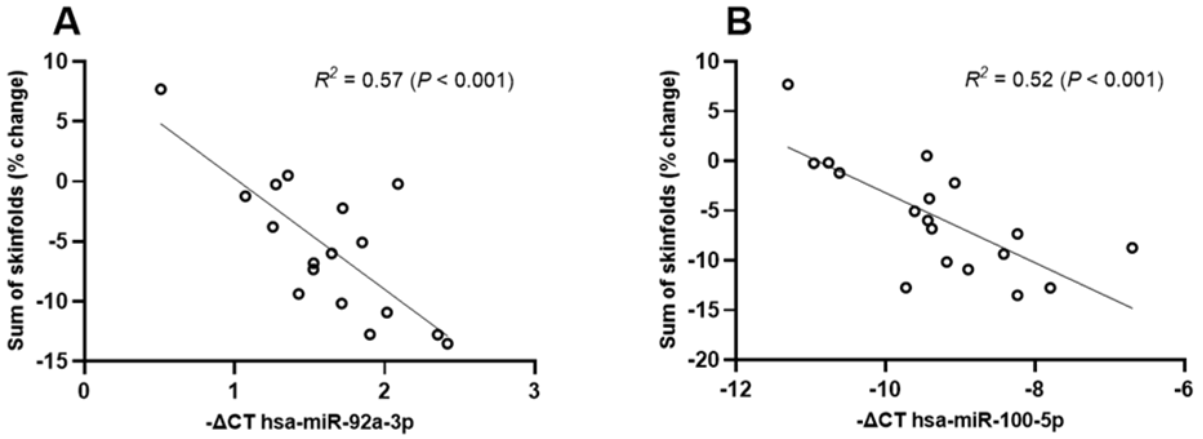

Figure 44. Relationships between changes in body composition (sum of skinfolds) following training and baseline miRNA expression levels. Note that higher levels of miRNA expression are to the right of the graph. (A) The Sum of skinfolds showed a significant negative correlation with levels of miR-100-5p (n = 18). (B) The Sum of skinfolds showed a significant negative correlation with levels of miR-92a-3p (n = 17).

7.3.3 Relationships between changes in fitness and strength-related performance measures and circulating miRNAs at baseline

As Figure 45 shows, there were significant correlations between basal ci-miRNA expression levels and performance changes. Baseline plasma ci-miR-208b-3p expression levels negatively correlated with percent change in absolute and relative 1 RM squat (R^2 = 0.45, P = 0.013 and R^2 = 0.50, P = 0.007, respectively, Figures 45A and 45B). Additionally, there was also a significant negative correlation between levels of ci-miR-221-3p and the increase in absolute and relative 1 RM squat (R^2 = 0.34, P = 0.018 and R^2 = 0.36, P = 0.014, respectively, Figures 45C and 45D). In both instances, a negative correlation was observed, suggesting an increased basal expression level is indicative of a lower observed change in squat and relative 1 RM score following the preseason training period. Negative correlations with individual responses in

strength are also seen for percent changes in absolute and relative bench press performance (R^2 = 0.27, P = 0.047 and R^2 = 0.31, P = 0.032, respectively, Figures 45E and 45F). None of the candidate baseline levels of microRNAs showed any correlation to a change in England anaerobic endurance performance (P > 0.05). Potential factors that could explain the differential regulation of miRNA levels were explored but no obvious patterns explained the response (Appendix G).

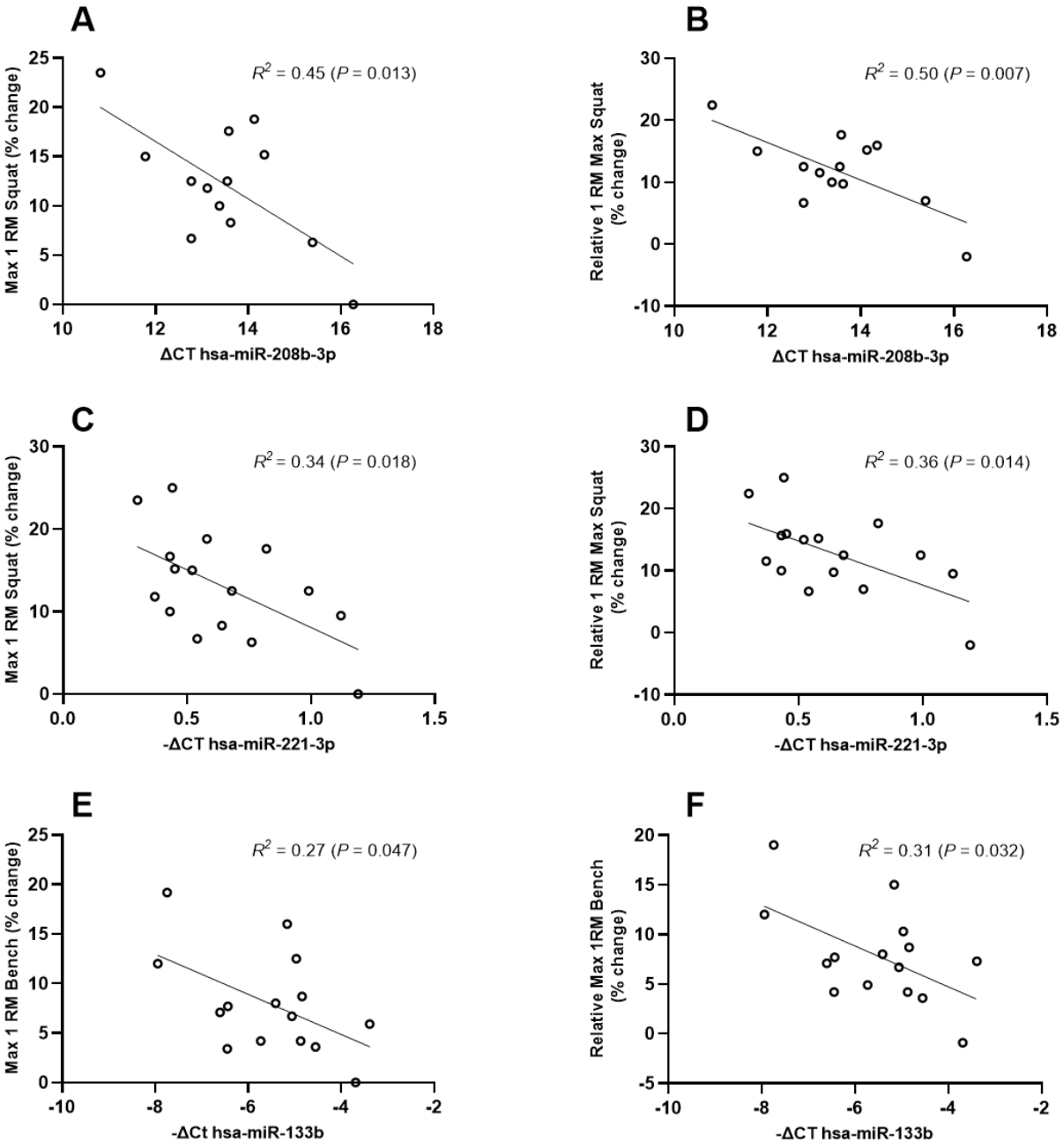

Figure 45. Relationships between changes in upper and lower body strength following training and baseline miRNA expression levels. Note that higher levels of miRNA expression are to the right of the graph. 1 RM = 1 repetition maximum (A) % change in 1 RM squat showed a significant negative correlation with levels of miR-208b-3p (n = 13). (B) % change in 1 RM squat showed a significant negative correlation with levels of miR-208b-5p (n = 13). (C) % change in 1 RM squat showed a significant negative correlation with levels of miR-221-3p (n = 16). (D) % change in 1 RM squat showed a significant negative correlation with levels of miR-221-3p (n = 16). (E) % change in relative 1 RM bench press showed a significant negative correlation with levels of miR-133b (n = 15). (F) % change in relative 1 RM bench press showed a significant negative correlation with levels of miR-133b (n = 15).

7.4 Discussion

The main findings of this investigation indicate that strength, fitness and body composition performance variables improve following a preseason period; however, the individual variability in response to training confirms that even in elite players, factors exist that affect the magnitude of training-induced response. Additionally, these findings present new novel biomarkers that suggest an ability to predict the magnitude of an individual's training response to preseason. To the best of our knowledge, this is the first study to use basal ci-miRNA profiles to predict potential training responsiveness in a cohort of elite team sport athletes. This study provides novel information that basal levels of ci-miR-100-5p and ci-miR-92a-3p may be useful biomarkers to predict body composition changes. The findings also observed associations between basal ci-miR-208b-3p, ci-miR-221-3p, ci-miR-133b with the magnitude of change in upper and lower body strength in response to preseason training in elite RU players.

7.4.1 Individual responses to preseason training

The optimisation of a player's body composition is a key aim of a preseason training program for the development of strength and power, as well as the relationships with game characteristics such as an improved work rate and an enhanced ability to repeat tasks (Smart *et al.*, 2014). This study's findings present a variety of individual changes in the sum of skinfold response to preseason training (Figure 42A), as values ranged from -13.5% to +7.7 %. Argus *et al.* (2010) observed a larger change in skinfolds following a short term preseason training programme (-11.5 %, pre; 93.4 ± 26.7 mm, post; 82.4 ± 22.5 mm) compared to the results of this study, however, their baseline measurements were greater. This could suggest that the subjects in this study may have been able to minimise unwanted body fat increases better over the off-season period. Although Bradley *et al.* (2015b) used the sum of 7 skinfolds, the observed percentage change in forwards and backs were similar to the findings in this study (forwards; -6%, backs; -7%).

