Mapping Adaptation In Football

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Abstract

Adaptation to the demands of football is a complex process. This is due to the intermittent and unpredictable nature of the game, which has numerous implications on the body (Drust et al., 2007; Reilly, 1997). Furthermore, the body is a complex biological system, where many different tissues respond in unique ways to ensure the body can cope with the demands placed upon it. These responses are also complex and begin with seemingly minor events that occur on the molecular scale. The magnitude of these responses will determine changes further up the system at the subcellular, cellular, tissue, organ system and organism scale (Wisdom et al., 2015). Consequently, practitioners and researchers attempting to understand adaptation in football are faced with a challenge that cannot be resolved with narrow views of adaptation. Broad views of adaptation are needed to capture the wide-ranging implications that occur across numerous levels in the body to this complex game.

The aim of study 1 was to explore how adaptation has been measured in football players. A scoping review was conducted. A scoping review was chosen over a systematic review as it was deemed more suitable for the exploratory nature of the study (Tricco et al. 2016; Arksey and O’Malley, 2005). In total 461 papers were analysed. From these papers, information was extracted that identifying the stimuli implemented, the study durations and frequency of measurements employed, the nature of the response to the stimuli, the outcome measures used to assess the response and the level these responses are measured at. This information was used to demonstrate that approaches to measuring adaptation seem to be dictated by pragmatic and conceptual views of adaptation, with the former approach being more common than the latter in football research.

Study 2 aimed to explore the potential for football training during the in-season phase to stimulate responses in the mechanical and physiological load-adaptation pathways. This study was an attempt to present the football stimulus in a broader way to reflect the potential implications of training on these two load-adaptation pathways. This was achieved by retrospectively classifying training sessions as 'physiologically' or 'mechanically' intense using positional GPS training data and a novel method for classifying intense sessions. This was conducted using positional data from a single season in an U23 squad in an elite football academy. For the purposes of this study it was proposed that the intensity of the training stimulus may provide the greatest opportunity for mechanical and physiological adaptations. Considering the highest volume of training players will face is during the pre-season phase, it was suggested that if physiological or mechanical adaptations were to occur in the in-season phase, it would be a product of intensity as opposed to volume.

Positional GPS data was captured across an in-season phase from an U23s squad in an elite football academy. This positional data was used to categorise and identify football training sessions that could be considered intense from a physiological and mechanical standpoint using different GPS metrics. The sessions with the highest mechanical & physiological outputs were identified for each playing position (Full backs, Centre Backs, Midfielders and Forwards). Different GPS-derived external load variables were used to categorise demands as either mechanical or physiological based on their potential to stimulate responses in these load-adaptation pathways. Sprint distance was selected as an indicator of 'physiological' load whilst the number of high-intensity accelerations and decelerations was selected as the indicator of 'mechanical' load. The 75th percentile was chosen as a separation line between intense and ‘baseline’ sessions. Therefore, any training session with an average sprint distance above the 75th percentile was considered a ‘physiological’ session.

Results demonstrated that sessions deemed intense from a mechanical and/or physiological perspective, had significantly higher demands than non-intense training sessions. Furthermore, non-intense training sessions occurred the most across the season. Thus, these significant differences may indicate that these intense sessions are different enough from more commonly occurring sessions and as such, have the potential to stimulate mechanical or physiological adaptive responses. Moreover, when these intense sessions were mapped across the season, it was apparent that players were exposed to intense mechanical or physiological sessions at different times during the season based on their playing position. Consequently, broader approaches to mapping responses are needed to understand the potential implications of training on mechanical and physiological adaptations in players of different playing positions during the in-season phase.

Study 1 acknowledged that the pragmatic views of adaptation generally favoured are understandable due to the difficulties of using time-consuming and invasive methods to measure adaptation in football players. As a consequence, many measures relating to adaptation are taken at the organism level. However, as alluded to throughout the thesis, adaptation occurs across all levels of organization in the body. Thus, more detailed insights into the processes that drive adaptive responses across numerous levels may be gained by taking measurements at the cellular level. Therefore, identifying ways to capture comprehensive insights into the complex responses to football at this level, using pragmatic approaches, is warranted.

Thus, study 3 acted as a pilot study that aimed to explore the sensitivity of change in the metabolomic response to a single intense training session in football using a novel methodology which involved capillary blood samples taken from the fingertip. These samples were analysed using untargeted metabolomics which provided insights into the energy metabolism pathways responsible for supporting the demands of a single football training session. Overall, this study demonstrated that this method was sensitive enough to detect changes in several metabolites related to energy metabolism, namely tRNA biosynthesis, glyoxylate and dicarboxylate metabolism, glycolysis, and gluconeogenesis following an intense football training session. From a methodological standpoint, this method may be an attractive avenue for future research due to the ease and speed at which these samples can be collected in the field, as well as the insights into the metabolic response to football that can be obtained.

The findings and approaches used throughout the thesis influenced the aim of Study 4. This study aimed to provide a protocol for an observational study in elite football that maps responses across multiple levels of organisation in both the physiological and biomechanical load-adaptation pathways. The aim of this study was to provide a protocol for an observational study in elite football that maps responses across multiple levels of organisation in both the physiological and biomechanical load-adaptation pathways. This study protocol demonstrates the detail required when mapping the stimulus, as well as the tissues that need to be observed and the level responses need to be measured at. This approach can help characterise the stimulus-response relationship in more detail and provide insights into the potential mechanisms behind changes in performance at the organism level. The key takeaways from this chapter are that the stimulus needs to be well defined and in detail. This detail will help characterise the stimulus-response relationship between training and responses measured across multiple levels of organization. Outcome measures need to be measured across multiple levels of organisation to help provide insights into the mechanisms behind changes on each level as well as the potential relationship between changes across levels. Ultimately if practitioners and researchers desire to be able to understand how individuals will respond to training, these types of comprehensive approaches are required. This approach also acts as a template whereby practitioners and researchers could ‘plug in’ different outcome measures.

Summary

In summary, this research program provides a novel approach to mapping adaptation in football. This work comprehensively examined how adaptation is measured in football and explored ways to progress in this research area. To the author’s knowledge, no study has attempted to map GPS training loads across a season from a physiological and biomechanical standpoint. This study revealed that training may stimulate responses in distinct load-adaptation pathways based on positional demands during the in-season phase. However, greater insights into the internal responses to training are needed to confirm this. Study 3 was, to the authors knowledge, the first study to assess the metabolomic response to football activities using capillary blood samples. This method has the potential to be a pragmatic way to comprehensively map responses to football activities. The final study in this chapter produced a protocol for comprehensively mapping adaptation across numerous tissues, levels, and time courses in football. Overall, it is hoped that the findings from this research project and level of originality will help practitioners and researchers in football think more broadly about adaptation to design more insightful studies in the future.

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“Learning is a gift, even if pain is your teacher” – Source Unknown.

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Contents

List of Abbreviations

Hi A/Ds	High-Intensity Accelerations & decelerations
CSA	Cross-Sectional Area
CB	Centre Back
FB	Full Back
MID	Midfielder
FOR	Forward
GPS	Global positioning systems
HR	Heart Rate (beats.min-1)
M	Metres
MD	Matchday
RPE	Rating of perceived exertion
HSD	High-Speed Running Distance
SD	Sprint distance
SSG	Small-sided games
TD	Total Distance
V̇O2max	Maximal oxygen uptake (L.min-1)

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Football is a game composed of varied actions that occur intermittently and unpredictably during the game. These actions require the coordination of multiple physiological systems (Drust et al., 2007; Reilly, 1997). Outperforming an opponent requires a training process that develops these physiological systems to support and augment the ability of the players to perform these actions. To develop these systems, it would therefore seem important to understand the implications of football on their adaptive processes. Understanding these adaptive processes requires a broad holistic view of adaptation.

These adaptive implications have been considered to occur in two distinct load-adaptation pathways. These pathways are considered either 'physiological' or 'biomechanical' (Vanrenterghem et al., 2017). Adaptations in the physiological load-adaptation pathway are those that augment the function of the cardiovascular and neuromuscular systems (Stølen et al., 2005; Christensen et al., 2011; Ali and Farrally, 1991; Bangsbo, 1994). These adaptations occur as a consequence of the running demands of the game. On the other hand, adaptations in the biomechanical load-adaptation pathway are related to those that augment the tissues of the musculoskeletal system (Hagman et al., 2018; Nicholas et al., 2000; Wragg et al., 2000; Vanrenterghem et al., 2017; Reilly, 1997). The adaptations occur due to high-force actions such as changes of direction and the subsequent high impact and ground reaction forces (Verheul et al., 2019).

Therefore, the training process in football will need to include activities that stress the physiological and biomechanical tissues and systems (Morgans et al., 2014). Research would suggest that the stimulus elite football players are exposed to leads to greater physiological and biomechanical adaptations when compared to amateur players (Rampinini et al., 2010; Dellal et al., 2011; Mohr et al., 2003; Beato et al. 2021; Carvalho et al., 2016; Murtagh et al., 2018). However, a complex biological system adapts because of changes that occur at the cellular, tissue, organ, organ system, and organism levels. Consequently, as part of a more holistic view of adaptation, the adaptive processes within the physiological and biomechanical load-adaptation pathways need to be explored across these levels.

1.2 Aims And Objectives

The main aim of this research programme was to critically evaluate approaches to investigating adaptation in football-related research and explore novel approaches to mapping adaptation.

The main objectives for achieving these aims are to:

1. Show how adaptation is measured in football and demonstrate the complexity associated with measuring adaptation.
2. Show how the physiological and mechanical load-adaptation pathways are impacted during a season by football training using external and internal load.
3. Show how metabolomics can capture the cellular responses involved in energy metabolism to a football training session.
4. Develop a comprehensive yet pragmatic protocol for mapping adaptation in football.

CHAPTER TWO

REVIEW OF THE LITERATURE

The following literature review will identify the match demands of football and the potential implications of these complex demands on adaptation, training design, and the monitoring of adaptive responses to training. Adaptations related to the cardiovascular and musculoskeletal system will then be explored as part of a broad view of adaptation. Finally, the implications of this broad view of adaptation will be framed in terms of their relevance to football training and the design of future studies aiming to map adaptation in football.

2.1 Demands of football

Adaptations are required across multiple physiological systems to effectively support the physical demands of football. This is a consequence of the sport's intermittent and unpredictable exercise pattern (Drust et al., 2007; Reilly, 1997). More specifically, a football game's exercise pattern can be viewed as a composition of running patterns that are of different intensities (sub-maximal to maximal), directions (forward, backward, sideways), and forms (straight line, changes of direction). These demands all occur within the tactical context of the game (Bloomfield et al., 2007; Bangsbo, 1994; Dupont et al., 2004). Figure 2.1 adopted from Bradley and Ade, demonstrates the influence of tactical factors on the physical requirements for different players. Consequently, different physiological systems are stressed in an unpredictable, complex, and demanding way throughout the game.

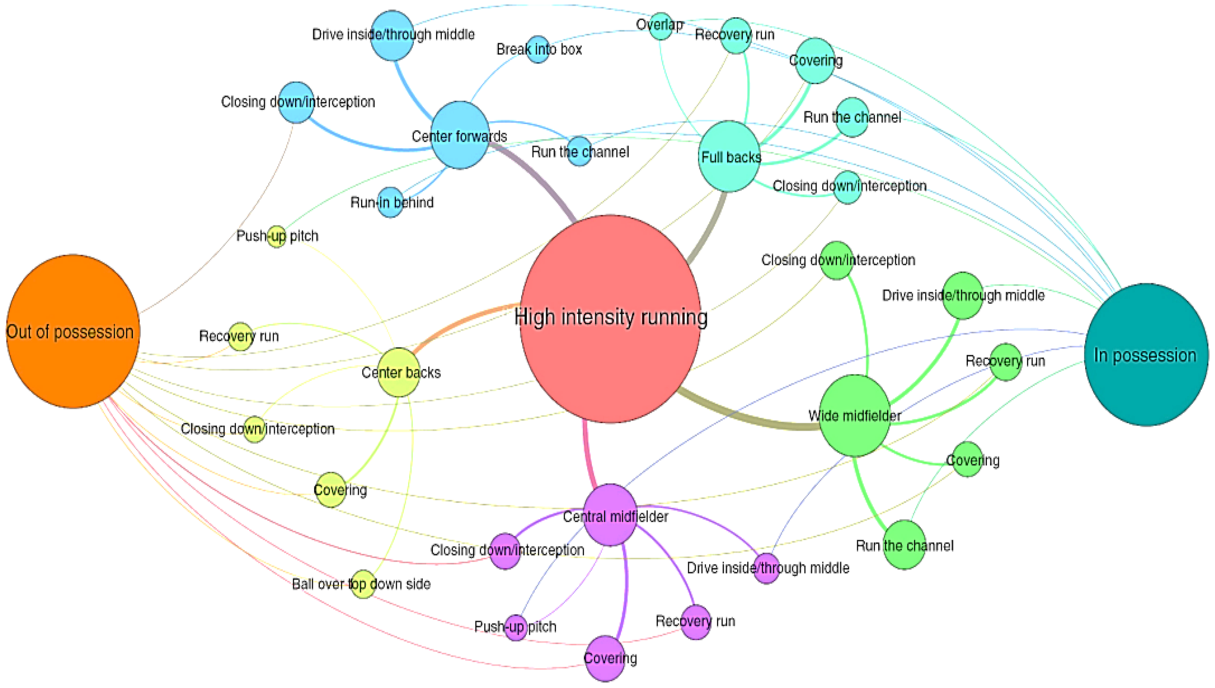


Figure 2.1 Position-specific application of the integrated approach in relation to physical–tactical activities. Note the node size has been adjusted to represent the distance covered in each position/activity and the edge thickness for the frequency of efforts (Adopted from Bradley and Ade, 2018)

Players do not just experience these stresses as single one-off infrequent events. Instead, they are repeated over time as games throughout what is typically a 48 week-long competitive calendar (Anderson et al., 2016). Accordingly, these repeated stresses can lead to many different adaptive and/or maladaptive responses within the body over these periods of exposure (Drust et al., 2000). Understanding the nature of these responses is important to impact football performance in several ways. For example, they may provide a better insight into the physical requirements of match-play and training, which may support a better talent selection process or game-specific preparation strategies. They may also help aid the design of appropriate training interventions that are more specific to the requirements of individual players (Reilly, 2005). Therefore, understanding the unique adaptive processes associated with the different physiological systems stimulated by football is an important practical consideration for those individuals working within the game.

An initial examination of the available detailed data from competitive performances provides an important starting point to understanding the potential adaptations associated with match-play. If the context of a single game is used to examine the physiological demands of football, the running demands require elite-level male outfield players to cover distances averaging between 9 and 13 km (Bradley et al., 2009; Sarmento et al., 2014, Bangsbo, 1994; Mohr et al., 2003; Krustrup et al., 2005). Standing, walking, and jogging have been observed to account for almost 80% of the distance covered in elite level football (Mohr et al., 2003). Critically though, the crucial moments during games, such as the prevention or conversion of goal scoring opportunities, are dictated by high-intensity activities (Gregson et al., 2010). The distance covered at higher speeds is approximately 600 metres to 1200 metres, with average sprinting values contributing between 150 metres to 360 metres of this amount (Di Salvo et al., 2009; Bradley et al., 2013).

Activities typically last for short durations (e.g. an average of 3-5 seconds for a sprint (Bangsbo,1991), leading to frequent changes in activity and direction of movement (around 1200 changes per game). These frequent changes highlight the need for players to almost continually alter their running speeds and movement patterns thereby requiring numerous accelerations and decelerations to enable changes in direction and activity rapidly during games (Abbot et al., 2018). However, only a small number of studies have investigated the change of direction and/or acceleration/deceleration demands in football.

Unlike the available information on the distance covered in diverse types of locomotor activity, there is no consensus on the frequency and intensity of these demands (Bloomfield et al., 2007; Nedelec et al. 2014; Baptista et al. 2018; Granero-

Gil et al. 2020; Morgan et al., 2021). Published observations indicate that change of direction can occur up to 700 times in a game (Bloomfield et al., 2007), whilst high-intensity accelerations greater than 2.78 metres per second can occur up to eight times more frequently than sprints (Varley and Aughey, 2013; Dalen et al., 2016). This may be influenced by contextual factors such as playing style, formation and the amount of possession one team has in a game (Barrett et al., 2018). Furthermore, the space required to sprint at high speeds may be more limited in a game than the space required to accelerate and decelerate.

This lack of consensus regarding these demands is a consequence of the different methods used to quantify these demands in the aforementioned research, which include video analysis, notational and qualitative analysis as well as GPS analysis. Whilst each of these methods provides useful information, they are not without limitation. Video and notational analysis is a time-consuming process and is relatively subjective, whilst the reliability of GPS at high change of direction velocities remains in question (Dos-Santos et al., 2022; Delaney et al., 2018). Nevertheless, these are important actions which may influence game outcomes and tissue adaptation and as such, developing accurately quantifying the magnitude and frequency of these actions, as well as recording the scenarios in which they occur from a tactical standpoint, would provide useful insights from a physical and tactical perspective.