It is important to consider that the training of elite athletes is different to the application of training to lose weight, as players will be prescribed goals to enhance changes in both fat mass and fat-free mass. This could result in not all players being required to lose body fat but a focus on attaining a greater lean muscle mass. This study reported a small significant increase in lean muscle index and peak increases of up to 4.3%. The values of LMI reported (pre; 58.6 ±

5.9 mm. kg ^{X}, post; 59.6 ± 6.2 mm. kg ^{X}), are greater than the original study by Slater *et al.* (2006) who observed LMI changes in 20 super 12 rugby union players following 10 weeks of intensive preseason training (pre; 53.1 ± 5.3 mm. kg ^{X}, post; 53.3 ± 5.3 mm. kg ^{X}). This suggests a shift in body types of a modern-day rugby player where more emphasis is placed on a greater lean muscle mass. The small changes in lean mass are to be expected as these professional individuals have a greater resistance training history making body composition improvements more difficult in this area (Bradley *et al.*, 2015b).

Following a period of preseason training (four weeks between pre and post measures), a small significant group change in the EAET was observed (Figure 43A). There are very few research papers that have observed changes in fitness pre and post an elite RU preseason (Bradley et al., 2015; McLaren et al., 2018), and the fitness qualities required for RU are broad, a variety of tests have been used to assess it. This makes it difficult to compare due to the output of different tests. McLaren *et al.* (2018) assessed championship RU players (8-week preseason) and Daniels *et al.* (2019), elite rugby league players (7-week preseason). They reported changes of 17.5 % and 29.9 % respectively, which is greater than the 1.89 % improvement observed in this study. Both studies used the Yo-Yo Intermittent Recovery Test, Level 1 as a measure of fitness which focuses on the ability to carry out intermittent exercise leading to maximal usage of the aerobic system (Bangsbo *et al.*, 2008), rather than a higher contribution from the anaerobic system as measured by the EAET in this study. Bradley *et al.* (2015b) used the 1 × 60 s shuttle and 3 × 60 s shuttle as fitness measures of elite RU players' response to a 10-week preseason, they reported a 1.5 and 2.1 % improvement for forwards and backs in the 3 × 60 s shuttle and a 3.6 and 2.8 % improvement in the 1 × 60 s shuttle. The changes in performance observed here are similar to the findings of this study. There are many factors that will influence the magnitude of change for example the physiological measure of the test and the length of the training period.

Six weeks separated the two maximal strength testing sessions within the preseason training block. At a group level, this training resulted in moderate improvements in upper (Bench press, 8%) and lower body strength (Squat, 13%). The individual responses observed in Figure 43, highlight a heterogeneous response that exists even in a homogenous group of elite players. The players' baseline 1 RM strength values (squat: 173 kg, bench: 132 kg) were within the ranges reported previously for professional rugby players (Table 20), confirming their trained

status. This data provides a reference point for expected strength scores following an off-season period.

Previous research has confirmed the positive impacts of a preseason training period on whole-body strength adaptations in rugby players. Positive improvements have been shown following periods of 4 – 10 weeks (Argus *et al.*, 2010; Bradley *et al.*, 2015b; Daniels *et al.*, 2019; Stokes *et al.*, 2020). The observed squat and bench changes in the current investigation produced similar, if not greater improvements in strength than in other studies undertaken during a preseason training phase (Argus *et al.*, 2010; McLaren *et al.*, 2018; Daniels *et al.*, 2019; Bagley *et al.*, 2020; Stokes *et al.*, 2020). Table 20 shows the magnitude of lower body strength change is greater than upper body following preseason training on return from the off-season. This could be due to greater atrophy of lower limb muscles during the off-season period as a result of disuse. Although players are advised to rest away from the club, the undertaking of maintenance training is still advised. Players may more regularly focus on the upper body during this period and may only train lower limb muscle groups occasionally. Additionally, as players are away from the club it may be harder for them to access the level of supervision and equipment at home or in a commercial gym compared to what is provided in a club environment.

Table 20. Preseason strength changes of professional rugby teams.

Participants	Professional Level	Intervention length	Average Gym sessions	Pre-training (kg)	Post-training (kg)	Strength change (%)	Weekly change (%)	Journal
18 players	English Premiership team	5 weeks	4 per week	Squat 1 RM: 173 ± 24	Squat 1 RM: 195 ± 28	13	2.6	Current Study
				Bench press 1 RM: 132 ± 15	Bench press 1 RM: 143 ± 15	8	1.6	
23 players	English Championship team	8 weeks	Unknown	Squat P1 RM: 184 ± 23	Squat P1 RM: 205 ± 21	11	1.4	(McLaren et al., 2018)
33 players	Super 14 team	4 weeks	5 per week	Box Squat P1 RM: 155 ± 26	Box Squat P1 RM: 172 ± 30	11	2.8	(Argus et al., 2010)
				Bench press P1 RM: 124 ± 19	Bench press P1 RM: 138 ± 20	11	2.8	
21 players	European Super League team	7 Weeks	4 per week	Squat 3 RM: 181 ± 21	Squat 3 RM: 199 ± 25	10	1.4	(Daniels et al., 2019)
				Bench press 1 RM: 125 ± 16	Bench press 1 RM: 130 ± 17	4	0.6	
45 players	Pro 12 League team	10 Weeks	4 per week	Squat P1 RM (Forwards): 201 ± 27	Squat P1 RM (Forwards): 215 ± 32	7	0.7	(Bradley et al., 2015)
				Squat P1 RM (Backs): 175 ± 12	Squat P1 RM (Backs): 196 ± 17	12	1.2	
				Bench press P1 RM (Forwards): 135 ± 12	Bench press P1 RM (Forwards): 141 ± 13	4	0.4	
				Bench press P1 RM (Backs): 122 ± 10	Bench press P1 RM (Backs): 128 ± 11	5	0.5	
35 players	Professional rugby union team	4 weeks	Unknown	Squat 1 RM: 167 ± 27	Squat 1 RM: 190 ± 28	14	3.6	(Stokes et al., 2020)
				Bench press 1 RM: 131 ± 13	Bench press 1 RM: 137 ± 13	4	1.0	

Note: 1 RM = One-repetition maximum; P1 RM = Predicted one-repetition maximum

7.4.2 The association between ci-miRNAs at a basal level and the magnitude of individual change in physical qualities

This is the first study to show a significant relationship between the individual rate of sum of skinfold change following a preseason with ci-miRNA-100-5p and ci-miRNA-92a-3p. This novel selection of ci-miRNAs displays associations with the rate of response of the sum of skinfold change in individuals; suggesting ci-miRNA-100-5p and ci-miRNA-92a-3p may be involved in complex molecular mechanisms important for weight loss via regulating pathways related to body composition homeostasis, lipid metabolism and metabolic processes. Primarily the research has focused on weight loss or body composition in obese and diabetic populations rather than elite rugby players (Milagro *et al.*, 2013; Parr *et al.*, 2016).

The findings of this study are the first to report a negative association between basal plasma levels of miR-100-5p in elite rugby players and the percentage change in body fat as measured by the sum of skinfolds pre and post a preseason training period. The relationship suggests a higher abundance of plasma miR-100-5p increases an individual's susceptibility to losing body fat. It is currently unknown directly how basal levels of ci-miR-100-5p could explain the variability of body fat loss in response to training, however, although sparse there are findings to support the putative role of miR-100 regulation in adipogenesis. Increased basal ci-miR-100 abundance has been reported in healthy and lean subjects compared to obese normoglycemic individuals (Pek *et al.*, 2016). This study demonstrated that miR-100-5p plays a key role in pre-adipocyte differentiation to mature adipocytes. A higher abundance of miR-100 decelerates this differentiation, whereas low levels accelerate this process, creating a greater lipid accumulation. A direct target of miR-100 is mTOR (Torres *et al.*, 2012; Pek *et al.*, 2016), which its kinase activity is required for preadipocyte differentiation (Kim and Chen, 2004). As an expression of mTOR kinase coexists with a down-regulation of miR-100 (Torres *et al.*, 2012), the reduction in miR-100 activity accelerates lipid accumulation due to greater preadipocyte differentiation, suggesting the putative role this specific miRNA has to mediate the process.

In addition to ci-miR-100-5p, an association between baseline ci-miR-92a-3p levels and a percentage change in the sum of skinfolds was also observed. Again, this is the first study to highlight this potential influential regulation in elite rugby athletes. Exercise induces beneficial adaptations upon white adipose tissue located in the visceral and subcutaneous regions as well as brown adipose tissue (Lehnig and Stanford, 2018), thus, is a beneficial tool to combat obesity as it is important for the regulation of body weight. As a result, a greater number of

studies have examined the impact of exercise on miRNAs in obese populations. Previous studies have highlighted a negative relationship between miR-92a-3p and brown adipose tissue activity (Chen *et al.*, 2016; Zhang *et al.*, 2019). The prevalence of brown adipose tissue has been correlated with the leanness of adults (van Marken Lichtenbelt *et al.*, 2009; Vijgen *et al.*, 2011), and the activity of brown adipose tissue is significantly lower in individuals with a higher body fat percentage (van Marken Lichtenbelt *et al.*, 2009). MiR-92a directly targets and inhibits the expression of SMAD7 which in turn constrains the promotion of brown adipocyte differentiation. Additionally, it was suggested that downregulation of miR-92a resulted in weight loss in rats training under hypoxic conditions (Cheng *et al.*, 2020). Research findings support a potential miR-92a regulation of adipogenesis and subsequent weight loss.

The findings of the papers discussed are contradictory to what was found in this chapter. Research in non-athletic and/or obese individuals has reported higher levels of miR-92a are associated with lower brown adipose tissue activity (Chen *et al.*, 2016), which subsequently a lower activity is found in individuals with a greater body fat percentage (van Marken Lichtenbelt *et al.*, 2009; Vijgen *et al.*, 2011). Additionally, following bariatric surgery, a treatment for weight loss, there was a significant reduction in the levels of miR-92a-3p. Although these studies have not directly shown the predictive capacity of this microRNA and body fat loss the implied findings appear contradictory. One such reason could be due to a significantly lower brown adipose tissue activity reported in endurance-trained individuals compared to lean sedentary men (Vosselman *et al.*, 2015). This implies findings from non-athletic individuals are not suitable. More research is needed to understand the role of circulating miR-92a-3p as a predictor of body-fat loss in athletes and there is a suggestion that these same findings may not be directly applicable in sedentary or overweight individuals.

This study reported no association between basal ci-miRNAs and the EAET. Although previous studies have reported the ability of baseline and change ci-miRNA signatures to be representative of an individual's current aerobic performance status (Baggish *et al.*, 2011; Mooren *et al.*, 2013), limited research exists on the prediction of fitness change via either baseline or change miRNA signatures. Of the limited studies that have measured ci-miRNAs and fitness changes as a result of team sports training, Domańska-Senderowska *et al.* (2017) observed 2 months of in-season football training and Li *et al.* (2018) studied 3 months of in-season basketball training. Levels of ci-miRNA-29a were found to positively correlate with $\dot{V}O_2$ max after completion of the training cycle but, no findings were reported on baseline

relationships correlating to a change in fitness (Domańska-Senderowska *et al.*, 2017). Li *et al.* (2018) observed only the change in ci-miRNAs and no baseline levels were related to parameters of cardiovascular adaptation. These findings are in agreement with this study, which found no significant relationship between baseline ci-miRNA levels and improvements in the fitness test performance.

This is the first study to report the abundance of ci-miR-133b, -208b, and -221 may play critical roles in the regulation of muscle response in elite RU players. Specifically, the baseline levels of ci-miR- 208b-3p and ci-miR-221-5p displayed a negative correlation with the percentage change in maximal squat strength and ci-miR-133b showed a negative correlation with the percentage change in maximal bench press strength. These findings suggest lower levels of these miRNAs are indicative of greater responsiveness, suggesting a role in regulation and implying their utility as a biomarker for the prediction of muscle response. Interestingly, these relationships were stronger when relative to body weight suggesting they are stronger per unit of muscle.

Currently, only a few studies have specifically explored the relationship between resting miRNA expression and the variability in muscle response, but they have confirmed that miRNAs are associated with the variability of training-induced hypertrophic response (Davidsen *et al.*, 2011; Ogasawara *et al.*, 2016). Their findings, however, are not comparable to this study as the miRNA expression levels were obtained via muscle biopsies pre and post a training period; this would not apply to elite athletes. The concept provided in this study proposes a biomarker that only requires one blood sample to predict an individual's training-induced response before the training intervention commencing has greater applicability. Although Ogasawara *et al.* (2016) did observe differentially expressed miRNAs at baseline dependent on a high or low hypertrophic response, they found no difference for miR-133b, -208b or -221; this could be due to miRNA being measured in muscle and not blood, as well as the participants being untrained and not elite athletes.

Prior research into the relationship between ci-miRNA expression and muscular hypertrophy remains equivocal. Zhang *et al.* (2015) included both ci-miR-133b and ci-miR-208b in their analysis, but only observed ci-miR-499 was able to predict changes in knee extensor strength in older adults. They did however report a strong correlation between changes in strength with the percent changes in levels of miR-133b obtained via a muscle biopsy. It has been suggested that a difference in phenotype prediction applicability exists between the various

sampling methods (D'Souza *et al.*, 2019). A further reason for this contrasting finding is that the participants were all aged between 65-80 years, commonly as you get older, muscle is characterised by a decline in performance and a reduced adaptability stage of atrophy sets in, which could be reflected by the expression of ci-miRNAs (Vinciguerra *et al.*, 2010). Horak *et al.* (2018) observed student-athletes and reported plasma levels of ci-miR-93 were a predictor of responder status to isometric strength, however further comparison to this study is limited as they only chose a subset of miRNAs which did not include the candidate miRNAs in this study.

The three ci-miRNAs in this study that were significantly associated with changes in strength have all previously been proposed as mechanistic regulators of phenotype (miR-133b, -208b, -221) and reported specific acute regulation in response to either a strength endurance, muscular hypertrophy or muscular strength protocol (Cui *et al.*, 2017). It is difficult to understand the precise roles that ci-miR-133b, -208b and -221 have in skeletal muscle adaptation, specifically their influence on gene expression and protein translation. This is complicated further due to the multiple levels of control that are required to initiate a hypertrophic response, resulting in many potential intervention opportunities. Evidence does however highlight the regulatory roles of these specific miRNAs (ci-miR-133b, -208b and -221) in muscle development via proliferation, differentiation (Koutsoulidou *et al.*, 2011; Horak *et al.*, 2016; Liu *et al.*, 2018) and the control of muscle myosin content (McCarthy *et al.*, 2009; Kirby and McCarthy, 2013; Horak *et al.*, 2016), suggesting they do play a role in strength heritability and warrants further attention. Figure 46 displays the target of miR-133b crucial for its role in myogenesis.

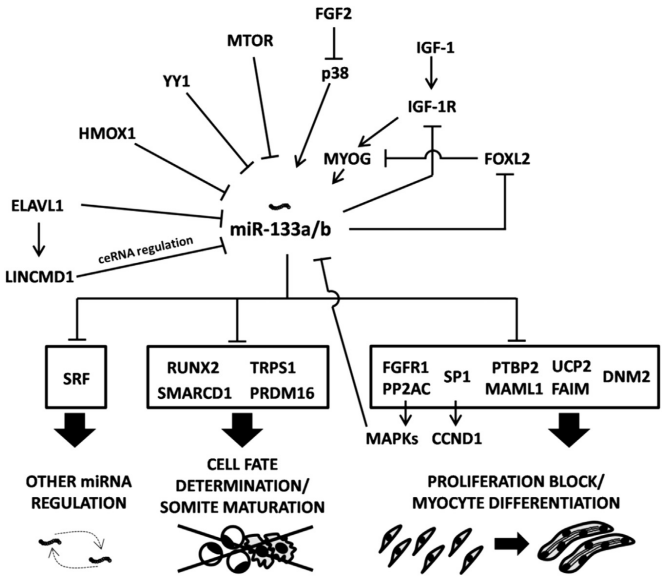

Figure 46 illustrates the factors that affect the expression of miR-133b and the targets of miR-133b related to the formation of muscle tissue. Arrows represent activation and blunt arrows represent inhibition (Horak *et al.*, 2016).

7.4.3 Limitations

As this study was undertaken in a professional setting, participant compliance was not an issue, however, the period of time between certain testing occasions may have been not long enough to induce a measurable difference.

Additionally, the tests chosen were solely rugby-performance based fitness tests and it may have been more applicable to examine specific physiological qualities, which may be more specific to how the athlete achieves performance.

It is plausible that the EAET used in this investigation lacks the sensitivity to detect a fitness change as a result of the preseason training. As this study was undertaken in an elite environment, it is a difficult balance to ensure a rugby-specific test, whilst ensuring a high degree of standardisation and reliability. It has been previously discussed that if a test is not entirely specific to the desired training-induced adaptation then it is not a surprise when clear relationships are not observed (McLaren *et al.*, 2018).

The number of participants although elite is low and larger studies need to take place to confirm the findings. Furthermore, unlike other studies that had a specific training programme, the participants in this study would have individually prescribed training programmes specific to their position and goals.

Although a high level of control around the blood collection was implemented, it proved more difficult to measure and account for the variety of outcome measures that may influence an individual's capacity to adapt. The study by Mann *et al.* (2014) discusses how measures such as sleep, diet, stress etc. may contribute to individual variation in training responses. Future studies should look at ways to account for these when wanting to understand training adaptation further.

7.4.4 Conclusion

In summary, this study demonstrated that there is large variability in training-induced responses in elite RU players following preseason training. Additionally, novel ci-miRNAs were shown to be associated with the magnitude of either a change in skinfolds (ci-miR-100-5p, -92a-3p) or strength (ci-miR-208b-3p, -221-3p, -133b). This suggests baseline ci-miRNA levels may be setting an individual's capacity to change via regulation of relevant signalling pathways. Although, currently there is not enough evidence to support the use of individualised training interventions based on ci-miRNA profiles to promote superior adaptations. Additional validation studies confirming the roles of these ci-miRNAs should be undertaken to investigate the mechanisms and what effects they may have on the individual adaptive training responses.

Chapter 8

8.0 General discussion

8.0 General discussion

The research presented in this thesis quantified the weekly external loads, examined the longitudinal training and competition demands and tracked diagnostic measures to uncover the tolerability of such demands in a cohort of elite RU players. This information is required to optimise training and enable effective load management throughout a season. Additionally, this thesis was the first to measure novel ci-miRNA biomarkers in elite players and determine their potential role in the observed variation in response to training. This chapter will consider and collate the findings of chapters 4 to 7 and incorporate the experiences I have had in an applied setting, as well as provide guidelines to practitioners, and future research directions.

8.1 Managing the individual; understanding the contextual variables

The measurement of training load and understanding the subsequent responses to this load is a key priority within elite RU (Quarrie *et al.*, 2017). Representatives of the game at the highest level have recognised the duty of care to monitor individual player loads (England Rugby, 2020, August 18; World Rugby, 2021).

Chapters 4 and 5 detailed the weekly training and match demands of commonly referred positional groups within a RU squad. The findings highlighted significant weekly locomotor (TD and THSR) and physical (AD and IS efforts) differences between positional groups and agreed with the findings of previous studies (Bradley *et al.*, 2015a; Dubois *et al.*, 2020a; Dubois *et al.*, 2020b). Collectively, the observed positional differences reflect the game activity differences and are a product of position-specific training. These findings are the first to provide an insight into the professional training and periodisation practices of an elite English Premiership team and support the findings from professional French rugby (Dubois *et al.*, 2020a; Dubois *et al.*, 2020b). The results presented underlie the necessity to prescribe training according to a player's position. It is therefore not appropriate to apply the same distances or physical efforts to every player as this could lead to under-loading or overloading of certain positions. This has important implications for the physical preparation (Mujika and Padilla, 2000) and injury risk to players (Cross *et al.*, 2016). These findings also promote the use of position-specific recovery strategies as backs experience higher explosive demands and forwards more contacts (Jones *et al.*, 2014).