This preliminary data provides a developing insight into the relative importance of these actions to the overall activity profile completed by players irrespective of their position. Of the activities completed by players, high-intensity actions are considered crucial components, as they are associated with creating and/or preventing goal-scoring opportunities or being first to the ball in other areas of the field (Ade et al., 2016; Carling et al., 2008; Garganta and Pinto, 1998). However, no studies have attempted to examine the role those non-linear activities may play in critical match events at the time of writing. This makes it currently difficult to interpret their importance in this way.

The demands placed on the aerobic system during football matches have been demonstrated via heart rates during match play, with average heart rates shown to be 85% of maximum, indicating a high aerobic demand (Stølen et al., 2005). Average oxygen uptake is estimated to be 70-75% of a player's $\dot{V}O_2$ max (Krustrup et al., 2011; Mohr et al., 2016). These values reflect the locomotor demands placed on the muscular system, which requires large amounts of oxygen delivery via blood pumped from the heart. The amount of blood pumped from the heart is dependent upon the body's oxygen demands and is a function of the Fick equation. On observing the Fick equation, oxygen consumption is a product of cardiac output (CO) and arterio-venous oxygen difference (a–$\dot{V}$ O2 dif). Cardiac output is the product of heart rate (HR) and stroke volume (SV) and is measured in liters per minute. The arteriovenous oxygen difference is a measure of the amount of oxygen taken up from the blood by the tissues. Consequently, the greater the amount of oxygen consumed by the tissues, the greater the arteriovenous oxygen difference (King & Lowery, 2022). Thus, as the body's oxygen demands increase, cardiac output will increase to match the oxygen consumption of the tissues ($\dot{V}$ O2 = CO x a–$\dot{V}$ O2 dif). Therefore, in order to meet the running demands of the game. football players require well-developed aerobic systems. The mechanisms behind aerobic adaptations will be explored in more detailed in subsequent sections.

The previous paragraphs have highlighted the aerobic demands of the game. However, the intermittent high intensity demands of the game will also tax the anaerobic system. This is evident from the high intensity running demands and explosive actions outlined previously, which would rely on the glycolytic and PCr energy pathway (Stølen et al., 2005). This can be evidenced by the high levels of muscle lactate during gameplay found by Krustrup et al. (2011). Moreover, in the same study, muscle creatine phosphate levels were shown to drop by approximately 30% post-game. Another previous study by Krustrup et al. (2006) also demonstrated that muscle fiber glycogen concentration was almost 50% lower than pre-match values by the end of competitive match play. Moreover, approximately 40% of individual muscle fibers were almost completely empty of glycogen in the same study. In a 90-minute test which simulated match activity profiles, Bendiksen et al. (2012), found that 80% of type I and type II muscle fibres were glycogen depleted. Whilst there does not seem to be a causal link between specific metabolites and fatigue during a game (Bangsbo et al., 2007), what this evidence does demonstrate is that the anaerobic system is taxed due to the demands of the game.

Overall, this provides a snapshot of the demands placed on both the aerobic and anaerobic systems because of the running demands during games. However, the implications of the high-velocity eccentric muscle contractions that occur during high-speed running and direction changes warrant further investigation. These demands are associated with greater levels of muscle damage (Chapman et al., 2006). This is evidenced by the reductions in hamstring and quadriceps maximum voluntary torque and sprint performance that have been demonstrated in response to 90-minute games and game simulations (Rampinini et al., 2011; Wilmes et al., 2021). Furthermore, decreases in repeat sprint performance following 90-

minute games have been demonstrated (Krustup et al.,2006), insinuating a high demand on the musculoskeletal tissues involved in sprinting. Based on the close interaction between muscles, tendons, and bones, the high demands on muscles will also have implications for these tissues (Warden, Fuchs and Turner, 2004; Bohm et al., 2015; Wisdom et al., 2015). However, no research exists on the effects of football games on tendons and bones.

An analysis of this description of activities within the game and the demands placed on different tissues/systems provides a basis to outline the implications for the adaptations that have the potential to occur as a consequence of regular exposure to this exercise. These adaptive implications are illustrated in figure 2.2 and can be broadly sub-categorized as either 'physiological' or 'biomechanical' (Vanrenterghem et al., 2017). Physiological adaptations are associated broadly with changes in the capacity to provide energy and to recover from the stress of demanding exercise. This would include energy system development and functions related to the support of exercise by the cardiovascular and neuromuscular systems (Stølen et al., 2005; Christensen et al., 2011; Ali and Farrally, 1991; Bangsbo, 1994).

Biomechanical adaptations may occur as a result of the high forces associated with ground contact during game-specific movements such as change of direction, braking, and jumping. These patterns of activity place stress on the tissues of the musculoskeletal system, leading to adaptations of the muscles, tendons, and bones. These adaptations occur as a result of tissue remodeling to allow these tissues to cope better with these demands in the future (Hagman et al., 2018; Nicholas et al., 2000; Wragg et al., 2000; Vanrenterghem et al., 2017; Reilly, 1997).

Together these categories would seem to provide a useful framework to understand the potential implications more fully for adaptations that are associated with football performance than more isolated system/tissue-specific approaches. A more holistic perspective would seem to more appropriately reflect whole-body physiology and the interactions that occur between systems to support exercise performance. It would also seem to provide a basis for the integration of different adaptive time courses that are potentially important for the overall adaptation of the individual that are not frequently considered. For example, changes in $\dot{V}O_2$ max, (which may be considered a physiological adaptation) occur within 1 to 4 weeks (MacInnis and Gibala, 2017) while a change in the morphology of tendon (a biomechanical adaptation) can take at least 8 to 12 weeks to develop (Bohm et al., 2015). Such models would therefore seem to have clear implications for both the conceptual understanding of the adaptations associated with football and the practical application of the training process.

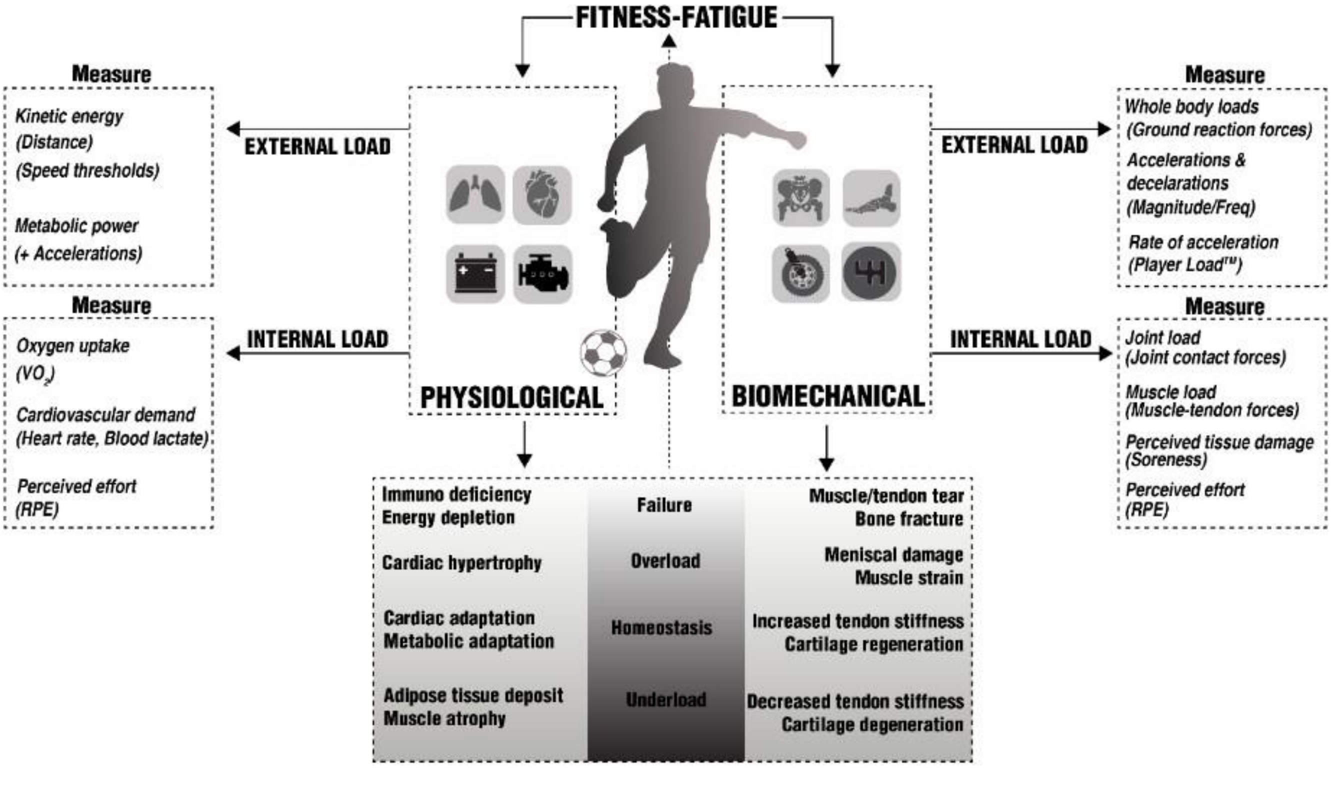


Figure 2.2 Framework proposed by Vanrenterghem et al., 2017 whereby physiological and biomechanical load adaptation pathways are considered separately. (Adopted from Vanrenterghem et al., 2017)

2.2 Training in Football

Adaptations occur as a result of the training process, which aims to develop the attributes required to perform sports-specific tasks (Stone et al. 2007). The training process in football will need to include activities that stress the physiological and biomechanical tissues and systems (Morgans et al., 2014). To what extent these tissues and systems adapt over time is dependent upon the systematic application of the training process time. Moreover, these tissues and systems adapt differently and over different time-courses and thus, the results of any training process in football are difficult to predict. Nevertheless, the management of the training process with respect to time, referred to as periodization (Cunanan et al., 2018), is commonplace in football as an attempt to manage adaptation. Therefore, the following section will explore the use the periodization in football and how the application of the training process is monitored.

The ability of an athlete to adapt and adjust to workloads imposed by the training process is crucial for the achievement of optimal levels of performance. Selye (1956) developed a model to explain the response to a physiological stimulus termed the general adaptation syndrome (GAS). This model proposes that all stressors result in similar responses. The process is based on a negative feedback principle in which physiological sensors regulate adaptive processes, to maintain homeostasis within the body. Bompa (2009) has described how the GAS model can be directly applied to exercise training. The author described the supercompensation cycle as having four separate distinct phases following a physiological stimulus: 1) exercise-induced fatigue, 2) restoration of various physiological systems to previous baseline levels, 3) a supercompensation of the physiological systems and 4) a repeated application of stimulus to maintain or increase the newly attained physiological level. However, whilst this GAS model fits nicely with respect to a controlled single stimulus, the extent to which this model fits when a chaotic stimulus such as football is applied, especially during the in-season phase is unknown. This renders this model too oversimplified to predict the training response.

Training periodization models have been utilized in football to control the stimulus throughout the season (Favero and White, 2018; Walker and Hawkins, 2018) and, thus, where adaptive responses may occur through the specific design and application of particular exercise stimuli. The potential for adaptation that accompanies these periodization models, in theory, is a direct function of the specific time courses of stress and recovery that are planned throughout these distinct phases of the season (Kiely, 2012; Gamble, 2006).

In football, the annual cycle is divided into three main phases; the pre-season, competitive in-season, and off-season; each of which has a specific training stimulus associated with it (Walker and Hawkins, 2018; Reilly, 2007; Gamble, 2006). Typically, the period of training with the highest volume and intensity occurs during the 4-to-8-week pre-season phase (Jeong et al. 2011; Fessi et al., 2016; Clemente et al., 2019; Clemente et al.,2020). During the pre-season phase, coaches seek to rebuild and develop players physically following the off-season in preparation for the competitive in-season phase that lies ahead (Reilly, 2007; Bangsbo, 1994; Bompa, 2009; Algroy et al., 2011). Since the pre-season phase does not contain competitive games, the stimulus can be controlled somewhat more than the in-season phase. Thus, the supercompensation in physical qualities sought by coaches in preparation for the season ahead can manifest more easily. This is due to the ability of coaches to plan the and control the stimulus, as well as rest periods to allow the potential for supercompensation to occur.

While scientific data that examines the differences in training demands between pre-season and in-season is scarce, evidence of higher levels of training demands during pre-season compared to in-season has been demonstrated by studies such as those published by Jeong et al. (2011). This study showed that RPE-derived external training loads and heart rate-based internal training loads were significantly higher during the pre-season phase than the in-season phase in elite professional football players. This is supported by Fessi et al. (2016) who demonstrated higher training session frequencies, mean session durations, and weekly RPE-derived training loads when comparing pre-season to in-season (Table 2.1). In some instances, players can also be faced with large increases in training frequency at these times (i.e. double the number of training sessions in the pre-season phase compared to the in-season phase (Impellizzeri et al., 2006). This may go some way toward explaining the higher volume of training in this period.

To examine the demands of training in-season, research has investigated the strategies used to periodize in-season weekly microcycles (Malone et al., 2015; Oliveira et al., 2019). Typically, microcycles during the in-season phase can be between 3 to 7 days. However, this varies depending on the team, competitive schedule, and manager's philosophy (Van Winckel et al., 2014; Morgans et al., 2014; Weston, 2018). For these reasons, various in-season microcycle periodization models appear in the literature (Anderson et al., 2016; Malone et al., 2015; Stevens et al., 2017; Akenhead et al. 2016; Impellizzeri et al., 2004; Owen et al., 2020).

Nevertheless, despite the different methods and models used during the microcycle, a pattern of post-game recovery, followed by high training loads of both volume and intensity at beginning of the week, and a ‘tapering’ of both volume and intensity as the gameday draws nearer to ensure readiness is high, is consistently reported (Table 2.1). For example, research shows that training loads, measured using RPE and HR (Impellizzeri et al., 2004; Owen and Wong, 2009) and GPS (Anderson et al., 2016; Malone et al., 2015), are reduced the day before and two days before a game (Table 2.2). This data also showed that sessions that occur three and four days before a game typically have the highest training volumes and intensity in the microcycle. As a result, the sessions completed on these days in the middle of the microcycle may present opportunities for an overload on specific physical components and allow time for recovery in the lead-up to a game. Therefore, whilst the overall stimulus is higher during the pre-season phase (Bompa, 2009), opportunities may occur to stimulate adaptive responses early in the training week during the in-season phase provided no more than two games are played in a week (Walker and Hawkins, 2017).

Table 2.1 Pre-season vs in-season loading trends in men's professional football.

Reference	**Standard**	**Method for measuring external load**	**Pre-Season vs In-Season**	**Other Measures**
Fessi et al., 2016	Qatar Stars League	BORGs RPE x Session Duration	Greater FRE & mean DUR in PRE vs IN Greater weekly TL, monotony & strain in PRE vs IN	Greater levels of sleep, fatigue, soreness, and stress in PRE vs IN using Hooper Index
Clemente et al., 2020	European First Division	GPS	Greater levels of monotony & strain in PRE vs IN	
Jeong et al., 2011	Korean First Division	BORGs RPE x Session Duration/ Heart Rate	Greater mean TL and HR higher in PRE vs IN More time in 80-100% max HR zones in PRE vs IN	
Clemente et al., 2019	Portuguese First Division	Fosters RPE x Session Duration/ Heart Rate	Highest weekly loads observed during PRE vs IN	
Malone et al., 2017	European First Division	BORGs RPE x Session Duration/ Heart Rate	Highest weekly loads observed during PRE vs IN	

Table 2.2 In-season microcycles utilised in men's professional football.

Reference	Standard	Typical Microcycle Structure	Method for measuring load	Main findings
Anderson et al., 2016	English Premier League	**1 GW** - OFF, OFF, MD-4, MD-3, MD-2, MD-1, MD. **2GW** - GD, OFF, MD-4, MD-3, MD-2, MD-1, MD. **3GW** - GD, OFF, MD-1, GD, OFF, GD-1, MD.	GPS	Highest loads on MD-3 for **1GW** & **2GW** with a reduction in load to MD-1
Malone et al., 2015	English Premier League	**1GW** - MD-5, OFF, MD-3, MD-2, MD-1, MD, OFF	GPS & RPE	Significant reduction in load on MD-1. No differences were observed (MD-2, MD-3, and MD-5).
Stevens et al., 2017	Dutch Eredivisie	**1GW** - MD-5, MD-4, OFF, MD-2, MD-1, MD, OFF	GPS	Highest loads on MD-4 with a reduction in load to MD-1
Owen & Wong, 2009	English Championship	**1GW** - OFF, MD-4, MD-3, MD-2, MD-1, MD, OFF	HR	Highest loads on MD-4 with a reduction in load to MD-1
Akenhead et al. 2016	English Premier League	**1GW** - MD-5, MD-4, OFF, MD-2, MD-1, MD, OFF	GPS/HR/RPE	Highest loads on MD-4 with a reduction in load to MD-1
Impellizzeri et al., 2004	Youth Football Players	**1GW** - MD-5, MD-4, MD-3, MD-2, OFF, MD, OFF	RPE	Highest loads on MD-4 with a reduction in load to MD-1
Owen et al., 2020	Chinses Super League	**1GW** - OFF, MD-4, MD-3, MD-2, MD-1, MD, OFF	GPS & RPE	Significant reduction in load on MD-1. Significant differences between MD-2 & MD-1, MD-3 & MD-1, MD-4 & MD-1, MD-3 & MD-2, MD-4 & MD-2, MD-3 & MD-4.