A key consideration to managing the load of players is the time point within a season. The careful manipulation of volume and intensity throughout the phases of a season aims to

prioritise performance (Robertson and Joyce, 2018). Within team sports, the longitudinal monitoring of players throughout a season is widely adopted, yet periodisation strategies remain relatively unexplored (Ritchie *et al.*, 2016). The data presented in Chapter 5 reported no significant differences in TD, TLSR or THSR between preseason and the competitive blocks 1 and 2. These findings were in disagreement with Dubois *et al.* (2020b) who reported the greatest workload occurred in the preseason. This suggests a potential difference in preparation strategy, where the aim is to achieve a high level of workload in preseason that can be maintained throughout the season. However, the reluctance of professional teams to publish training data has resulted in limited knowledge of elite training practices, thus making it difficult to understand how best to optimise rugby-specific periodisation. Future research should focus on the strategies to understand an optimum training dose that can be applied to maintain fitness and performance whilst avoiding injury.

Tapering towards the end of the season is also an important strategy to understand. Findings from Chapter 5 reported a reduction in training load to ensure high performance at the latter stages of the season, although both performance and haematological parameters deviate significantly from baseline. This raises questions over whether this method achieved the desired performance/fatigue managing benefit. Appropriate rugby-specific training interventions need to be explored whereby at relevant in-season periods methods can be introduced to maintain/improve performance whilst minimising fatigue.

In Chapter 4, there was a large variation in the weekly demands for the external load metrics. These can be seen from the figures in the respective chapter where individual weeks are plotted alongside the average over the competition period. Due to the unpredictable nature of rugby matches and high between-player variability (McLaren *et al.*, 2016) coaches would individually prescribe training on a week-by-week basis. There are additional contextual factors that play a role in training design throughout a week (Figure 47). The impact of a player's match starting status has previously been observed in football (Anderson *et al.*, 2016), but this was the first study to quantify the differences in a Premiership rugby team. English Premiership rugby squads are large (~ 40) and only 15 of these players will start at a game. A challenge for practitioners is the need to manipulate the training load to take into account additional conditioning for players who have not played a match within that training week and also moderate for those who may have had a considerably high training and match week.

Therefore Chapters 4 and 5 provide important findings on the influence of match starting status on the weekly locomotor and physical demands.

The findings demonstrate clear differences in weekly locomotor and physical effort demands between differing match statuses. Starting players reported the highest running distances and the greatest PL and IS efforts compared to bench or non-squad players. In this regard, the data have important implications for the management of players and training programme design. Top-up conditioning is often prescribed to ensure compensatory adjustments to those who did not play or had limited game time. The findings provide evidence to advise what these top-up sessions need to comprise. Due to the higher reported weekly demands of 'starting players' they need to be appropriately managed, whether that be during the training week to ensure recovery or through appropriate squad rotation policies. Additionally, consideration needs to be given to players returning from injury. Practitioners rely on quantitative running load data to progress a player's return to sport protocol (Taberner *et al.*, 2019). The regular starting status will have an influence on the previous data and practitioners need to be aware of the increased training and match load associated with being a starter to avoid re-injury. Furthermore, players that may be on the fringes and fluctuate between different match statuses need to be monitored carefully from an injury risk perspective to avoid spikes in load (Gabbett, 2020).

This thesis provides insight into the quantification of elite rugby training and match demands and, takes into account integral influential factors such as position and match starting status. A one size fits all approach is simply not appropriate to the prescription of training when prioritising performance and the health of the players. Focusing on the quantification and prescription of training load, future research should:

- Explore rugby-specific periodisation techniques.
- Focus on the effects of match status on training load in a wider population of teams
- Understand the effectiveness of prescribing off-feet training top-ups to maintain fitness

Figure 47. A variety of contextual factors which influence a player's in-season training week.

8.2 Optimising the player monitoring process; aiding decision making

Appropriate monitoring of the training and match load that players are exposed to can offer insights into whether a player may be positively or negatively responding to the accumulated stresses of training and matches (Halson, 2014; Thorpe *et al.*, 2017). Regular quantification of the workload is fundamental to better understand this dose-response relationship (Dubois *et al.*, 2020b). Throughout the competition phase, professional rugby works on a week by week basis to promote consistent performance and to ensure recovery for an upcoming game. To achieve this players' will need their training load adjusted accordingly. A combination of the quantification of prior training load and diagnostic indicators of fatigue supports this decision-making process. The primary goal is to optimise training and recovery and maximise performance and health (Robertson *et al.*, 2017). With the findings from this thesis, I have suggested an approach to optimising player performance whilst minimising the risk of injury and fatigue associated with underperformance (Figure 48).

A key step to applying this programme is to ensure valid and reliable measures of fatigue in elite rugby players. In Chapter 5, a holistic approach was applied due to the multifaceted nature of fatigue; measures of self-reported wellbeing, performance and biochemical markers

were recorded. The performance measures of CMJ and RSI in Chapter 5 were consistent throughout the season, other than a reduction at the end of the season for CMJ. This supported previous findings where performance was observed throughout a rugby season (Dubois *et al.*, 2017; Dubois *et al.*, 2020b). Interestingly, however, significant differences were observed between high and low game exposure groups at time-points in-season 1 (a block of consistent premiership fixtures and typically limited squad rotation) and at the end of the season (Figure 32). This could suggest an impaired performance is associated with greater game minutes. Additionally, an association was observed between a reduction in weighted jump mean velocity performance and 4-week THSR distance and IS effort loads, which suggests these measures could identify players experiencing high loads. However, no clear evidence suggests which performance measures are superior at identifying a player responding negatively to an increased or adverse loading pattern. Future research should aim to determine the most valid and reliable performance measure of neuromuscular function. It should be stressed that this measure needs to be non-invasive and quick and easy to administer within an elite sports setting (Thorpe *et al.*, 2017).

Results of the self-reported wellbeing questionnaire demonstrated no differences throughout the season. A limitation to the study detailed in Chapter 5 was that it may not have been appropriate to average the weeks as part of a phase block in the season, as this could have decreased the sensitivity of this tool. Additionally, players' load may have been adjusted in response to reported results, providing the opportunity to enhance recovery throughout the week. Prior research has supported the use of regular custom questionnaires to understand a holistic approach to players' responses (Hills and Rogerson, 2018). It must be stressed that players are provided with the specific reasons they are performing these tests to ensure valid and reliable results. Therefore, both performance and self-reported wellbeing measures are included in the proposed training design plan (Figure 48). This approach is not uncommon in elite sports and would enable more support to the decision making process when prescribing and modifying training (Burgess, 2017).

Prior research has reported the difficulty in using biochemical markers for observing non-functional overreaching (Coutts *et al.*, 2007) in rugby league players. The incorporation of haematological analysis throughout a season was a novel concept, and the results of Chapter 5 were the first to report this data from elite Premiership rugby players. Findings demonstrated significant game exposure group differences for HCT and the significant

changes that occurred at the end of the season for CK, HB and MCV. The findings suggest these markers may support the need for rotational policies and periodisation but are unlikely to replace other common measures of training load management (e.g., wellbeing). Most of the aforementioned biochemical measures responded to acute rather than chronic measures of external load, which questions their utility as markers of chronic fatigue. Additionally, it would not be appropriate to take these measures weekly due to their invasive nature. Further research is needed to assess the usefulness of biochemical markers of chronic fatigue in RU. It could be proposed that infrequent testing provides limited information on the accumulated tolerability of training.

It is important to optimise consistent player monitoring without creating an additional player and staff burden. It is advised to select a minimum number of validated assessments required to understand injury risk, fatigue status and readiness to perform (Burgess, 2017). Data from Chapter 5 presents an opportunity to use GPS as a non-invasive and useful marker based on the observed associations between external workload metrics and biochemical measures. This could provide an indicator of fatigue status following both training and matches (Varley *et al.*, 2017). This would then enable a decision to be made on whether certain players may need training modification and informs individual recovery protocols straight after a game or high volume/intensity training day. Future research would benefit from uncovering further methods to measure the responses of players to optimise training prescription and personalise recovery strategies without the need for additional testing.

Effective player monitoring is a continuous, individualised, contextualised, evidence-based, decision-making process.