Nevertheless, to strike a balance between stimulating adaptive responses and ensuring this stimulus does not lead to a level of fatigue that compromises gameday performance, holistic approaches are required when monitoring the stimulus-response relationship in football (Foster, 1998; Morgans et al., 2014). Periodization offers a theoretical plan of where stress and recovery may occur, which is difficult to predict in reality. This results from any stimulus-response curve being system-specific and governed by a complex array of biological factors (Kiely, 2012). Consequently, even with a periodized training plan, a training stimulus may not be appropriate for an individual or the team at a specific time. Furthermore, due to the unique process of the physiological and biomechanical load-adaptation pathways, any reduction in load may help some systems recover for gamedays but not others. Overall, this reiterates the need to understand the mechanisms behind multiple adaptive responses to the demands of football.

Consequently, a more holistic approach is required when monitoring the stimulus-response relationship in football. The training process requires consistent monitoring as the same stimulus does not lead to the same response in different athletes, or indeed the same athletes in different circumstances (Sands and Stone, 2005). Therefore, monitoring both sides of the stimulus-response relationship has the potential to optimize the training process. Recent technological advances have led to GPS (global positioning system) derived training loads becoming a standard indicator of the stimulus, or 'external load' (Casamichana et al. 2013; Impellizzeri, 2019). As evidenced previously, this has led to a detailed insight into the demands of games and training, allowing for a more precise prescription of the training stimulus. Furthermore, from a practical standpoint, this information is the easiest to collect (Sands and Stone, 2005), and may help identify periods during the training week where the stimulus is high enough to elicit adaptive responses. Nevertheless, as conceptualised in figure 2.3 by Impellizzeri et al. (2019), the internal response or ‘internal load’ drives adaptation. Furthermore, many actions that have implications on internal responses such as ball striking, jumping, and tackling cannot be quantified well using GPS (Rebelo et al., 2012; Mallo and Navarro, 2008). Consequently, practitioners and researchers have been warned against the shift towards examining the stimulus instead of the response (Impellizzeri et al., 2019).

The acute responses to any training stimulus begin on the molecular scale and move through the subcellular, cellular, and tissue scales over time, eventually leading to adaptations at the organism level (Wisdom et al., 2015; Hawley et al., 2014; Zierath and Wallberg-Henriksson, 2015). Therefore, solely measuring the external stimulus placed on the organism ignores the intricacies of this complex process. For example, a stimulus may be enough to stimulate an acute response on the cellular level yet not permeate the levels of the body over time to lead to a chronic change at the organism level (Balagué et al., 2020; Pol et al., 2020). Furthermore, this journey from stimulus to an observable change in physical characteristics may look different for the multiple components within the physiological and biomechanical load-adaptation pathways. Therefore, whilst focusing on the internal response is important, it seems logical to take a broader and more conceptual view of adaptation, which involves understanding the internal response of multiple tissues and systems to the demands of football.

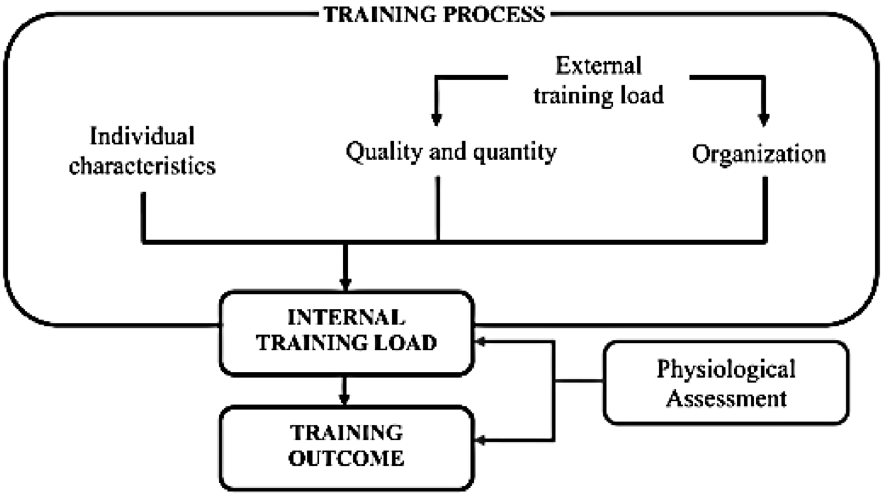


Figure 2.3 The training process as conceptualised by Impellizzeri et al. (2005) emphasizes the importance of the internal training load for training outcomes.

2.3 Adaptation

In the spirit of taking a broad view of adaptation, it would seem pertinent to first consider adaptation relative to its overall purpose for living organisms, before focusing on exercise and football. Charles Darwin (1858) introduced the theory of natural selection, the chief process by which evolutionary change occurs. This process is a naturally occurring mechanistic manner in which organisms develop traits, allowing them to adapt to their environment, making survival more likely (Darwin, 1858). This process is a systemic response triggered to maintain homeostasis or improve the ability of an organism to cope with the stressors encountered in its environment. Adapting to exercise relies on a similar process, exercise after all is just a different type of stressor.

However, from a physiological perspective, the emphasis is focused specifically on ensuring the body's tissues and systems adapt to support and improve exercise efficiency instead of developing traits to aid survival (Hughes et al., 2018). These adaptations will occur if the body is subjected to stress within tolerable limits. The purpose of any training stimulus is to stress the body to a point within these tolerable limits, where it must adapt accordingly to tolerate said stimulus more effectively. These adaptations will reflect the training stimulus (Fahey and Chicho, 1998). In football, adapting to the training stimulus allows players to perform optimally and cope with the physiological and biomechanical components of the game. Physiological and biomechanical adaptations are dependent upon unique adaptive processes. Therefore, it is imperative to explore the mechanisms behind these processes. However, it would be pertinent to first outline how these pathways are stimulated by the demands of football.

Research would suggest that the stimulus elite football players are exposed to leads to physiological and biomechanical adaptations over time. Indeed, there are examples in research that demonstrate that elite players generally show higher levels of fitness, strength, and augmented musculoskeletal properties when compared to their amateur counterparts. However, there seems to be more evidence relating to physiological adaptations. On the side of the physiological load-adaptation pathway, Rampinini et al. (2010) demonstrated that elite football players covered more distance in the YOYO intermittent running tests than their amateur counterparts. Furthermore, in the same study, the elite players showed a lower physiological cost (RPE and blood lactate levels) when performing a standardized high-intensity running protocol.

Similarly, it has been shown that elite players demonstrate the ability to cover more distance and high-speed running at a lower physiological cost (heart rate, RPE, and blood lactate levels) during various small- sided games compared to amateur players (Dellal et al., 2011). Moreover, Mohr et al. (2003) also demonstrated that elite level footballers cover more high-intensity and sprint distances during competitive games when compared with other footballers of a lower standard, even though they were professional.

On the side of the biomechanical load-adaptation pathway, Beato et al. (2021)

showed that professional and elite academy players had higher concentric and eccentric hamstring and quadriceps strength compared to amateur players, despite the average age of the amateur group being higher than the academy group. Additionally, Carvalho et al. (2016) showed that first division football players demonstrated greater eccentric hamstring and quadriceps strength when compared to second division players. Moreover, evidence has shown that elite academy football players have superior patellar tendon CSA, elongation, and strain compared to amateur counterparts (Murtagh et al., 2018).

Overall, this evidence suggests that favourable physiological and biomechanical adaptations occur due to the training habits of elite football players. However, our understanding of the complex mechanisms that dictate these changes over time in football players remains somewhat elusive. This lack of understanding is due to the complex adaptive processes that occur within the physiological and biomechanical load-adaptation pathways. It has been argued by Balagué et al., (2020), that reductionist approaches to mapping adaptation in research are to blame for this lack of understanding. Consequently, they propose that more complex approaches are required to understand complex problems. These complex approaches to mapping adaptive responses are necessary due to the interactions that occur on and across the levels of organisation in the body dictate the future response of an organism, as demonstrated in figure 2.4 (Balagué et al., 2020; Gershenson and Fernández, 2012). Therefore, a complex biological system adapts because of numerous interactions that occur on and across multiple levels; these include the cellular, tissue, organ, organ system, and organism levels, as illustrated in figure 2.4 (Balagué et al., 2020; Carmichael and Hadzikadi, 2019; Walleczek, 2000).

Consequently, as part of a more holistic view of adaptation, research in football must explore the mechanisms behind the adaptive responses in each of these pathways across multiple levels.

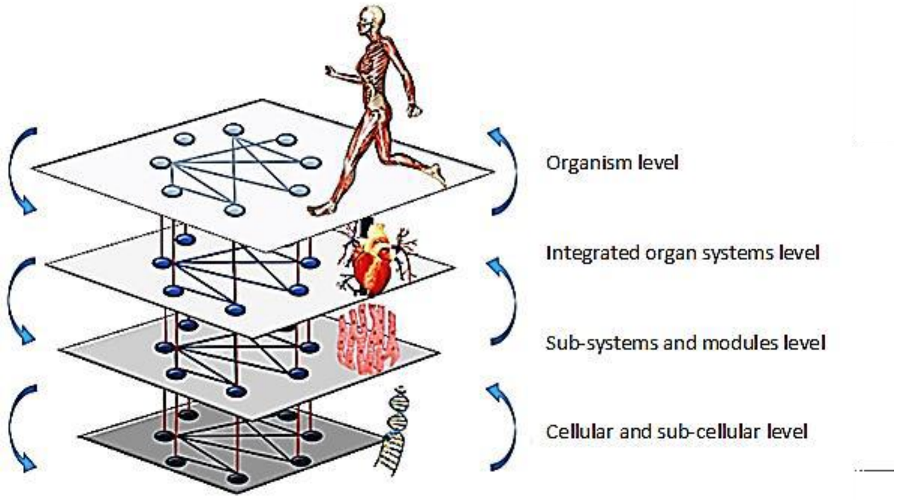


Figure 2.4 Representation of the interaction across levels and between levels through circular causality. Vertical interactions represent interactions between levels. Horizontal interactions represent the interactions that occur on a single level (Adopted from Balagué et al., 2020).

Adaptations exist beyond the physiological and biomechanical load-adaptation pathways. There is of course adaptations of the nervous system (neuroplasticity) which allows for the successful transfer of learned movement patterns, which have the potential to increase the impact of any physiological or biomechanical adaptations (Grooms et al., 2018). For example, improved running technique is a learned motor skill, which would optimise the force capabilities of the biomechanical load-adaptation pathway.

However, it is beyond the scope of this review to deeply explore these complex processes in a completely holistic manner. Therefore, the following section will provide a ‘helicopter-view’ outline of some of the key central and peripheral cardiovascular adaptations in the physiological load-adaptation pathway. Whilst key adaptations of the major tissues involved in producing force and locomotion, such as muscle, tendon**,** and bone, will be explored on the side of the biomechanical load-adaptation pathway. Whilst not exhaustive, these adaptations will be framed to help the reader appreciate the ‘tissue-specific’ array of interactions that occur across multiple levels in the body in response to exercise. This detail will also help the reader appreciate how broadly adaptation needs to be considered in football.

2.3.1 Physiological Adaptation

From a metabolic standpoint, the energy needed to sustain running over a 90-minute game comes through aerobic metabolism, whilst high-intensity running efforts rely on anaerobic metabolism (Stølen et al., 2005). Aerobic metabolism provides the necessary energy for running performance and improved recovery following high-intensity actions (Reilly, 2007; Impellizzeri et al., 2006). The net effect of aerobic training adaptations is an improved capacity of the cardiovascular system to transport and utilise oxygen and fuels. These adaptations support the metabolic processes required to perform the aerobic and anaerobic running demands of football (Joyner and Coyle, 2008; McKay et al., 2009). Consequently, a multitude of structural and functional adaptations occur in the cardiovascular and muscular system to achieve this improvement in oxygen and transport. These implications need to be considered when designing studies investigating responses to the demands of football.

However, it is essential to note that improvements in oxygen transport and consumption result from two distinct central and peripheral adaptive processes (Hellsten and Nyberg, 2016). Central adaptations relate to the structure and function of the heart. Cardiac muscle, like skeletal muscle, undergoes morphological adaptations as a result of the haemodynamic stress imposed by training (Unnithan et al., 2018). On the other hand, increases in the muscles’ ability to consume and utilize oxygen also occur, termed 'peripheral adaptations' (Hellsten and Nyberg, 2016;

Hostrup and Bangsbo, 2017). These adaptations increase the amount of oxygen-rich blood transported by the heart to the working muscles during exercise. However, the adaptations that manifest are dependent upon the training exposure (mode, intensity, duration, and volume) (Weiner and Baggish, 2012; Unnithan et al., 2021).

2.3.1.1 Central Structural and Functional Adaptations

In terms of central adaptations specifically to football training, the majority of research is cross-sectional and demonstrates augmented left ventricular adaptations relating to mass, end diastolic volume and end systolic volume in professional football players when compared with matched controls. This has been demonstrated using cardiac magnetic resonance imaging (Tahir et al., 2015) and echocardiography Unnithan et al. (2018). The increase in left ventricular chamber size would allow for increased left ventricular filling and consequently an increase in stroke volume, which means more blood can be pumped from the heart to the working muscles per contraction (Hughes et al., 2018; Hellsten and Nyberg, 2016). Indeed, greater left ventricle morphology this has been suggested to account for the differences in aerobic fitness between soccer players and controls (Unnithan et al., 2018).

Furthermore, it has been demonstrated in youth soccer players that soccer training leads to increases in left ventricular function during submaximal exercise (45% $\dot{V}O_2$ peak). These adaptations have been shown to occur yearly across a 3 year period, independent of the effects of growth and maturation (Unnithan et al., 2022). These findings would suggest that positive cardiovascular adaptations occur in response to what could be considered a relatively low training exposure when considering the amount of training youth athletes would have accumulated compared to adults. This has also been supported by McLean et al., (2018), who’s systematic and meta-analysis provided evidence of exercise-induced cardiac re-modelling in the pre-adolescent years. Further research is needed to establish the time-course of central structural and functional adaptations in elite soccer players in terms of the minimum time needed to observe changes in these parameters.

2.3.1.1 Peripheral Structural and Functional Adaptations

The net effect of central adaptations is an improved capacity of the cardiovascular system to transport oxygen, ultimately improving aerobic performance (McKay et al., 2009). However, whilst the adaptations mentioned previously have focused on oxygen delivery, there are also adaptations that augment oxygen utilization in trained subjects. These ‘peripheral’ adaptations are needed to transform an increased cardiac output into aerobic performance (Gliemann, 2016). As mentioned in preceding sections oxygen consumption is a product of cardiac output (CO) and arterio-venous oxygen difference (a–$\dot{V}O2$ dif). Thus, in order to increase the arterio-venous oxygen difference adaptations such as increases muscle mitochondrial content and capillary volume are needed to optimize the use of transported oxygen in the working muscles (MacInnis and Gibala, 2017).

The energy needed to support aerobic metabolism originates in the mitochondria, where adenosine triphosphate (ATP) is generated through the electron transport system (Egan and Zierath, 2013; Dunn and Grider, 2022). The processes required to generate ATP require sufficient oxygen levels to be transported to the active muscles for consumption in the mitochondria. This increase in oxygen consumption stimulates processes that lead to mitochondrial biogenesis (Jornayvaz and Shulman, 2010). Consequently, an increased number of mitochondria, or an increase in their functional ability, provide the capacity for increased ATP production. In terms of capillary volume, high muscle capillary volume means that a greater area is present over which the diffusion of oxygen and nutrients into the working muscles can occur, creating a more efficient process (Hellsten and Nyberg, 2016; Liang et al., 2006; Hawley et al., 2018).

This efficiency manifests as an increase in V̇O2 kinetics. Indeed, it has been suggested that both oxygen delivery and utilization improvements that explain the faster V̇O2 kinetics observed in trained versus untrained subjects (Marwood et al., 2010). This has also been demonstrated in football by Rampinini et al. (2010), who demonstrated that V̇O2max did not distinguish elite from amateur players, but faster V̇O2 kinetics, measured by the time constant of the transition to moderate intensity exercise, was evident in the elite players. Moreover, Doncaster et al (2016) demonstrated that faster V̇O2 kinetics were related to higher YOYO IR1 scores and high-speed running distance in matches in a group of highly trained youth soccer players.

In terms of skeletal muscle capillarization, the time-course of this adaptation requires weeks to months to manifest in response to exercise training (Andersen & Henriksson, 1977). A recent meta-analysis conducted by Liu et al. (2022) found that in untrained subjects, capillarization is induced after training interventions of 2-4 weeks. Whereas these changes would seem not to occur in already well-trained subjects within a time frame of training of 2-8 weeks. Thus, it would seem longer training durations would be needed to see these adaptations manifest in elite soccer players.

Importantly, increases in capillarization and mitochondrial biogenesis can also lead to reductions in muscle and liver glycogen utilization. A depletion of muscle glycogen stores has been attributed to a reduction in repeated sprint performance during match-play (Krustup et al., 2006). Therefore, muscle and liver glycogen sparing means these fuels are available for high-intensity anaerobic outputs that require near-maximal force production in football (Coggan, 1991; Coggan and Swanson, 1992; Mendenhall et al., 1994; Reilly, Drust and Clarke, 2008).

Furthermore, increases in capillarization and mitochondrial biogenesis lead to more efficient buffering of metabolic waste products, such as lactate and hydrogen ions, during anaerobic exercise (Gliemann, 2016; Hellsten and Nyberg, 2016; Hawley and Nader, 2008).

Overall, this section provides a snapshot of the complex array of adaptive processes that occur in the physiological load-adaptation pathway to support aerobic and anaerobic exercise. These processes occur at various levels of organisation in the body to support performance at the organism level. Consequently, there are processes that require various durations to manifest and impact performance. However, it must be noted that any stimulus that aims to improve the physiological load-adaptation pathway through football or running-based activities will also affect the biomechanical load-adaptation pathway. These implications also need to be considered as part of a broad view of adaptation in football.

2.3.2 Biomechanical Adaptation

Musculoskeletal adaptations are triggered by mechanical loads. These adaptations manifest as a consequence of a continuous cycle of adaptive iterations, referred to as ‘turnover', where musculoskeletal tissues adapt by altering their size, shape, and strength relative to the mechanical forces they encounter (Warden, Fuchs and Turner, 2004; Bohm et al., 2015; Wisdom et al., 2015). These adaptations, and the time courses over which they occur, depend on the frequency and intensity of the training stimulus (Farup et al., 2012; Kubo et al., 2010; Kraemer et al., 2004; Aagaard et al., 1996; Schoenfeld, 2010; Martone et al., 2017). However, the responses and the implications of the interactions between these tissues are not as appreciated in football as components of the physiological load-adaptation pathway (Vanrenterghem et al., 2017), as evidenced in the previous section. The mechanical demands of football and their implications on the tissues of the biomechanical load-adaptation pathway result in responses that permeate the molecular, subcellular, cellular, tissue, and organ scales (Wisdom et al., 2015). Consequently, it would seem important to understand how these tissues respond across multiple levels to mechanical load.

While musculoskeletal tissues such as muscle, tendon, and bone respond to mechanical load similarly, they each have specific structures that determine how they adapt to these loads (Kalkhoven et al., 2019). Musculoskeletal tissues strive to better tolerate mechanical loads by improving load tolerance and force production. From a football performance standpoint, the potential for muscle to improve performance comes from its ability to produce force compared to non-muscle cells (Krivickass et al., 2011; Wackerhage et al., 2019). Increasing a footballer’s ability to produce force may augment their explosive abilities, improving vital actions such as accelerating and sprinting (Bangsbo, 1994; Thorlund et al., 2009).

However, changes in muscle adaptation also have implications on tendons since they transmit the forces generated from the muscle to the skeleton during locomotion (Kannus, 2000; Mersmann et al., 2017). These muscle forces deform tendinous tissue, eventually causing structural changes (Wang, 2006). In addition to muscle and tendon, bone is also subjected to the mechanical loads associated with ground reaction forces from running activities and the contractile forces transmitted by the muscles and tendons (Klein-Nulend et al., 2012). However, the function of bone adaptation, unlike muscle and tendon adaptation, strictly relates to load tolerance.

2.3.2.1 Basic mechanisms of muscle adaptation to mechanical load

At the organ scale, changes in muscle CSA correlate with changes in load tolerance, strength/force production, and RFD (Häkkinen and Komi, 1986; Vigotsky et al.,2015).

Changes in CSA are, in part, a result of an increase in net protein accretion, which comes as a result of muscle protein synthesis outweighing muscle protein breakdown (Damas et al., 2016). Mechanical load disturbs myofibers and the related extracellular matrix on the subcellar level. This can lead to an increase in the expression of regulatory growth hormones such as Insulin-like growth factor-1 (IGF-1). IGF-1 promotes anabolism by increasing the rate of protein synthesis in differentiated myofibers when stimulated (Schoenfeld, 2010). This increase in protein synthesis is part of the remodelling process of contractile and structural proteins (McGlory et al., 2017). Albeit oversimplified, over time, this remodelling process increases muscle CSA (Damas et al., 2016).

Generally, increases in CSA and muscle strength can occur within 8 to 12 weeks (Folland and Williams, 2007), with some studies showing this can occur within 4 weeks (DeFreitas et al., 2011; Brook et al., 2015; Damas et al., 2016). However, this variation is dependent upon the methods used to measure the response, as it has been acknowledged that short terms increases in muscle CSA (2-4 weeks) may be a consequence of exercise-induced swelling (McGlory et al., 2017). This highlights the importance of understanding the processes behind observed changes when considering the adaptive time courses of specific tissues. As a consequence of any increase in CSA, there is also an increase in contractile force of the muscle at the organ level (Russell et al., 2000; Attwaters and Hughes, 2022; Wisdom et al., 2015). Which will have implications for tendon adaptation.

2.3.2.2 Basic mechanisms of tendon adaptation to mechanical load

Overall, the force transmitting abilities of tendons play a crucial role in explosive actions at the organism level, such as sprinting. Furthermore, the force transmitting abilities of tendons have been estimated to account for half of the energy cost of locomotion (Voigt et al., 1995; Biewener and Roberts, 2000; Hoff et al., 2002;

Bohm et al., 2015). Increasing tendon stiffness improves the ability of the tendon to stretch and recoil quickly. Consequently, muscular forces can be transmitted through the tendon and into the ground faster than a more compliant (less stiff) tendon (Docking and Cook, 2019; Arampatzis et al., 2007). Adaptations of the tendon properties, such as material (Young's modulus) or morphological (CSA), account for increases in tendon stiffness (Bohm et al., 2015).

Tendons are primarily made of collagen, which increases its synthesis in response to the forces placed on them by muscles (Kjær et al., 2009). These forces are transmitted through the extracellular matrix through integrins, G-protein receptors, and protein kinases (Wang, 2006). This leads to the expression of genes and molecular responses, such as IGF-1, which stimulate collagen synthesis. This increase in synthesis is a part of the tendon remodelling process, which can lead to positive functional and structural adaptations (Magnusson et al., 2010). This process is analogous to the role of protein synthesis in muscle adaptation, as increased collagen synthesis over time can lead to mechanical (stiffness), material (Young's modulus), and morphological changes (CSA) in tendon (Heinemeier and Kjaer, 2011; Galloway et al., 2013; Kjaer, 2004; Wang, 2006; Kjær et al., 2009). Interestingly, a meta-analysis conducted by Bohm and colleagues (2015) found several studies demonstrated a significant increase in tendon stiffness and Young's modulus without changes in the tendon CSA, suggesting unique adaptive time courses. As a result, it is postulated that material changes in the tendon may lead to increased stiffness instead of morphological changes. However, this could also result from the study durations employed, which were generally less than 12 weeks. The meta-analysis by Bohm and colleagues (2015) suggests that studies lasting at least 12 weeks may be necessary to observe changes in tendon CSA.

Curiously, despite the intertwined role muscle and tendons play, differences in metabolic activity seem to play a role in the different rates and time courses of muscle and tendon adaptation (Mersmann et al., 2014). However, further evidence is needed to demonstrate this phenomenon. Nevertheless, these apparent differences highlight the importance of considering the unique adaptive processes of muscles, tendons, and bones.

2.3.2.3 Basic mechanisms of bone adaptation to mechanical load

Primarily, the load tolerance of bone is determined by its strength, mass, structure, and interaction with connecting tissues (Seeman, 2013; Burr, 2011). Stronger and robust bones, with high levels of bone mineral density and greater surface area to distribute loads over, can tolerate higher stress levels before damaging strains than slender, weaker bones (Hart et al., 2017: Burr, 2011; Beck et al., 1996). Changes in bone mineral density and size occur due to the net effects of bone resorption and formation during the bone remodelling process (Kenkre and Bassett, 2018; Crocket et al., 2011; Papapoulos and Schimmer, 2007; Burr, 2011). Changes in morphological muscle properties occur three to four times faster than bone. This has implications for bone as it develops during the remodelling process (Hart et al., 2017).

The process of creating stronger bones is initiated by mechanical loads, which trigger mechanosensitive cells in bone. If these loads are large enough to elicit an adaptive response, the bone remodelling process is triggered, which helps mould the structure of bone relative to these demands (Klein-Nulend et al., 2012; Bakker and Klein-Nulend, 2009; Robling and Tuner, 2009; Kenkre and Bassett, 2018). Bone remodelling, or bone turnover, is the process by which bone is repairs fatigue damage and renews itself to maintain bone strength and mineral homeostasis (Mellon and Tanner, 2012; Rodan, 1998; Burr, 2011, Crocket et al., 2011; Hadjidakis and Androulakis, 2006). Changes in bone mineral density occur as a result of the net effects of bone resorption and formation during this remodelling process (Kenkre and Bassett, 2018; Crocket et al., 2011; Hadjidakis and Androulakis, 2006; Papapoulos and Schimmer, 2007; Feng and McDonald, 2011). Bone cells (osteocytes, osteoblasts, and osteoclasts) collaborate to mould the structure of bone relative to the mechanical demands it is subjected to (Florencio-Silva et al.,2015; Wittkowske et al., 2016; Hemmatian et al., 2017; Klein-Nulend et al., 2012; Bakker and Klein-Nulend, 2009, Robling and Tuner, 2009). Osteoclasts attach to the surface of micro damaged bone and begin to dissolve the structure (Hughes et al., 2017; Romani et al., 2002; Florencio-Silva et al.,2015; Clarke, 2008).

Once these cells have accomplished their function, they undergo apoptosis. Transition signals are then sent that halt bone further resorption and stimulate the bone formation process (Raggatt and Partridge, 2010; Rucci, 2008). Once the resorption process is complete, several growth factors are released, such as bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs) and transforming growth factor β (TGF β). This triggers osteoblasts to move into the resorption space left by osteoclasts. The osteoblasts begin to produce and deposit organic matrix called osteoid. Some osteoblasts become trapped in the matrix and become osteocytes, others will die or will revert to lining cells covering the surface of bone, bringing an end to the remodelling process (Hughes et al., 2017; Raggatt and Partridge, 2010; Parra-Torres et al., 2013; Hadjidakis and Androulakis, 2006; Crocket et al., 2011).

Interestingly, slender bones with lower CSA develop higher density cortices and are stiffer than larger bones. This is a compensatory adaptation due to a lack of cross-sectional area (Wallace et al., 2012; Beck et al., 2000). This increased stiffness, however, does not result in increased strength. On the contrary, this stiffness comes at the expense of ductility (the ability to stretch under tensile stress) and robustness. As a result, slender bones have an increased risk of accumulating microdamage and are less resistant to overload than their larger counterparts (Tommasini et al., 2005; Beck et al., 2000). Therefore, increasing cross-sectional area would seem to be an optimal strategy to improve the tolerance of bone to mechanical load (Fan et al., 2011; Seeman, 2008; Bouxsien and Karasik., 2006; Pearson and Leiberman., 2004). Furthermore, this demonstrates how the underlying structure of bone determines how it responds to load, highlighting an adaptive process that is highly individualized. Curiously, the remodelling process exists as a paradox. The process of replacing fatigue-damaged bone is necessary to strengthen bone and prevent future damage (Hart et al., 2017). However, the risk of further damage is high during this negative bone-balance phase, as there is an acute increase in vulnerability during the stages between old bone being removed and new bone being formed (Hughes et al., 2017).

2.4 Perspective - What does this mean for adaptation in football?

Overall, there is a complex interplay between different tissues in the biomechanical load-adaptation pathway. The separation of responses into 'physiological' and 'biomechanical' load-adaptation pathways may help further our understanding of the mechanisms behind the adaptive responses to football. However, as evidenced in this review, even within these load-adaptation pathways, different tissues have unique adaptive processes and time courses. This further highlights the complex nature of adaptation. Figure 2.5 below demonstrates a theoretical time-course for the systems and tissues that have been reviewed. These differences could have important implications for training interventions as increases in muscle strength will occur over shorter periods than increases in tendon properties (Mersmann et al., 2017).

This could result in more force being transmitted through a tendon that has not adapted to these new demands yet. If the muscle continues to get stronger, the tendon may end up in a continuous state of "catching up", which could overwhelm the tendon due to higher levels of strain, especially during maximal muscular contractions (Mersmann et al., 2017; Vanrenterghem et al., 2017). A further noteworthy example relates to the fact that processes involved in mediating the physiological response to aerobic exercise, such as AMPK and PGC- 1α, can blunt signalling pathways involved in muscle protein synthesis (Schoenfeld, 2012; Enright et al., 2015). This blunted response has implications for managing concurrent training protocols, such as strength training and football activities. Attenuations in adaptive responses can occur if these different training methods are performed in close proximity (Enright et al., 2015).

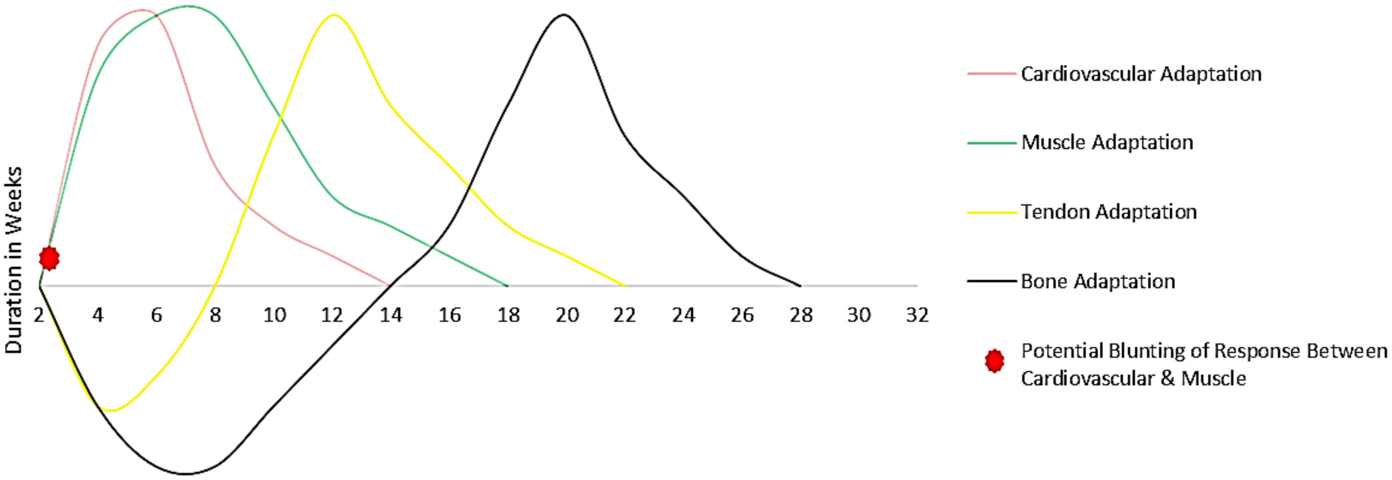


Figure 2.5 Theoretical time courses for physiological and biomechanical load-adaptation pathways. Even within the biomechanical load-adaptation pathway, there are unique adaptive processes and time courses. Improvements in central and peripheral cardiovascular adaptations, as well as increases in muscle strength, could occur over similar time courses. However, at the same time tendon and bone could be going through their respective remodelling processes (this would be the negative phase in the figure above). An increase in a stimulus to ‘chase’ further cardiovascular and muscular adaptations may have negative implications on tendons and bones.

Despite the abundant knowledge on the mechanisms behind physiological and biomechanical adaptations, questions remain regarding how these mechanistic processes permeate throughout each level of the body. Furthermore, the mechanisms and time courses related to these adaptations remain unclear. These examples illustrate the complex nature of adaptation and how broadly it must be considered when designing interventions and research studies. Therefore, a deeper understanding of the mechanisms that generate patterns of adaptation to the demands of football is required (Kiely, 2018; McLaren et al., 2018; Weston, 2013).

2.5 Summary

A review of the literature demonstrates that footballers need to possess multiple physical qualities to perform at an elite level and cope with the demands of training and games across a competitive season. However, evidence suggests that adaptation has not been considered broadly in football, considering the lack of research on the demands and implications in the biomechanical load-adaptation pathway. Furthermore, there is a complex array of adaptive processes that occur across levels of organisation in both the physiological and biomechanical load-adaptation pathways. It would therefore seem that more holistic views of adaptation need to be incorporated in future studies. Such approaches would provide novel and comprehensive insights into how the body and its diverse components adapt to generate a change in football performance (Balagué et al., 2020; Pol et al., 2020). A pertinent first step would be to map how adaptation is currently measured in football.