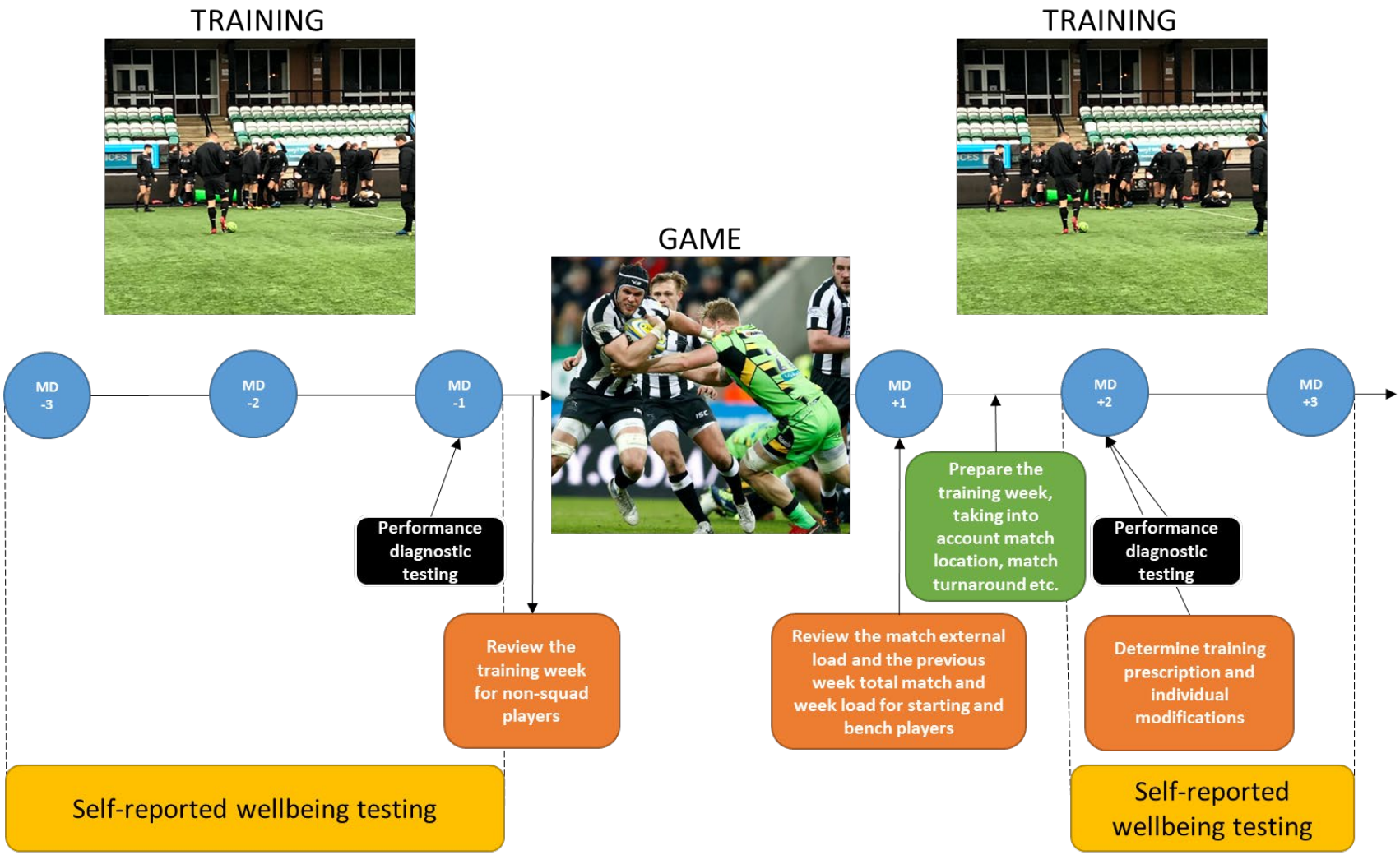

Figure 48. An approach to the weekly management of players adopting monitoring tools and GPS to ensure performance and minimise fatigue.

8.3 Can circulating miRNAs enhance the performance process in elite sport?

The studies featured in this thesis were the first to investigate the potential role of ci-miRNAs in elite RU players. Findings from this thesis have supported the value of ci-miRNAs as biomarkers and indicated the role they may play in phenotypic change. Chapter 6 demonstrated a significant difference in ci-miRNA-149-5p between forwards and backs and highlighted a possible role for 11 ci-miRNAs, which were correlated with anthropometric and performance variables important for rugby. Initial evidence from Chapter 7 suggests basal levels of ci-miR-100-5p and ci-miR-92a-3p may be useful biomarkers to predict body composition changes. In addition, upper and lower body strength changes were associated with basal ci-miRNA expression (ci-miR-208b-3p, ci-miR-221-3p, ci-miR-133b). These findings suggest that ci-miRNAs may play a role in regulating key gene networks for muscle strength adaptation.

Elite sport is continuously progressing and exploring methods to enhance the physical potential of athletes. New and exciting research is now exploring how genetic and epigenetic testing can complement existing training practices (Varley *et al.*, 2018). Within an elite sport setting the ability to identify individual training needs and prescribe specific training based on a player's epigenetic profile could have great benefits. This information would enhance individualised training. Previous research suggests this strategy of optimised training, promotes superior training improvements (Jones *et al.*, 2016; Simpson *et al.*, 2020). Additionally, ci-miRNA profiling could benefit a more efficient training process, understanding of injury susceptibility, athlete trainability, precise training top-ups and an improvement to in-season training. These benefits would not only improve a player's performance and availability but also their health.

Despite all this potential, I believe that whilst the findings of this thesis present support for a potential role of ci-miRNAs in an elite sport setting, currently support staff and elite athletes are not prepared for the use of ci-miRNA testing. Presently, more research is required to provide coaches and practitioners with evidence-based guidelines for its utility. Without these guidelines, there is the potential for negative ramifications in areas such as player recruitment and talent identification (Webborn *et al.*, 2015; Varley *et al.*, 2018). This raises both moral and ethical issues as coaches could ignore players that do not have a 'desired' epigenetic profile. Additionally, due to the lack of research in elite sport, studies are often underpowered due to small sample sizes of players in a club so definitive evidence is limited on specific profiles that

associate with superior performance traits (Bray *et al.*, 2009). To ensure confidence in the practice of ci-miRNAs in sport, education should be provided to coaches hence more research is needed. Future research should explore how the manipulation of training variables impacts ci-miRNA profiles particularly for those potentially labelled as 'non-responders' and explore how utilising ci-miRNA fits into an elite sport setting.

It is also important to highlight that these findings also have a wider application as regular exercise has proven to be very important for physical and mental health and the prevention or management of diseases (Williams *et al.*, 2021). Exploring phenotypic and exercise response associations with ci-miRNAs can also translate to the general population. This could enable better health care provisions; for example, the usage of resistance training to offset age-related physical decline and how appropriate exercise prescription could be as a preventative method of treatment. Research is increasing in areas of obesity (Parr *et al.*, 2016), diabetes, cancer treatment (Hagstrom and Denham, 2018) and the prediction of disease risk (Kho *et al.*, 2018).

8.4 An informed-practice approach

This thesis was conducted whilst I was immersed within the elite RU environment. The conversations I have had with coaches and practitioners have led me to explore questions that arose and ultimately influenced this body of work. A key aim of this thesis was to bridge the gap between applied sporting environments and research, to highlight the current challenges faced by practitioners and, to conduct applied research.

Currently, there is a lack of elite-level RU research on external training and match-play load, so little is known about the current practices and the challenges of this rapidly changing environment. Although prior research has discussed the common usage of Session Rating of Perceived Exertion in RU monitoring (West *et al.*, 2019), this tool is a measure of response to training load and provides no contextual element of the work done. Previous research, therefore, provides limited evidence on the actual work done by players. GPS metrics were confirmed as the most important monitoring tool by conditioning staff from all 12 English Premiership rugby teams (West *et al.*, 2019). Despite this, there is limited understanding of how these measures are used in individual training prescription and throughout a season. Prior research in football has discussed the reluctance of professional teams to share their practices due to the prospect of enabling rival teams with a competitive edge (Akenhead and

Nassis, 2016). I believe it is crucial to work in conjunction with teams and practitioners to enable research that connects current practice with scientific evidence (Buchheit, 2017).

Throughout this thesis, a key emphasis has been placed on the individual responses of players and highlighting the variable nature of competitive weeks. I have tried to promote this via visualisations and research into the novel ci-miRNAs, which specifically focus on individuality. Too often within research, this viewpoint can be neglected. Population-based analysis is used in most sports and exercise science literature. This method does not enable the identification of individual responses (Timmons, 2010; Mann *et al.*, 2014; Buchheit, 2016). Additionally, the graphical representation of this data is typically presented as a bar graph which hides the distribution and response of each individual (Halperin *et al.*, 2018). Therefore, the response to an exercise intervention is often represented as a typical response for individuals in order to determine its overall efficacy. Ultimately this method fails to highlight the wide range of individual training-induced responses, often referred to as the phenomenon that is 'high responders' and 'low responders' (Mann *et al.*, 2014). Figure 49 highlights how in response to a training intervention there can be large inter-individual variations that are masked when the focus is the 'average' benefit within a population. Future research should adopt some of these methods to enable a clearer picture, especially in an elite team sport where individuals are at the centre of the process.

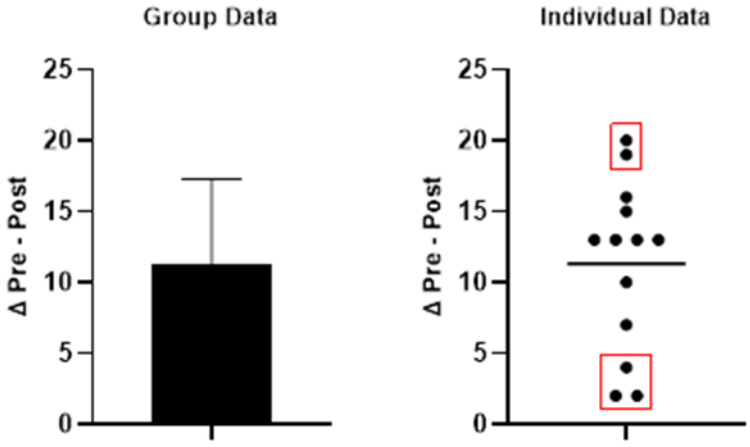

Figure 49 illustrates how variations in graphing can provide a better representation of the data. The mean change in both graphing options is the same, however 'Group Data' fails to represent the individuals that did respond to an intervention and others didn't. The red boxes highlight the extremes as high and low responders.

8.5 Limitations of the studies presented in this thesis

There were several limitations throughout this thesis and these were discussed in individual chapters (Chapters 4 - 9). Primarily the key limitations of this research were the sample size and the usage of only one team. However, due to the scarcity of data in elite RU, this information will greatly benefit and progress the area. The findings in this thesis are principally observational studies, which require larger team reviews and more control studies to support the evidence base.