CHAPTER THREE

MAPPING ADAPTATION IN FOOTBALL – A SCOPING REVIEW

3.1 Introduction

Chapter 2 demonstrates that the demands of football are complex due to the intermittent and unpredictable arrangement that encompasses the physical demands, such as accelerations, decelerations, running, sprinting, changes of direction and jumping (Bangsbo, 1994; Drust et al., 2000; Hagman et al., 2018; Nicholas et al., 2000; Reilly, 1997). This creates a challenge when assessing adaptation, as the inherent complexities associated with a complex biological system and the demands of football make measuring both stimulus and response accurately challenging (Impellizzeri et al., 2019; Pol et al., 2020). The complexity of the body's response relates to the diversity of levels and the multitude of interactions, nonlinearities, and feedback loops that occur across these levels. This creates a system that is not only complicated but emergent (Tzafestas, 2018; Walleczek, 2000; Carmichael and Hadzikadi, 2019; Pol et al., 2020).

Emergent behaviour in complex biological systems occurs in situations where the properties of the system at one level cannot explain the properties on another level (Walleczek, 2000). An example of emergence at the tissue level is the different cells that make up a muscle. These cells on their own cannot perform a muscle contraction, nor can the strength of a muscle be determined by analysing its properties on the cellular level. However, these cells come together through complex interactions across levels to form a muscle that can contract. Therefore, a complex biological system performs because of numerous interactions and emergent behaviours that occur on and across multiple levels; these include the cellular, tissue, organ, organ system and organism levels (Balagué et al., 2020; Carmichael and Hadzikadi, 2019; Walleczek, 2000). Consequently, adaptation can be measured using a variety of approaches involving numerous levels in the body to help explain any change in the performance of the organism (Robling and Turner, 2009; Turner et al., 1995; Manolagas, 2000; Parfitt, 1994; Wisdom et al., 2015; Bohm et al., 2015; Rio and Docking, 2017). However, to date, no study has attempted to comprehensively map the approaches used to measure adaptation in football. Mapping how adaptation is measured in football could highlight gaps in football research that if filled, could expand our knowledge of how the body adapts to the demands of football. Therefore, this scoping review aims to provide insight into how adaptation is measured in football, specifically focusing on:

1. Identifying the stimuli implemented.
2. Identifying the study durations and frequency of measurements employed.
3. Identifying the nature of the response to the stimuli implemented.
4. Identifying the outcome measures used to assess the response to these stimuli and the level they are measured at.

Scoping reviews have developed due to evolving research objectives and questions, leading to new approaches to synthesising evidence. Although these reviews are systematic, they lend themselves to being more suitable for less precise research questions, thus making them more ideal for exploring the extent of the literature, mapping concepts, or informing future practice in a research field (Tricco et al. 2016; Arksey and O'Malley, 2005). This scoping review does not aim to assess the feasibility or effectiveness of any methodology, as this would be more suited to a more specific research question, meaning a systematic review would be more appropriate. Instead, this scoping review aims to map the methodological trends used to determine the relationship between stimulus and response in football.

3.2 Methods

3.2.1 Search Terms & Information Sources

A scoping review of the available literature was conducted per the PRISMA Extension for Scoping Reviews (PRISMA-ScR) Checklist and Explanation (Tricco et al., 2018). Ethical approval was not sought as the data collected for this study is available in the public domain. SportDiscus, Pubmed, Web of Science and Scopus were researched for relevant publications before 19/06/2020. These databases were deemed to represent most of the relevant literature. The search terms involved using the keywords Soccer and Football and each associated with the terms: Bone OR Mechanical OR Physiological OR Muscle or Tendon AND Soccer or Football AND Change* OR Increas* OR Improve* OR Adapt* OR Respons* OR Decreas*.

3.2.2 Eligibility Criteria

Peer-reviewed articles in the English language that measured an adaptive response to a training stimulus in healthy, uninjured male professional, semi-professional, youth, and university level football players were eligible for analysis. Conference proceedings, book chapters, abstracts and reviews were excluded from the analysis.

3.2.3 Screening & Data Extraction

Results from all databases were combined, and any duplicate studies were removed. Studies were initially screened by title and abstract by three authors (AS, BD & MR). Included studies were examined by full text. A two-step approach was adopted for the extraction and organisation of data.

1. An excel spreadsheet (Microsoft Corporation, Redmond, WA) was used to collect and organise the extracted data from each study under the following headings: Title, duration of the study, stimulus, no. of measurements in the study period, outcome measure, method of obtaining outcome measure, and any additional relevant details.

2. Outcome measures were then categorised by response type, level, and measurement category. Details of how the outcome measures were categorised can be found further on in the methods section. Outcome measures were extracted from the methods and results section of each paper. In some cases, variables may not have been mentioned in the methods section as they are sub-variables of the primary outcome measure (i.e. peak power during a countermovement jump); these sub-variables were included as an outcome measure provided it was clear how they have been measured. Outcome measures mentioned in the results with no indication of how they have been measured were disregarded. However, in the rare case that a reference was made to a paper from which the method was replicated, this paper was reviewed, and the methods were extracted.

The following assumptions and simplifications were made to organise the extracted data under the following headings: Stimulus, Response Type, Duration and Frequency of Measurements, Level and Outcome Measures. Figure 4 illustrates the logical flow of information that the results section will follow. This is to familiarise the reader with the terminology used in this section and how the results section will be segmented.

3.2.4 Stimulus

The stimulus was separated into categories; football, strength, metabolic conditioning, detraining, and combinations of these categories, such as football and metabolic conditioning. Football interventions refer to any intervention or observation based on football activities, such as small-sided games or matches. Strength interventions refer to any intervention aimed at improving players' musculoskeletal characteristics through strength, explosive, or plyometric training protocols, either gym or pitch-based. Metabolic conditioning intervention refers to any intervention involving conditioning protocols aimed at improving cardiovascular fitness, either through pitch or gym-based interventions. Examples of such interventions include speed endurance interventions on pitch or spin bike sessions in the gym. Detraining interventions refer to any intervention where responses were measured during a period of inactivity. Combinations of both protocols were used in studies where the stimulus of multiple interventions was quantified, i.e. comparing responses to football training vs speed endurance training. This was labelled as ‘football & metabolic conditioning’. Based on the eligibility criteria of the studies included in this review, all subjects would have performed football activities alongside interventions, for example, strength interventions. However, if the football stimulus was not quantified in detail (i.e. quantification of training demands), the study intervention was labelled solely as ‘strength’ instead of a combination of ‘strength & football’.

3.2.5 Response Type

Outcome measures were defined by the nature of their response and were defined as a transient response or an adaptive response. A transient response was defined as a temporary change in a physiological process that occurs in response to an external stimulus. An example according to this definition is a creatine kinase response to intense exercise, which rises with intense training and then returns to baseline levels at rest. An adaptive response was defined as a physiological change in outcome in response to an external stimulus. An example according to this definition is a change in testosterone levels or a change in lactate threshold ; these responses change over time and stabilise to ‘new’ baseline levels in response to stimuli, i.e. higher testosterone levels or an ability to run at a faster speed before hitting lactate threshold. These definitions were also used to differentiate between studies that only measured transient responses or adaptive responses, not both. For example, a study that only used one outcome measure, a creatine kinase response to football training, would fall under transient only. A study that solely measured a change in testosterone levels would fall under adaptive only. Studies that used both transient and adaptive responses in their design were labelled as adaptive & transient. An example of this would be a study that measured creatine kinase responses to football training and a change in countermovement jump height in response to football training.

3.2.6 Duration & Frequency. of Measurements

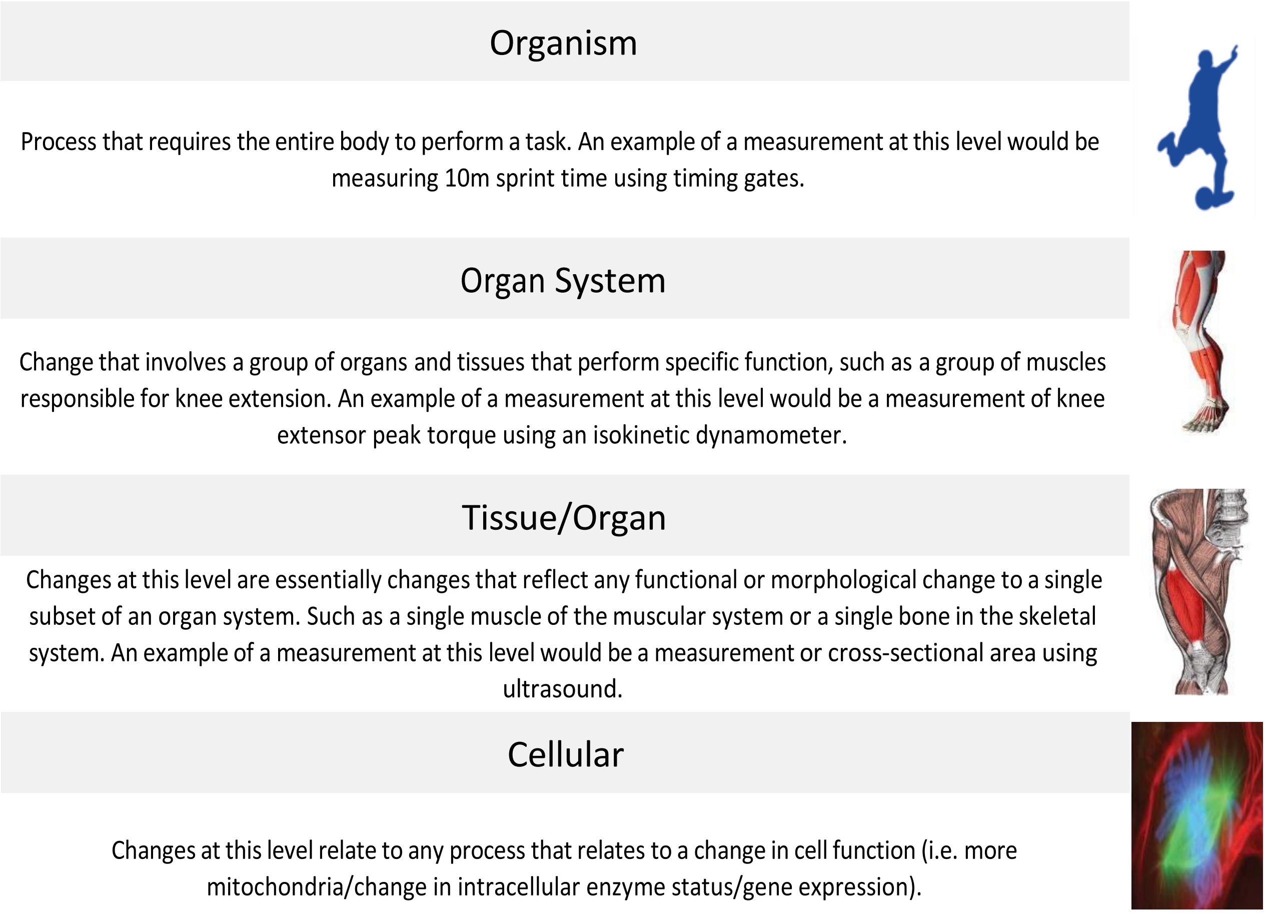
In studies where the duration was specified in months or days, this was converted into weeks (i.e. 11 months or 7 days was converted to 48 weeks and 1 week, respectively). In studies where only the name of the months was mentioned as measurement time points, such as October and January, it was assumed the measurement took place at the same time in each month (i.e. 1st of October and 1st of January, leading to a duration of 3 months or 12 weeks). Studies that took place over a year or longer were labelled “52 Weeks+”. Studies that performed measurements in response to a single test, training session or match, such as responses to a simulated football protocol, small sided-games intervention, or football match, were labelled as 1 day. Furthermore, studies that performed measurements in response to multiple tests, training sessions or matches, without specifying the duration between each intervention, were also labelled as 1 day. Studies of this nature measured responses to multiple matches, tests, or training sessions but were not measuring the change in response to accumulated interventions over a specified time. Instead, these studies measured responses to a single match, test, or training session and repeated these protocols several times. As a result, the duration for studies of this nature were labelled as 1 day.

Additionally, various study designs were employed where studies took measurements at different frequencies over similar periods. For example, some studies took measurements during a training session, whereas others took measurements before and after. Furthermore, the same study may have taken measures pre- and post-training, whilst other measures may have been collected during a training session. This is a consequence of the nature of the outcome measure. Therefore, the frequency of measurements during a study was classified into five categories: during, post, pre & post, pre, mid & post and numerous. Studies that focused on measuring responses during single training sessions or games were categorised as 'during'. Studies that solely measured responses at the end of a study period were categorised as 'post'. Studies that measured responses before and after the study period were categorised as 'pre & post'. Studies that measured responses before, at the halfway point, and after the study period were categorised as 'pre, mid & post'. Studies that took more than three measurements during a study period or at irregular intervals that could not be labelled 'pre, mid and post' were labelled as numerous.

3.2.7 Level

The study of anatomy categorises the body into multiple levels; subatomic particles, atoms, molecules, macromolecules, cells, tissue, organ, organ system and organism (Shier et al., 2019). However, for the purposes of this study, the focus will be on the cellular, tissue, organ, organ system, and organism levels; with the tissue and organ levels combined into one system. The definitions of each system are outlined in Figure 3.1. A decision tree (Figure 3.2) demonstrates the process for categorising an outcome measure by level. If a measure can be obtained without using the whole body, then it was categorised by the outcome measure's level, not the test's level.

For example, if bicep femoris activity is measured during a running test, the outcome measure is of the tissue, not the running test. Thus it is taken at the tissue/organ level. Any test that isolates a group of muscles, such as a leg press 1RM, was categorised as an outcome measure taken at the organ system level. Whilst it can be argued that the leg press involves the entire body, the purpose of this outcome measure is not to evaluate the synchronized performance of the whole body specifically.



Organism

Process that requires the entire body to perform a task. An example of a measurement at this level would be measuring 10m sprint time using timing gates.

Organ System

Change that involves a group of organs and tissues that perform specific function, such as a group of muscles responsible for knee extension. An example of a measurement at this level would be a measurement of knee extensor peak torque using an isokinetic dynamometer.

Tissue/Organ

Changes at this level are essentially changes that reflect any functional or morphological change to a single subset of an organ system. Such as a single muscle of the muscular system or a single bone in the skeletal system. An example of a measurement at this level would be a measurement or cross-sectional area using ultrasound.

Cellular

Changes at this level relate to any process that relates to a change in cell function (i.e. more mitochondria/change in intracellular enzyme status/gene expression).

Figure 3.1 Classification of levels in the body

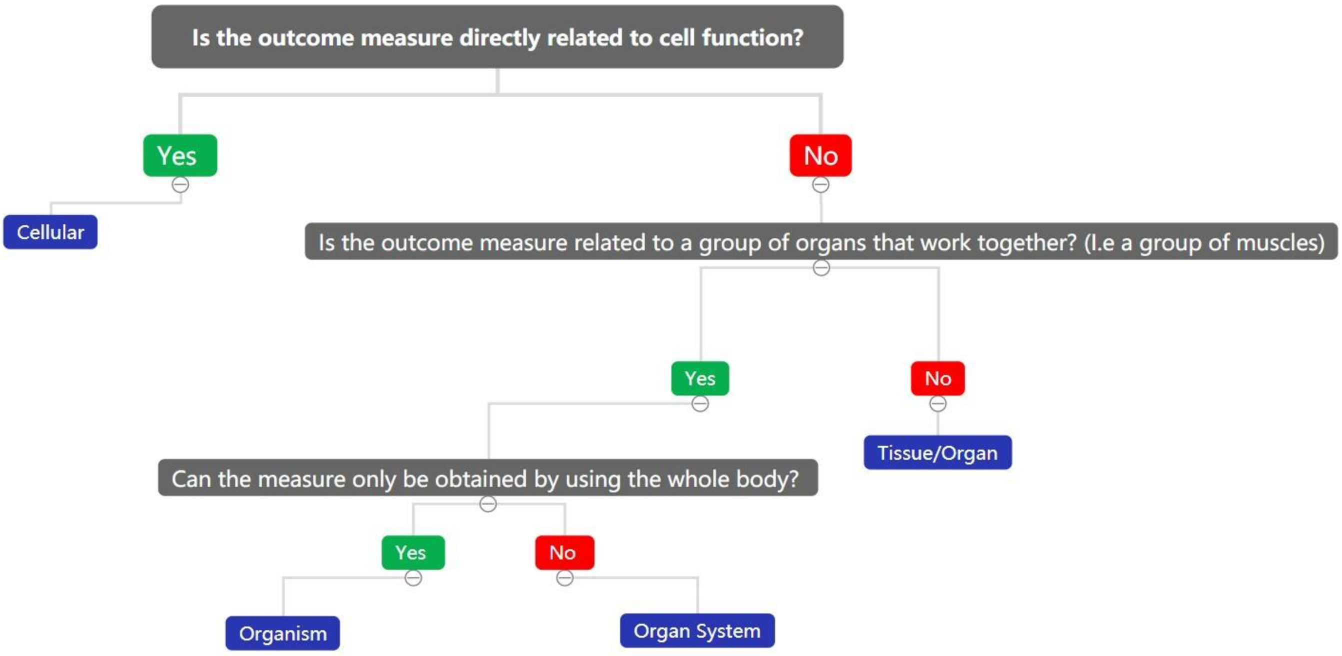


Figure 3.2 Decision tree used to categorise outcome measures into levels.