8.6 Future studies

Despite the limitations of this programme of work, the findings provide novel insights into season long training prescription and the player responses. Additionally, this is the first work to explore and understand epigenetic regulation in a cohort of elite rugby union players. It is no doubt however that these outcomes raise further questions and warrant further investigation.

i) Research has shown how prior training load may impact a variety of monitoring markers, however, the additional effect on rugby union match performance remains unexplored. Knowledge of this would provide further information on the impact of training prescription on not just an individual's fatigue level but also on the potential effect to match performance.

ii) Findings from Chapters 4 & 5 report season long training prescription via GPS distance and effort loads. This evidence is rarely provided in research due to the difficulty to obtain this information, but the contextual findings are key to pushing forward in this area. Studies observing more than one team or, more studies providing a measure of external load in addition to an S-RPE volume would help develop periodisation techniques and weekly structures. This information would help to optimise and manage the training loads of elite rugby athletes.

iii) The ability to control for a multitude of factors that may influence an individual's responsiveness to training is difficult in an elite sports setting and is discussed as part of the limitations section in Chapter 7. A similar study design with a cohort of well trained individuals who are not in an athletic programme would be beneficial to determining the unique effect that ci-miRNAs have on physiological adaptations.

iv) As discussed ci-miRNAs have great potential to integrate into an athletic programme to inform individual training prescription and specific adaptation. Although the outcomes of Chapters 6 & 7 were very interesting and novel, the evidence is not enough to support the use of ci-miRNAs in personalising training programmes. Currently, within research, the term 'non-responder' is used if an individual does not adapt with sufficient magnitude. With the current findings it could be assumed if an athlete does not possess a specific ci-miRNA profile they will not adapt to the training, however, this is assuming that the training is appropriate and adequate to promote a response at an individual level. The research would therefore benefit from studies exploring further if it is the training that is not appropriate for the individual rather than the individual thought to not have the appropriate genetic material to adapt. Again, other factors for individual variation need to be controlled such as sleep and diet.

8.7 Conclusions

The findings of this thesis present longitudinal data for an entire season of elite RU. Results have quantified the training and match loads undertaken within a professional Premiership club and illustrate the positional, match status differences and variances throughout the season. The data adds to a limited literature base and explores the potential physiological and well-being consequences of undertaking the required demands of being a professional player. Recommendations have been provided to ensure effective individual monitoring and training prescription can be utilised and, has highlighted the need to pay particular attention to those players with a high game exposure. Novel ci-miRNA insights were also presented. Whilst the findings support a current understanding of the role they play in the regulation of gene expression, more research is needed to support their use in personalising training programmes.

Chapter 9

9.0 References

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Chapter 10

10.0 Appendices

Appendix A: Participant information sheet for all studies (Chapters 4, 5, 6 and 7)

Biomarkers and their Assistance in Monitoring Individual Athlete Status in Professional Rugby Union to Inform Decision Making and Improve Athlete Wellbeing

Information Sheet for Study Participants

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You are being invited to take part in a research study. Before you decide whether or not you wish to take part it is important that you understand why the research is being done and what it will involve. Please read this information carefully and discuss it with others if you wish. Please do not hesitate to contact us if anything is unclear, or if you require more information. Take time to decide whether or not you wish to take part. Details about the conduct of the study are also explained which will help you to decide whether or not you wish to take part.

What is the purpose of this study?

The purpose of this study is to determine whether measuring certain markers of physiological stress in your blood (particularly changes relating to hematology, hormones and gene expression) are sensitive to changes in training load and training demands throughout specific elements of a season and therefore useful for monitoring your response to different types of training. Three studies will be running throughout the season to determine this. A season long study to observe the evolution and change in biomarkers and physiological tests as a response to training load. A pre-season study to observe how a pre-season prepares you for the competitive season and again your responses to the weekly training load. Finally, a prediction study, looking at how well baseline microRNA analysis can predict performance change. These studies hope to enable a more precise prediction of an athlete's response to a specific training loads enabling better athlete management with the aim to increase athlete wellbeing and reduce injuries.

Why have I been chosen?

You have been chosen because you are part of the Newcastle Falcons first team squad, and the aim this study is to investigate physiological stress in elite Rugby Union players.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to do so, you will be asked to sign a consent form. However, you will still be free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

We will than take a small amount of blood from your arm at time points throughout the season from June 2018 to May 2019.

What will I have to do?

You will be given this information sheet and briefed on the full study procedures at which point you will be invited to ask any questions or raise any concerns. Your participation will then depend on the satisfactory completion of a medical and exercise history questionnaire. If you are considered eligible and agree to take part, you will sign an informed consent form. You will then have to see one of the research team on the pre-determined testing days before training to have a small blood sample taken from a vein in your arm (24 ml).

Blood samples required:

-Pre-season study: *5 samples (1 baseline (start of preseason), 1 midway through preseason 1 following a post fitness test start and end of preseason and 1 final rested sample end preseason)*

-Season long study: *5 samples (samples will be used from the pre-season study but other key time points are, 'mid-season', 'less competitive period', 'period of intense competition' and 'penultimate stage of the season')*

What are the possible benefits of taking part?

You will learn how your body responds too and recovers from different training loads throughout a season. For example, you will learn whether your body is more tired after high intensity training periods or at the end of the season for example. These measures will be objective, that is, they will tell us how your body is feeling (physiologically) without having to rely on subjective ratings of wellbeing, whereby you inform your coach how you feel (psychologically). We can then relay this information to the high performance team who can use it to better design your training to your needs; e.g., do you need more rest at certain periods of the season. Ultimately, the aim is to help you perform consecutively and recover better. Additionally, to this the aim is to determine what GPS variables contribute most to fatigue during a game or training so we can better understand individual response to specific training loads enabling better decisions on athlete management with the aim to increase athlete wellbeing and reduce injuries.

Will my participation involve any physical discomfort?

You may experience some mild discomfort when blood samples are being taken. This is not a painful procedure but you will feel a slight prick when the needle is inserted and may have

some very light, small bruising following, which is a completely normal response. However, all samples will be collected by appropriately trained personnel to minimize this possibility.

What will happen if anything goes wrong?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you wish to make a complaint you can contact the principal investigator Joe Kupusarevic whose details are on the front page on this information sheet.

Will my taking part in this study be kept confidential? Yes. All information that is collected about you during the course of this research will be kept strictly confidential.

What will happen to the study results?

The overall results of the study may be presented at scientific meetings or published in a scientific journal. All data is anonymized and you will not be identified in any of these presentations or publications.

Will I be reimbursed for my time?

No, there will be no reimbursement for your time.

Contact for further information

If you have any further questions, then please contact Joe Kupusarevic

Telephone: 07772321132
Email: J.Kupusarevic2@newcastle.ac.uk

And finally…

Thank you for having taken the time to read this information sheet and your interest in the study. If you do decide to take part in the study, you will be given a copy of the information sheets and a signed consent form for you to keep.

Appendix B: Informed consent form for all studies (Chapters 4, 5, 6 and 7)

Newcastle University

ID:_____

CONSENT FORM

I agree to participate in the study: **'Biomarkers and their Assistance in Monitoring Individual Athlete Status in Professional Rugby Union to Inform Decision Making and Improve Athlete Wellbeing'** being carried out by Newcastle University.

- I have had adequate time to consider whether or not to take part in the study ☐
- I understand that the data collected for this study will be stored in a secure location in the Human Nutrition Research Centre at Newcastle University. ☐
- I understand that the data will be used only for research purposes. ☐
- I understand that I will not be mentioned by name on any documents or in any presentations about the research. ☐
- I understand that I can withdraw from the study at any time without needing to give a reason. ☐
- Withdrawing from the study will not affect any services I am receiving now or might receive in the future. ☐

Please delete as appropriate - I Would/ Would not like to take part in the sub-study which requires collection of blood samples.

Signature of participant…….....

Name (in capitals) ……Date…….

Contact details…….

Date of Birth: ……

Signature of researcher……

Name (in capitals).....

Appendix C: Health screening form for all studies (Chapters 4, 5, 6 and 7)

HEALTH-SCREENING QUESTIONNAIRE

Participant ID: ……

Name:

Date: ……

Date of birth: ……

All information within this questionnaire is considered confidential.

Where appropriate please circle your selected answer.

1. How would you describe your current level of activity?
   Sedentary / Moderately Active / Highly Active

2. How would you describe your current level of fitness?
   Very Unfit / Moderately Fit / Trained / Highly Trained

3. How would you describe your current body weight?
   Underweight / Ideal / Slightly Overweight / Very Overweight

4. Smoking Habit: -
   Currently a non-smoker Yes / No
   Previous smoker Yes / No
   If previous smoker, how long since you stopped? ……Years
   Regular smoker Yes / No of …… per day
   Occasional smoker Yes / No of …… per day

5. Alcohol Consumption: -
   Do you drink alcohol? Yes / No

   If yes then do you - have an occasional drink Yes / No
   have a drink every day? Yes / No
   have more than one drink per day? Yes / No

6. Have you consulted your doctor within the last 6 months?
   Yes / No

   If yes, please give details …….

7. Are you currently taking any medication (including anti-inflammatory drugs)?
   Yes / No

   If yes, please give details …….

8. Do you, or have you ever suffered from:-
   Diabetes Yes / No
   Asthma Yes / No
   Epilepsy Yes / No

Bronchitis	Yes / No
Elevated cholesterol	Yes / No
High Blood Pressure	Yes / No

9. Do you suffer from, or have you ever suffered from any heart complaint or pains in your chest, either associated with exercise or otherwise?
   Yes / No

10. Is there a history of heart disease in your family?
    Yes / No

11. Do you feel faint or have spells of severe dizziness when undertaking exercise or otherwise?
    Yes / No

12. Do you currently have any form of muscle joint injury?
    Yes / No

    If yes, please give details ……

13. Have you ever suffered from any knee joint injury or thigh injury?
    Yes / No

    If yes, please give details ……

14. Do you currently take any form of nutritional supplement (e.g. creatine, whey and casein protein, HMB, etc)?
    Yes / No

    If yes, please give details ……

15. Do you have any food allergies or intolerances that you know of?
    Yes/No

    If yes, please give details ……

16. Are you currently on any special diet or have dieted in the past? (e.g. weight loss/ high protein)
    Yes/No

    If yes, please give details ……
    ……

17. Is there anything to your knowledge that may prevent you from successfully completing the test that has been explained to you?
    Yes / No

    If yes, please give details ……..

Please provide any further information concerning any condition/complaint that you suffer from and any medication that you may be taking by prescription or otherwise.

……
……

Is the participant eligible?

Yes / No

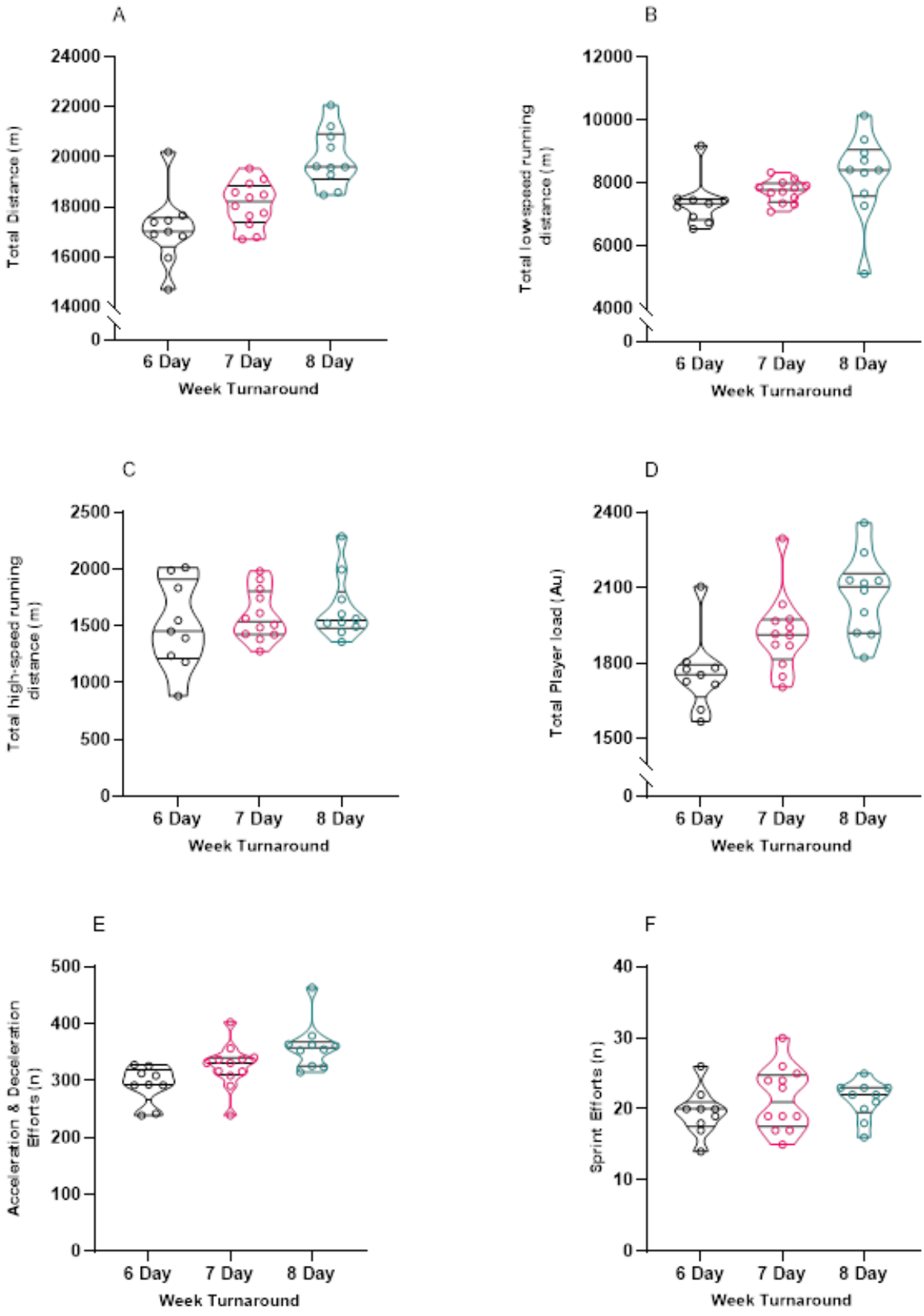
Name of participant: …….

Signature of participant: ……. Date: ……..

Name of researcher: …….

Signature of researcher: …… Date: ……..

Appendix D: The influence of game turnarounds on external training load (Chapter 4)



Mean ± SD violin plots for, A - total distance, B - total low-speed distance, C - total high-speed distance, D - player load, E - acceleration and deceleration efforts, F - sprint efforts.

Appendix E: Daily wellness questionnaire that players were required to complete prior to training (Chapter 5).

Question
How is your upper body today?*
How is your lower body today?*
How many hours did you sleep last night?*
How well did you sleep last night?*
How is your mood today?*
How are your energy levels today?*
How motivated do you feel to train today?
How has your diet been during the previous 24 hours?
How are your stress levels today?
How has your previous days training been?
Do you have any muscle pain or tightness this morning?
Describe your muscle pain or soreness
Describe the location of your tightness or pain

Note: * represents the questions that were included in the wellbeing analysis

Appendix F: Relationships between anthropometric measurements and ci-miRNAs (Chapter 6).

ci-miRNA	Anthropometric - related					
	Height (cm)		Body mass (kg)		Sum of eight skinfolds (mm)	
	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*
hsa-miR-1-3p	0.01	0.670	0.04	0.421	0.01	0.661
hsa-miR-100-5p	0.08	0.245	0.11	0.172	0.06	0.308
hsa-miR-126-3p	0.01	0.645	0.03	0.461	0.12	0.166
hsa-miR-133a-3p	0.00	0.807	0.09	0.216	0.05	0.352
hsa-miR-133b	0.01	0.637	0.07	0.259	0.02	0.554
hsa-miR-144-3p	0.06	0.320	0.01	0.711	0.14	0.133
hsa-miR-146b-5p	0.00	0.935	0.03	0.481	0.05	0.380
hsa-miR-149-5p	0.00	0.858	**0.27**	**0.049**	**0.36**	**0.018***
hsa-miR-150-5p	0.04	0.449	0.02	0.580	**0.26**	**0.030***
hsa-miR-18b-5p	0.01	0.757	0.01	0.753	0.01	0.656
hsa-miR-206	0.00	0.852	0.03	0.459	0.00	0.799
hsa-miR-208b-3p	0.24	0.052	0.00	0.870	0.03	0.553
hsa-miR-221-3p	**0.34**	**0.011***	0.07	0.291	0.03	0.507
hsa-miR-301a-3p	0.00	0.910	0.00	0.791	0.03	0.458
hsa-miR-421	0.13	0.135	0.00	0.867	0.00	0.864
hsa-miR-885-5p	0.16	0.126	0.08	0.291	0.07	0.327
hsa-miR-92a-3p	0.02	0.625	0.04	0.439	0.07	0.307

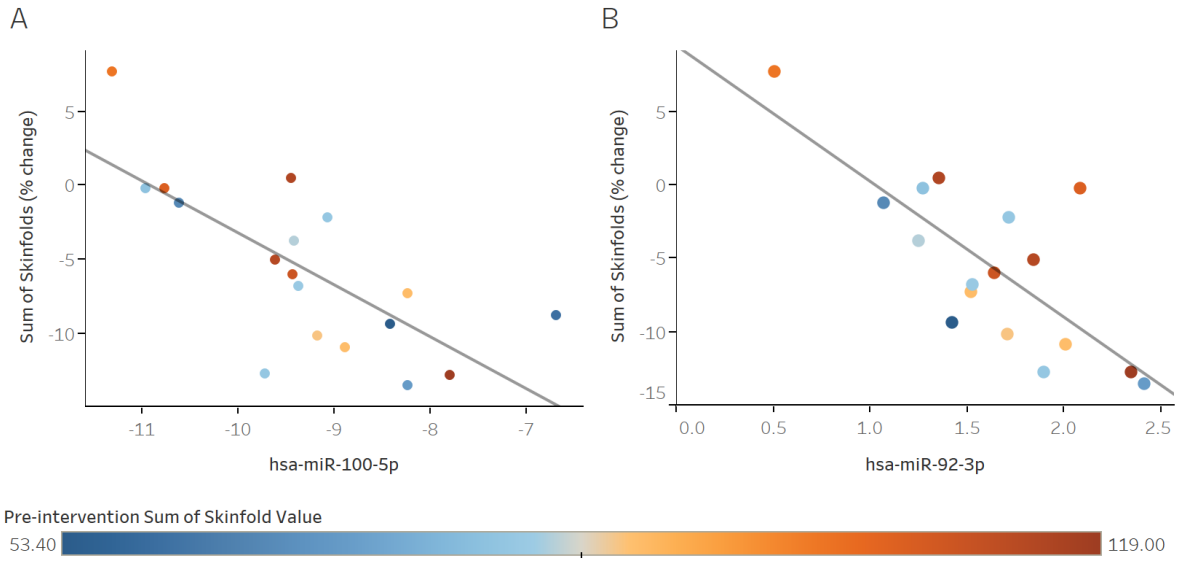
* Significant $P < 0.05$.