3.2.7 Outcome Measure

Outcome measures were categorised by the outcome measure only, not the scenario in which the outcome measure occurred in. For example, if heart rate was taken during a running test, the outcome measure was simply referred to as heart rate. If an outcome measure was deemed too vague on its own, for example: ‘hip angular velocity, the context of this measure was included for clarity, leading to the example outcome measure being labelled ‘hip angular velocity during kicking test’. Measures of sprinting speed or agility were categorised based on the distance used in the study or the type of agility test (i.e., 10m sprint time/agility t-test time). Outcome measures of jump performance were categorised based on the kind of jump being measured and the measurement variable taken from that jump (i.e., drop jump height/drop jump RSI). Outcome measures associated with muscle force or toque measured using an isokinetic dynamometer were categorised based on the role that group of muscles has on joint movement (i.e., hamstring peak force would be knee flexor peak force).

3.2.8 Outcome Measure Category

Outcome measures were grouped under broad descriptive categories in the results section for the reader’s convenience. Many of the categories may be intuitive; in some papers, the purpose of an outcome measure was specially mentioned, i.e. lactate was used as a marker of muscle damage, or countermovement jump height was used to measure lower-body power. However, some papers failed to categorise specific markers on the cellular level. In this case, molecules and metabolites that were unfamiliar to the authors were referenced against the open chemistry database PubChem (Kim et al., 2021) or The Human Metabolome Database (HMDB) (Wishart et al., 2018), which guided the categorisation.

Organism Level

Agility: measures related to measures assessing change of direction performance.

Anatomical: measures related to fat, lean tissue, and bone mass of the full-body, such as total body bone mineral density.

Balance: measures using tests designed to examine lower limb balance, such as a Y balance test.

Fitness: measures used to assess running performance, such as a YOYO IR1 test.

Power: measures used to assess the ability to produce force rapidly, such as countermovement jump height.

Speed: measures used to assess acceleration and top speeds, such as 5m or 30m speed.

Strength: measures used to assess the ability to produce or absorb force, such as a 1RM Squat

Subjective: measures used to assess participants’ feelings or opinions on a task, such as RPE.

Organ System Level

Anatomical: measures related to fat, lean tissue, and bone mass at specific sites in the body.

Contractile properties: measures used to assess the ability of a group of muscles to contract, such as hamstring muscle activity.

Fitness: measures used to assess the performance of systems in the body related to cardiorespiratory fitness, such as V̇ O2 max.

ROM: measures used to assess muscle flexibility or joint range of motion, such as a Thomas test.

Strength: measures used to assess the ability of an isolated muscle group to produce force, such as concentric knee flexor peak torque.

Temperature: measures used to assess the temperature of systems in the body, such as core temperature.

Tissue/Organ Level

Anatomical: measures related to fat, lean tissue, and bone mass of the entire body at specific tissues in the body, such as bone mineral density.

Contractile properties: measures used to assess a specific muscle's ability to contract and produce force, such as vastus lateralis muscle activity.

HR/Cardiovascular: measures used to assess the function and performance of the heart, such as Max HR

Cellular Level

Antioxidant Status: measures of damaging species, such as reactive oxygen, nitrogen, and chlorine species.

Blood Status: markers of blood acid balance/blood cell status such as red blood cell count.

Bone and Collagen Tissue: markers that reflect the status of bone/collagen turnover.

Cardiovascular: markers reflecting the status of cardiovascular cell structure and function.

Endocrine Response: markers that reflect hormone levels, such as testosterone or cortisol.

Immune Response: markers related to immune responses/inflammation, such as Immunoglobulin G levels.

Muscle Status: markers related to muscle structure, function and muscle damage, such as muscle fiber type, creatine kinase or lactate.

Nutrient Metabolism: markers related to macro (fats & carbs) and micronutrient metabolism, such as Iron levels.

3.3 Results

Due to the extensive nature of the results section, figure 3.3 below is presented to provide clarity for the reader. This figure outlines how the information will flow in the results section and demonstrates the terminology used in the methods section under the headings; stimulus, response, duration, level, and outcome measure will appear.

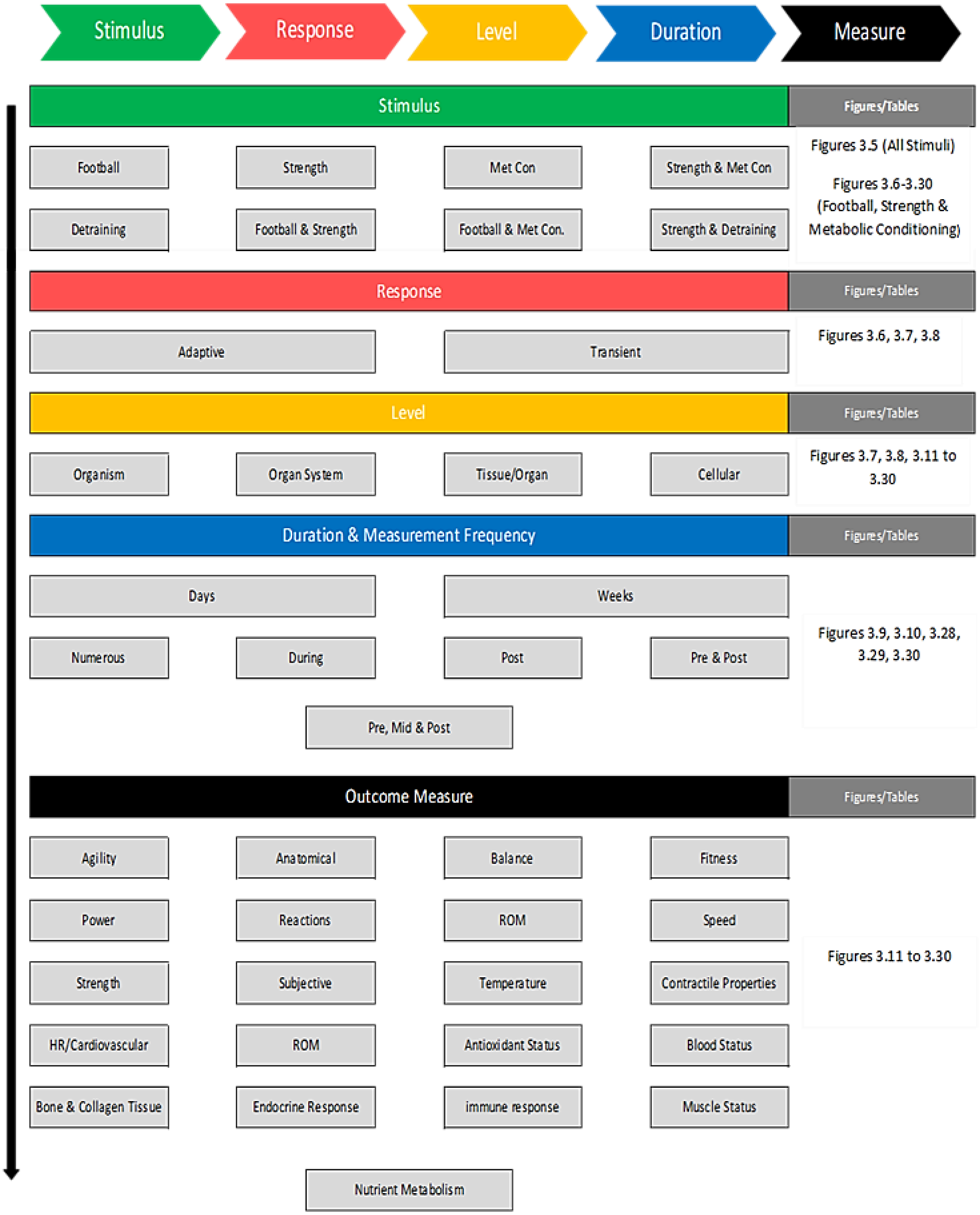


Figure 3.3 The logical flow of information and terminology used throughout the results section.

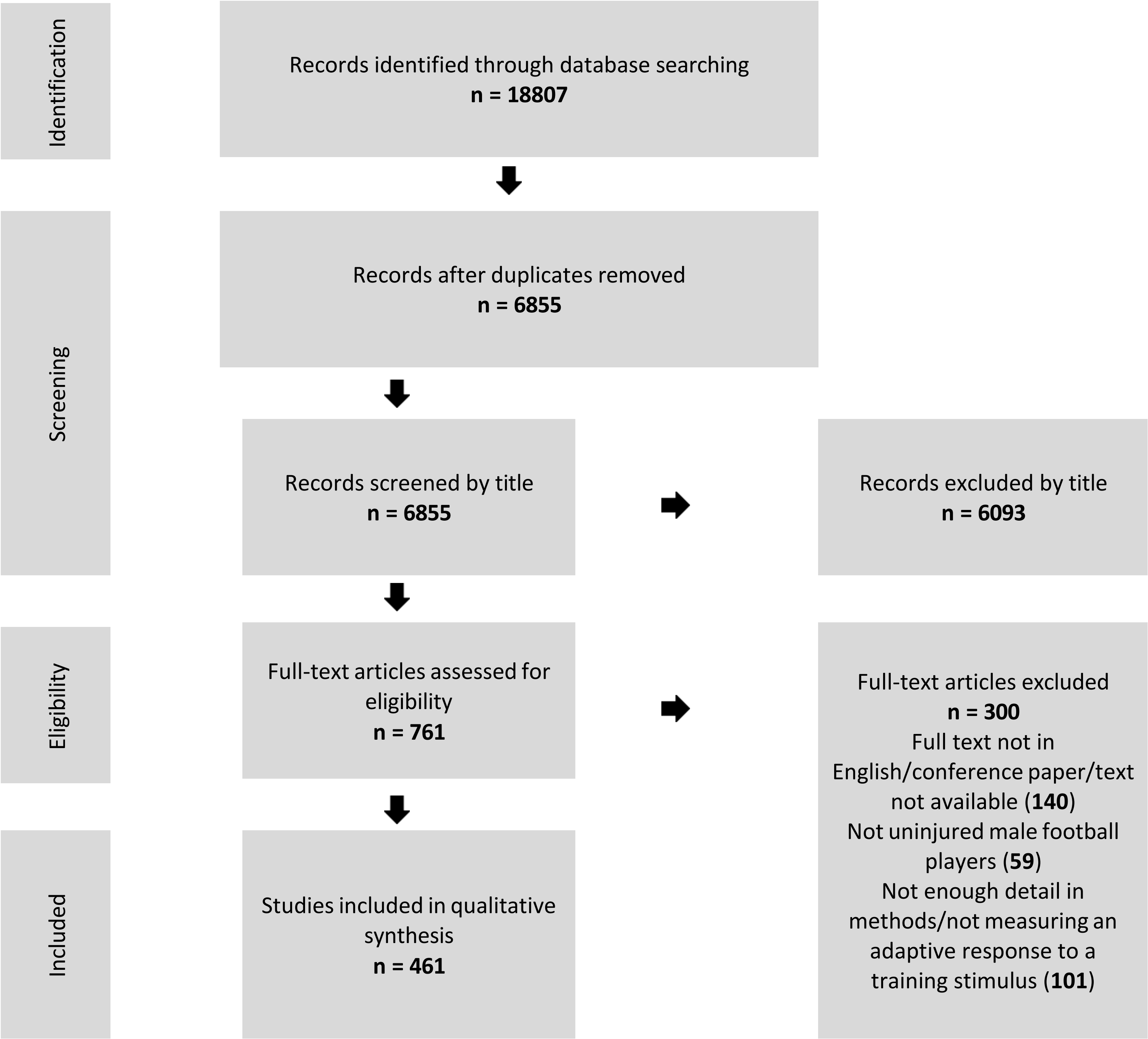


Figure 3.4 Prisma diagram of the study selection process

3.3.1 Study Design

3.3.1.1 Stimulus

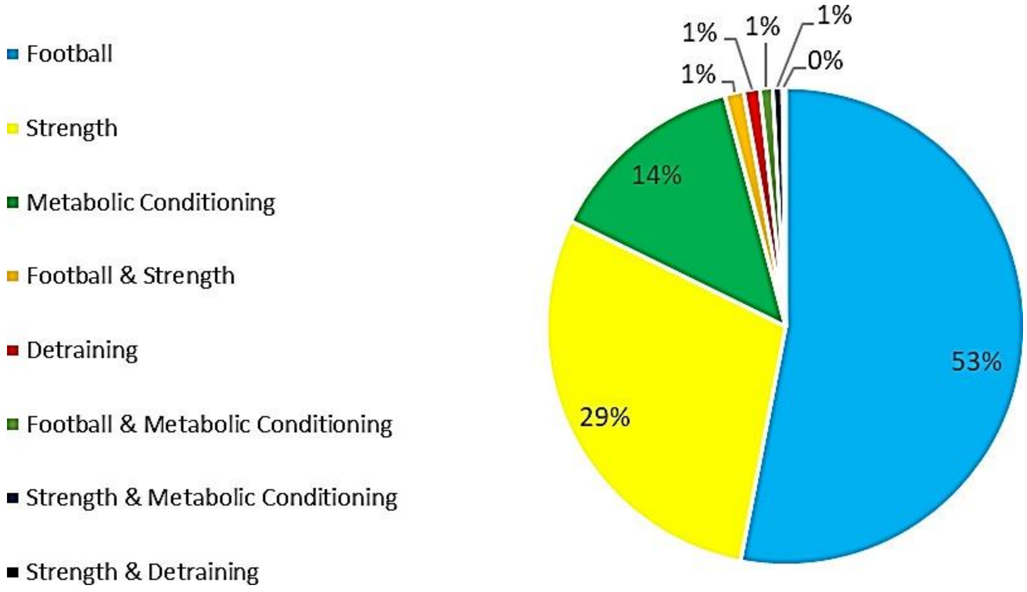
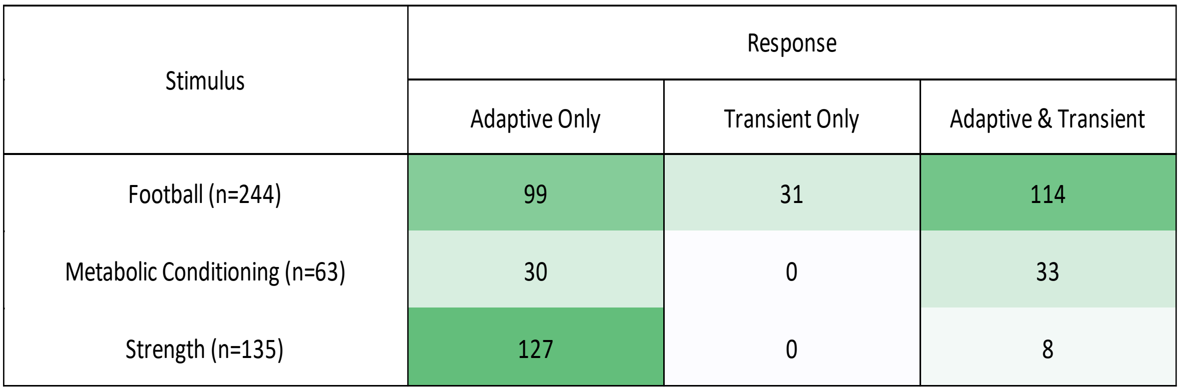


Figure 3.5 Breakdown of studies by stimulus

Figure 3.5 demonstrates the breakdown of stimuli used in the 461 studies. The most common stimuli were categorised as football, strength, and metabolic conditioning, used in 244, 135, and 63 studies. Based on this breakdown, the results section will now focus on the responses to these three predominant stimuli.

3.3.1.2 Response



Stimulus	Response		
	Adaptive Only	Transient Only	Adaptive & Transient
Football (n=244)	99	31	114
Metabolic Conditioning (n=63)	30	0	33
Strength (n=135)	127	0	8

Figure 3.6 Breakdown of the number of studies using adaptive, transient or a combination of both adaptive & transient responses relative to a stimulus.

A breakdown of the number of studies using adaptive, transient or a combination of both responses relative to a stimulus is shown in Figure 3.6. Of the 244 studies using football as a stimulus, 118 studies used a combination of adaptive and transient responses, 87 studies focused solely on measuring adaptive responses, whilst 39 studies focused exclusively on measuring transient responses. 127 of the 135 studies using strength as a stimulus solely focused on measuring adaptive responses, with 8 studies using a combination of adaptive and transient responses. Of the 63 studies using metabolic conditioning as a stimulus, 36 studies used a combination of adaptive and transient responses, 23 studies focused solely on measuring adaptive responses and 4 studies focused solely on measuring transient responses.