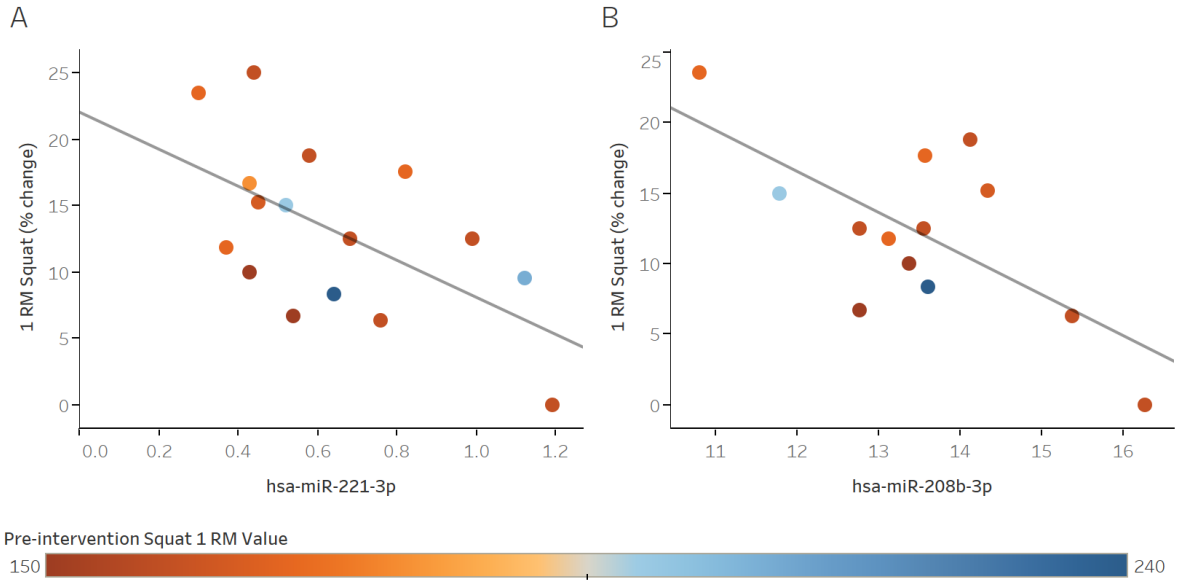
Appendix G: Relationships between performance-related measurements and ci-miRNAs (Chapter 6).

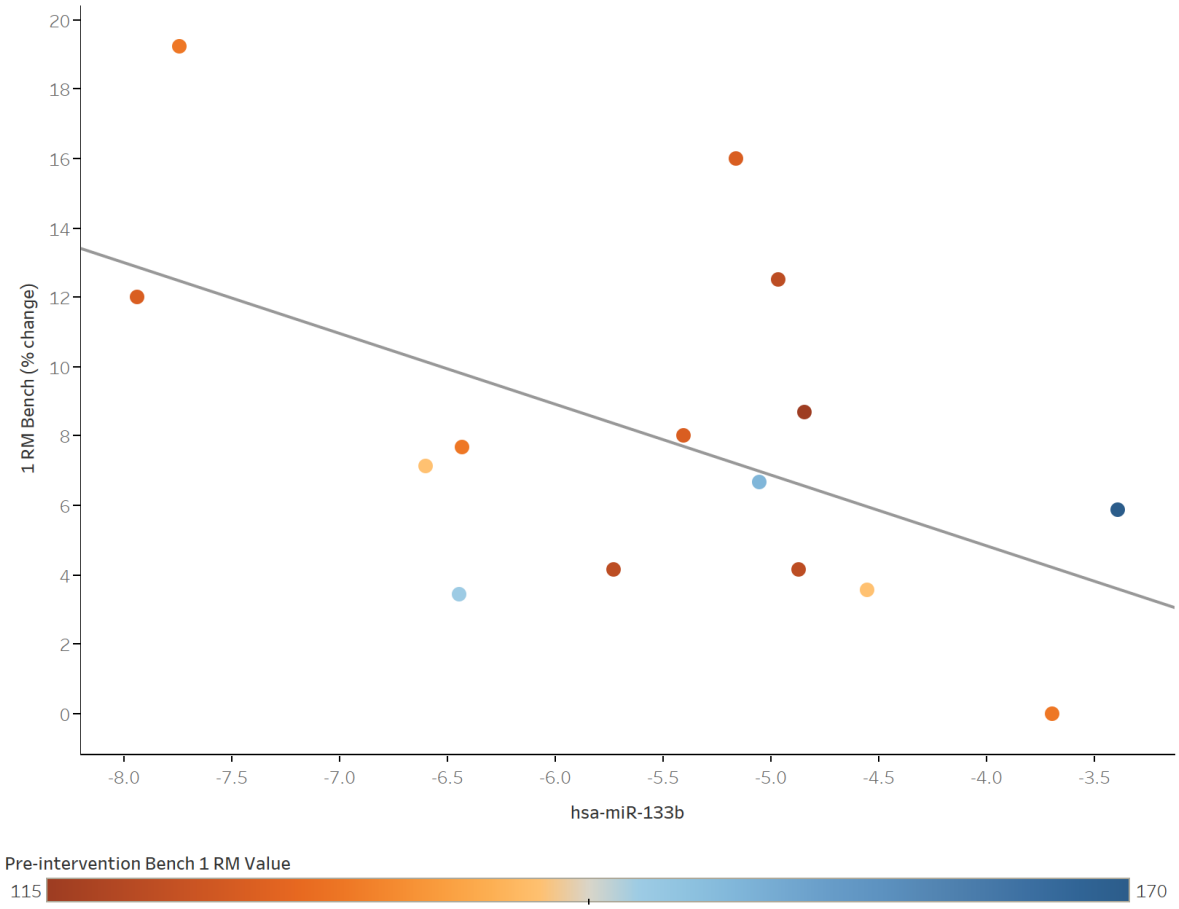
ci-miRNA	Fitness - related				Strength - related				Speed and Power - related									
	EAET (s)		Bronco (s)		1RM Squat (kg)		1RM Bench (kg)		10 m sprint (s)		CMJ (cm)		SJ (cm)		40 kg jump velocity (m·s)		RSI (cm·s^{-1})	
	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*	R^2	*P Value*
hsa-miR-1-3p	0.00	0.854	0.03	0.499	0.16	0.097	0.04	0.459	0.07	0.280	0.07	0.279	0.06	0.309	0.20	0.060	0.00	0.984
hsa-miR-18b-5p	0.15	0.149	0.01	0.667	0.05	0.396	0.00	0.843	0.06	0.324	0.01	0.635	0.02	0.566	0.17	0.087	0.04	0.520
hsa-miR-92a-3p	**0.21**	**0.019***	0.22	0.068	0.11	0.186	0.17	0.111	0.00	0.942	0.07	0.290	0.03	0.511	0.01	0.777	0.04	0.555
hsa-miR-100-5p	0.00	0.985	0.02	0.628	0.07	0.281	0.07	0.313	0.00	0.781	0.02	0.533	0.02	0.580	**0.27**	**0.028***	0.04	0.505
hsa-miR-126-3p	**0.31**	**0.038***	0.02	0.579	0.03	0.529	0.03	0.513	0.05	0.386	0.01	0.671	0.00	0.951	0.06	0.343	0.01	0.806
hsa-miR-133a-3p	0.00	0.889	0.05	0.407	**0.26**	**0.031***	0.10	0.225	0.02	0.608	0.04	0.405	0.03	0.481	0.16	0.098	0.01	0.777
hsa-miR-133b	0.00	0.960	0.04	0.421	0.20	0.063	0.07	0.305	0.05	0.396	0.07	0.299	0.05	0.367	0.20	0.060	0.00	0.850
hsa-miR-144-3p	0.02	0.664	0.01	0.769	0.02	0.584	0.01	0.746	0.00	0.908	0.00	0.946	0.01	0.649	0.14	0.132	0.08	0.374
hsa-miR-146b-5p	0.13	0.187	0.00	0.914	0.06	0.357	0.07	0.337	**0.43**	**0.004***	0.06	0.323	0.10	0.195	**0.26**	**0.032***	0.03	0.589
hsa-miR-149-5p	0.30	0.082	0.02	0.613	0.15	0.176	0.13	0.219	**0.31**	**0.040***	0.04	0.482	0.10	0.261	0.00	0.863	0.02	0.720
hsa-miR-150-5p	0.05	0.430	0.00	0.966	0.12	0.165	0.06	0.346	0.00	0.988	0.14	0.145	0.04	0.438	0.10	0.213	0.20	0.146
hsa-miR-206	0.01	0.699	0.04	0.450	**0.29**	**0.021***	0.03	0.534	0.00	0.798	0.04	0.399	0.07	0.296	0.01	0.761	0.05	0.466
hsa-miR-208b-3p	0.01	0.734	0.00	0.932	0.00	0.932	0.03	0.553	0.09	0.282	0.00	0.864	0.00	0.868	0.05	0.416	**0.43**	**0.027***
hsa-miR-221-3p	0.00	0.995	0.00	0.948	0.02	0.598	0.03	0.546	0.02	0.545	0.03	0.499	0.11	0.196	0.03	0.540	0.10	0.325
hsa-miR-301a-3p	0.07	0.334	0.00	0.843	0.02	0.571	0.04	0.423	0.20	0.060	0.01	0.661	0.00	0.814	0.12	0.157	0.01	0.768
hsa-miR-421	0.00	0.905	0.09	0.234	0.03	0.489	0.00	0.852	0.00	0.830	0.22	0.050	**0.22**	**0.048***	0.01	0.662	0.00	0.915
hsa-miR-885-5p	0.22	0.108	0.01	0.721	0.03	0.540	0.01	0.690	0.05	0.411	0.02	0.620	0.01	0.669	0.03	0.540	0.16	0.223

* Significant P < 0.05.

Appendix H: Figures exploring the possibility that ci-miRNA abundance is influenced by having a greater sum of skinfolds or being stronger but, no patterns are visually apparent






20
18
16
14
12
10
8
6
4
2
0
1 RM Bench (% change)
-8.0
-7.5
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
-4.0
-3.5
hsa-miR-133b
Pre-intervention Bench 1 RM Value
115
170