3.2.1.3 Level

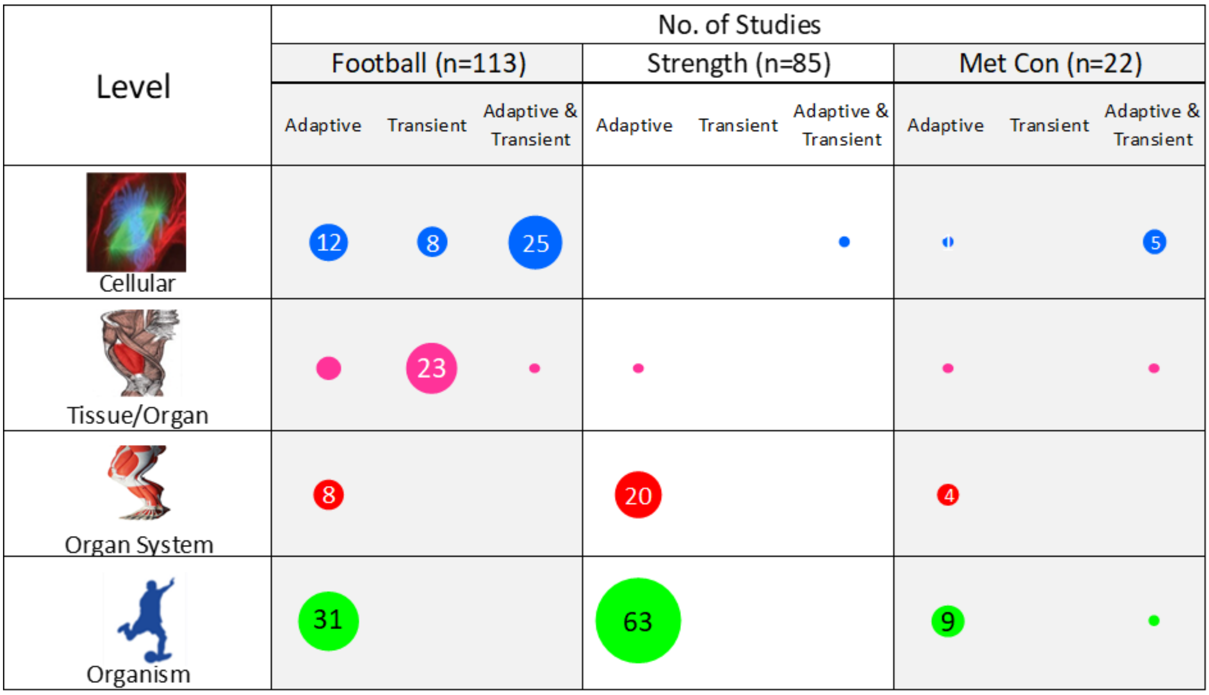


Figure 3.7 Number of studies measuring responses at one level, by stimulus and response (n=220). Individual dots of a lower value are labelled, whilst some are not; this is to help with size differentiation.

From the 113 studies measuring responses to football on 1 level, 31 studies measured adaptive responses at the organism level. The second most common level measured was the tissue/organ level. 23 studies measuring at this level focused on measured transient responses. The cellular level was measured in 23 studies using a combination of adaptive and transient responses. 85 out of 135 studies using strength as a stimulus, measured responses on 1 level. The most common level observed when measuring responses to strength on 1 level was the organism level, with 63 of the 85 studies measuring adaptive responses at this level. Similarly, the most common level observed when measuring responses to metabolic conditioning on 1 level was the organism level, with 9 of the 22 studies measuring adaptive responses at this level.

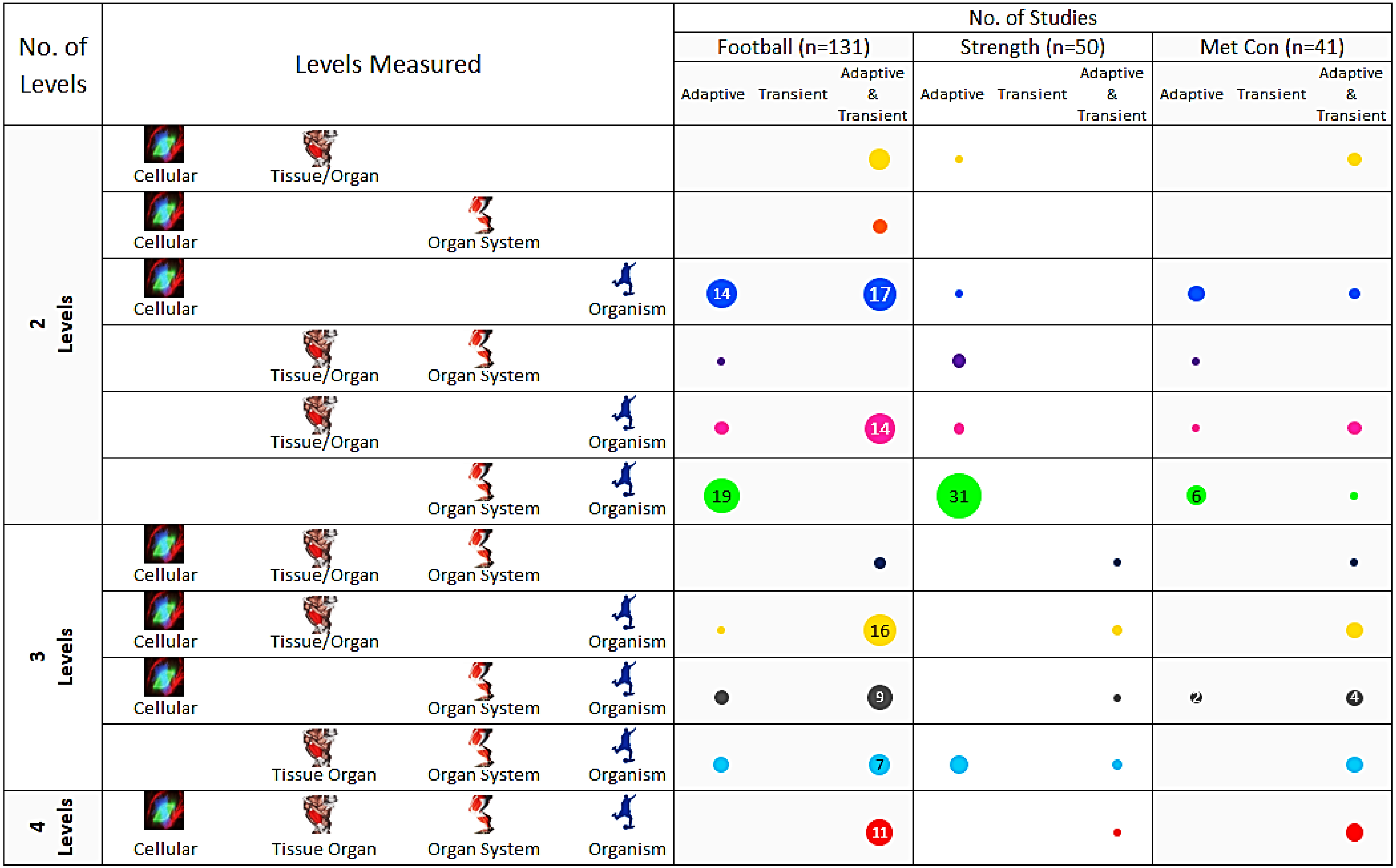


Figure 3.8 Number of studies measuring responses at multiple levels, by stimulus and response (n=222). Individual dots of a lower value are labelled, whilst some are not; this is to help with size differentiation.

The breakdown of the remaining 222 studies, which measured responses across multiple levels, is illustrated in Figure 3.8. 78 of the 131 studies used football as a stimulus and measured responses across 2 levels, 42 studies measured responses across 3 levels, and 11 studies measured responses across 4 levels. The majority of football studies measuring responses across multiple levels did so using a combination of adaptive and transient responses.

Of the 50 studies using strength as a stimulus, 38 studies measured responses across 2 levels, 11 studies measured across 3 levels, with only 1 study measured responses across 4 levels. 41 studies using metabolic conditioning as a stimulus measured responses across multiple levels. 21 studies measured responses across 2 levels, 15 studies measured across 3 levels, and 5 studies measured responses across 4 levels. Like football studies, most studies measuring responses across multiple levels did so using a combination of adaptive and transient responses.

3.2.1.4 Duration & No. of Measurements

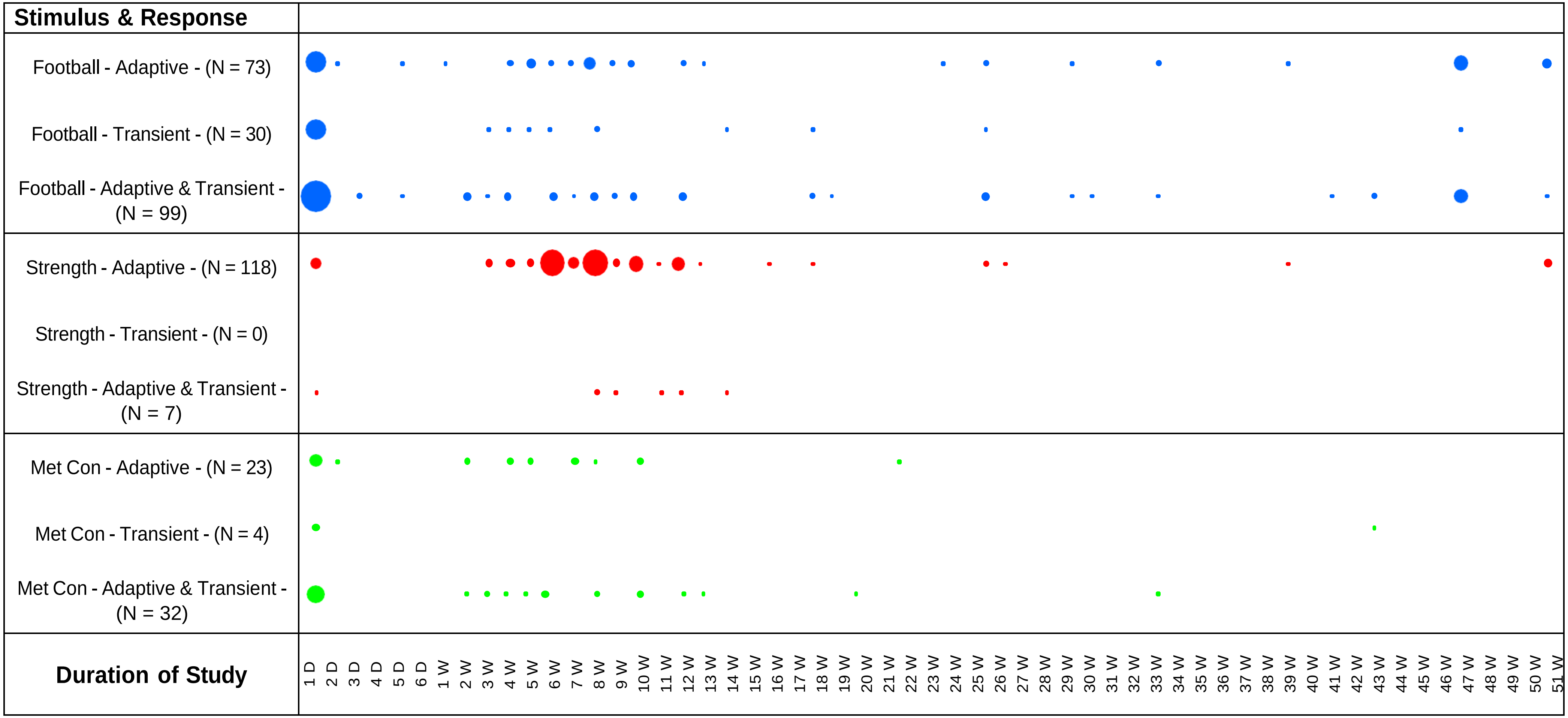


Figure 3.9 Illustration of the most common study durations used, depending on the nature of the stimulus and response.

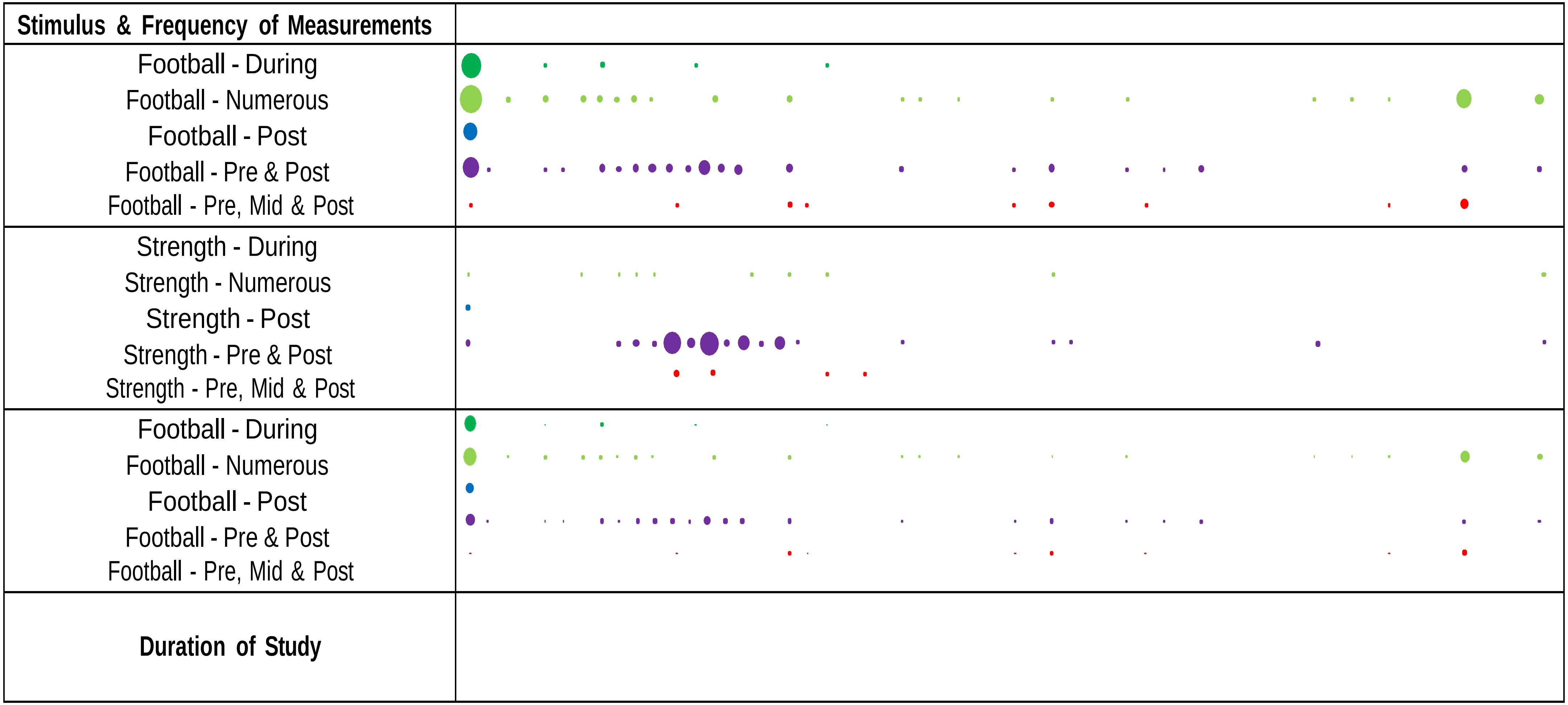


Figure 3.10 Durations used by studies against the stimulus and frequency of measurements**.**

The most common durations employed by studies measuring responses to football were 1 day, 48 weeks, and 8 weeks. In total, of the 244 studies measuring responses to football, 101 studies measured responses over 1 day, 30 studies measured responses over 48 weeks, and 15 studies measured responses over 8 weeks. Figure 3.10 demonstrates that the most common measurement frequencies used during football studies were during, numerous and post.

The most common durations employed by studies measuring responses to strength were 8 weeks, 6 weeks, 10 weeks, and 12 weeks. In total, of the 135 studies measuring responses to strength, 38 studies measured responses over 8 weeks, 36 studies measured responses over 6 weeks. 13 studies measured responses over 10 weeks, and 11 studies measured responses over 12 weeks. Figure 3.10 demonstrates that the most common measurement frequency used during strength studies measuring responses was pre & post.

The most common durations employed by studies measuring responses to metabolic conditioning were 1 day, 4 weeks, and 7 weeks. Of the 63 studies measuring responses to metabolic conditioning, 27 studies measured responses over 1 day. 5 studies measured responses over 10 weeks, and 4 studies measured responses over 6 weeks. Figure 3.10 demonstrates that the most common measurement frequencies used during metabolic conditioning studies measuring responses were during, and pre & post.

3.3.1.2 Summary of Study Design Section

Studies using football as a stimulus focused on measuring adaptive and transient responses in combination, followed by adaptive responses. The majority of studies measuring adaptive responses only measured responses on the organism and cellular level independently. In contrast, adaptive and transient responses to football were primarily measured on two levels, using a combination of measures taken at the cellular and organism levels. Most studies took measurements at numerous timepoints throughout the intervention, during, or pre & post-intervention. Most football interventions took place over 1 day.

Studies using strength as a stimulus focused on measuring adaptive responses. The majority of studies were measured on the organism level when measured on one level alone. Responses measured on two levels primarily used a combination of measures taken at the organ system and organism level. Most studies took measurements pre & post-intervention, with the most common study durations employed lasting 8, 6, 10 or 12 weeks.

Studies using metabolic conditioning as a stimulus focused on measuring adaptive and transient responses, followed by adaptive responses. The majority of studies measuring adaptive responses on one level measured responses at the organism level. Adaptive and transient responses were measured primarily on three levels. The majority of studies took measurements during the intervention or pre & post-intervention. Most metabolic conditioning studies took place over 1 day.

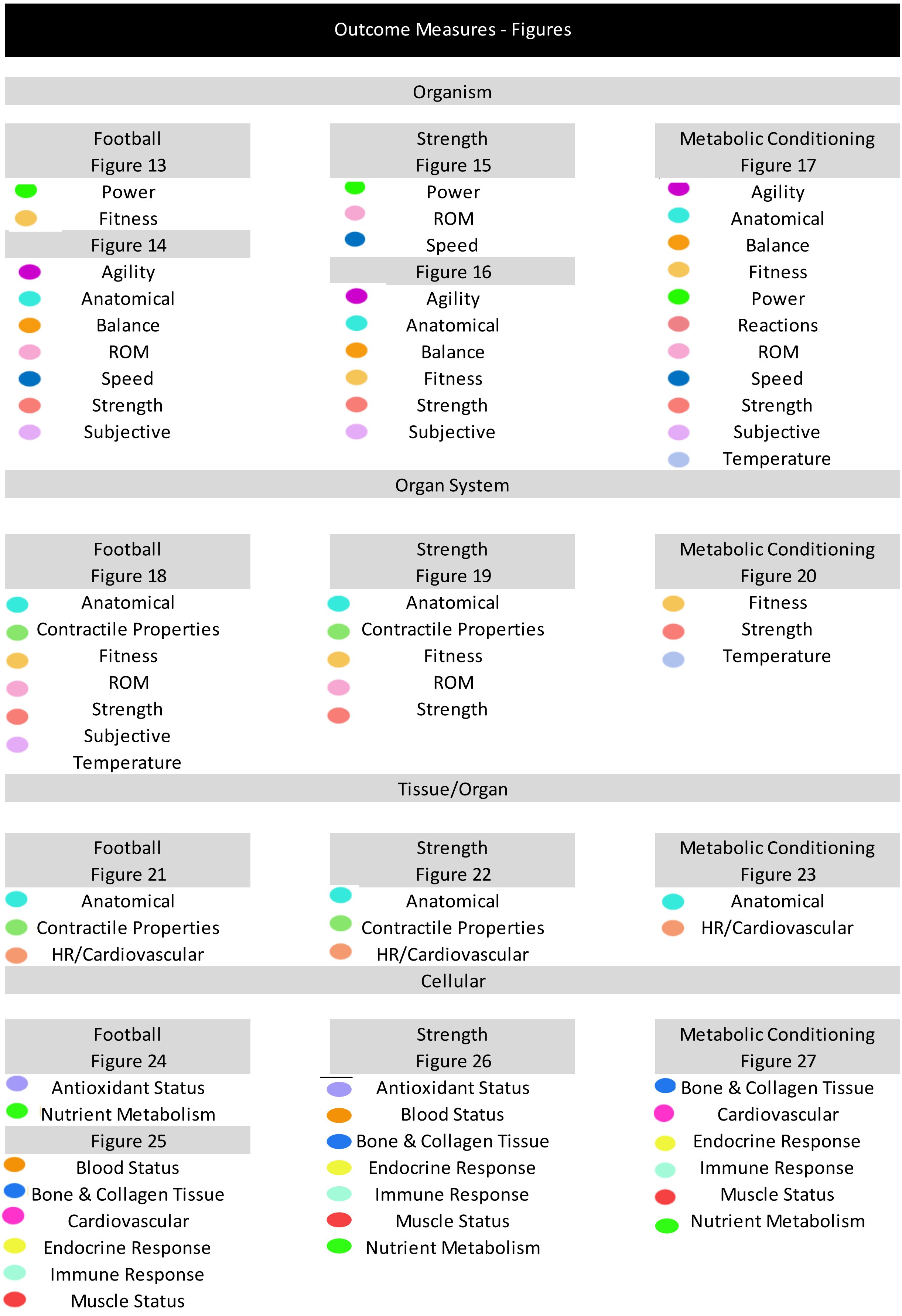
The following section will transition away from the design of studies to focus on the outcome measures used across all studies. These outcome measures will be categorised by the levels in the body they are measured at. Each level will then be dissected to identify the most common outcome measures used by football, strength, and metabolic conditioning studies. Furthermore, at each level, the outcome measures will be grouped into the outcome measure categories outlined in the methods section.

3.3.2 Outcome Measures

The outcome measures used to assess responses in all 461 studies resulted in 1170 unique measures. The subsequent figures were created using Gephi software (Bastian, Heymann & Jacomy, 2009). Due to the high volume of outcome measures, many figures will follow that display the outcome measures taken at each level under the stimuli football, strength, and metabolic conditioning. Furthermore, the outcome measures will be grouped under their respective outcome measurement category, as outlined in the methods section. However, at some levels, the information is too extensive to visualise. Therefore, two figures display all outcome measures for a particular stimulus, at a certain level, as required. Furthermore, to provide clarity for the reader, table 3.1 below provides the abbreviations used in the figures. Moreover, figure 3.11 outlines each figure that will follow and the information that will be displayed. This figure also acts as a legend for the outcome measure categories, as mentioned previously.

Table 3.1 Abbreviations used in outcome measure visuals.

Term	Abbreviation	Term	Abbreviation
Abduction	ABD	Immunoglobulin M	IgM
Active Knee Extension Range	AKE	Isokinetic	ISOK
Adductor	ADD	Isometric	ISO
Aerobic Threshold	AT	Knee Extensor	KE
Agility Test Time	AGTT	Knee Flexor	KF
Anaerobic Threshold	ANT	Lactate	LAC
Anterior-Posterior	Ant-Pos	Lactate Threshold	LT
Areal Bone Mineral Density	aBMD	Max Voluntary Contraction	MVC
Biceps Femoris	BF	Max voluntary Iso contraction	MVIC
Bone Mineral Apparent Density	BMAD	Mean Power	MP
Bone Mineral Content	BMC	Modified	Mod
Bone Mineral Density	BMD	Multiple Five Bounds Test	M5BT
Carboxy-Terminal Collagen Crosslinks	CTX	Peak Force	PF
Change of Direction	COD	Peak Power	PP
Concentric	CON	Peak Torque	PT
Contact Time	CT	Power Output	PO
Countermovement Jump	CMJ	Procollagen Type 1 Amino-Terminal Propeptide	P1NP
Countermovement Jump with Arm Swing	CMJA	Quadriceps	Quad
Creatine Phosphate	CP	Reactive Strength Index	RSI
Cross Sectional Area	CSA	Rectus Femoris	RF
Distance	Dist	Root Means Squared	RMS
Drop Jump	DJ	Single-Leg Horizontal Countermovement Jump	SL HCMJ
Eccentric	ECC	Squat Jump	SJ
Estimated	Est	Squat Jump with Arm Swing	SJA
Fascicle Length	FL	Total Body Less Head	TBLH
Forced Expiratory Volume	FEV	Tumour Necrosis Factor	TNF
Forced Vital Capacity	FVC	Vastus Intermedius	VI
Hamstring	Ham	Vastus Lateralis	VL
Heart Rate	HR	Vastus Medialis	VM
Heart Rate Variability	HRV	Velocity	vLT
High-Density Lipoprotein	HDL	Ventilation	Vent
Horizontal Countermovement Jump	HCMJ	Visual Analogue Scale	VAS
Horizontal Countermovement Jump with Arm Swing	HCMJA	Volumetric Bone Mineral Density	vBMD
Immunoglobulin A	IgA	Watts	W
Immunoglobulin G	IgG		



Outcome Measures - Figures

Organism

Football
Figure 13
● Power
● Fitness
Figure 14
● Agility
● Anatomical
● Balance
● ROM
● Speed
● Strength
● Subjective

Strength
Figure 15
● Power
● ROM
● Speed
Figure 16
● Agility
● Anatomical
● Balance
● Fitness
● Strength
● Subjective

Metabolic Conditioning
Figure 17
● Agility
● Anatomical
● Balance
● Fitness
● Power
● Reactions
● ROM
● Speed
● Strength
● Subjective
● Temperature

Organ System

Football
Figure 18
● Anatomical
● Contractile Properties
● Fitness
● ROM
● Strength
● Subjective
Temperature

Strength
Figure 19
● Anatomical
● Contractile Properties
● Fitness
● ROM
● Strength

Metabolic Conditioning
Figure 20
● Fitness
● Strength
● Temperature

Tissue/Organ

Football
Figure 21
● Anatomical
● Contractile Properties
● HR/Cardiovascular

Strength
Figure 22
● Anatomical
● Contractile Properties
● HR/Cardiovascular

Metabolic Conditioning
Figure 23
● Anatomical
● HR/Cardiovascular

Cellular

Football
Figure 24
● Antioxidant Status
● Nutrient Metabolism
Figure 25
● Blood Status
● Bone & Collagen Tissue
● Cardiovascular
● Endocrine Response
● Immune Response
● Muscle Status

Strength
Figure 26
● Antioxidant Status
● Blood Status
● Bone & Collagen Tissue
● Endocrine Response
● Immune Response
● Muscle Status
● Nutrient Metabolism

Metabolic Conditioning
Figure 27
● Bone & Collagen Tissue
● Cardiovascular
● Endocrine Response
● Immune Response
● Muscle Status
● Nutrient Metabolism

Figure 3.11 Outline of figures in the outcome measures section. As well as a legend for outcome measure categories

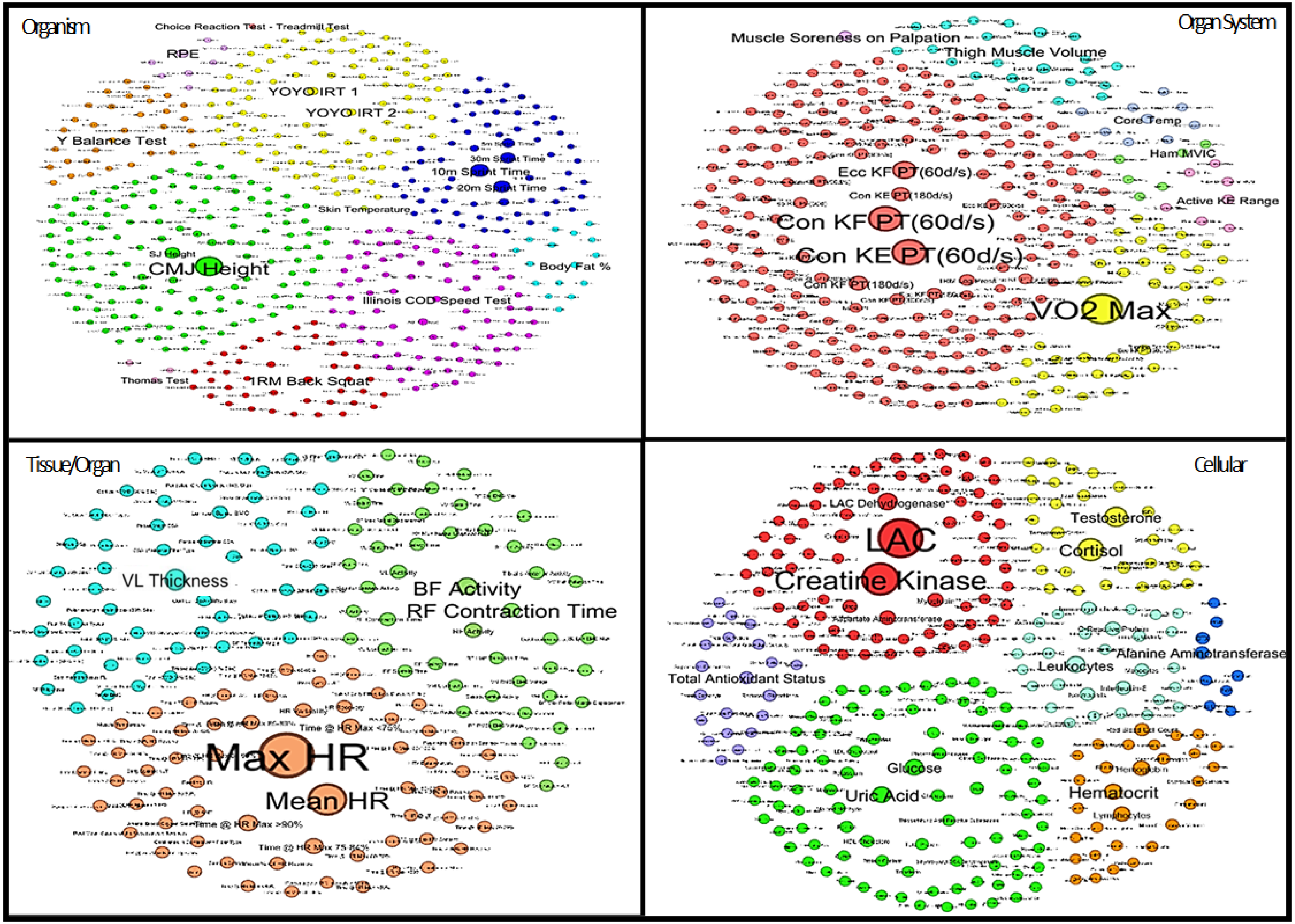


Figure 3.12 Outcome measures across all 4 levels of organisation in the body, specific measures have been highlighted for illustrative purposes.

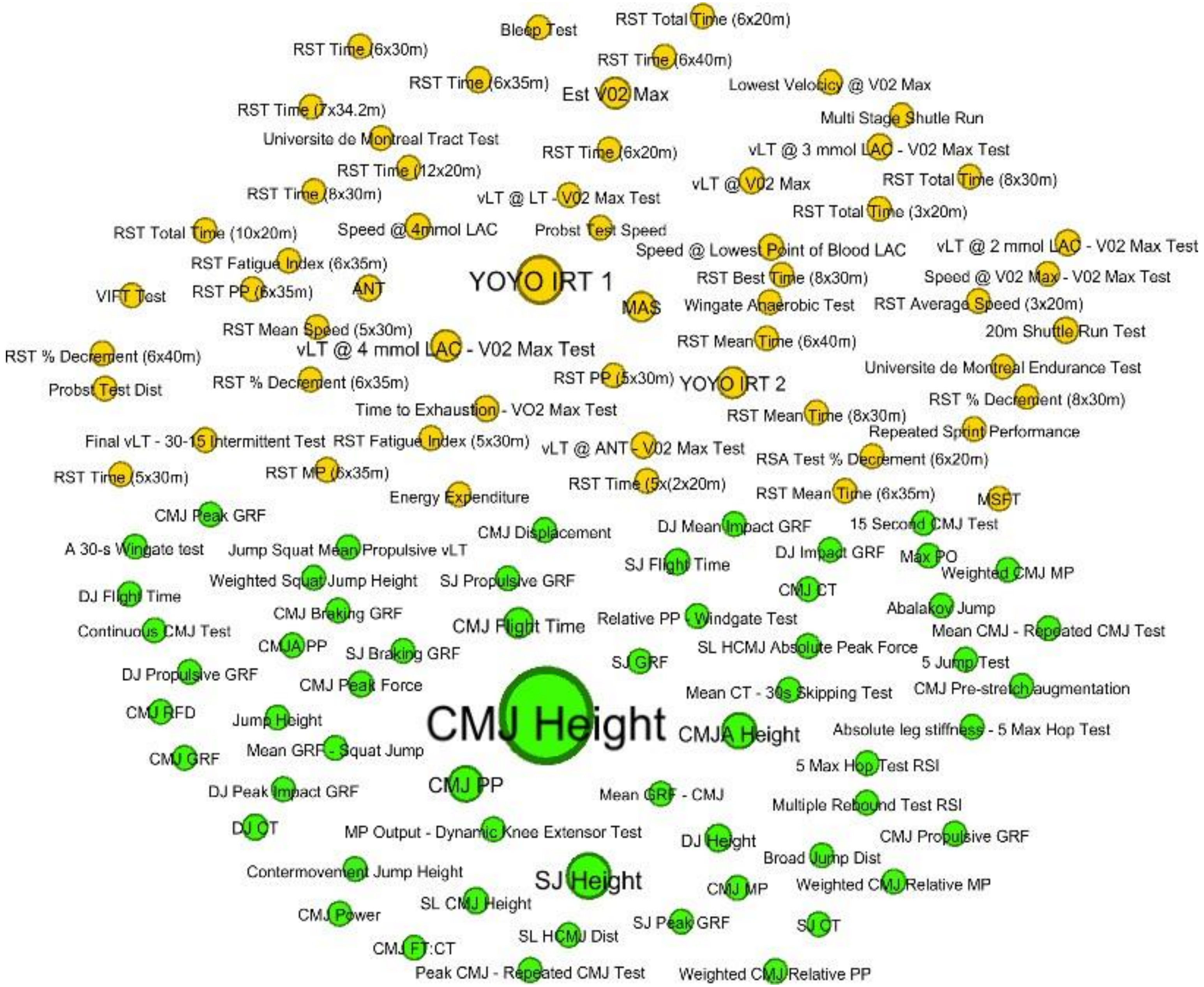


Figure 3.13 Fitness and power measures at the organism level using football as a stimulus.

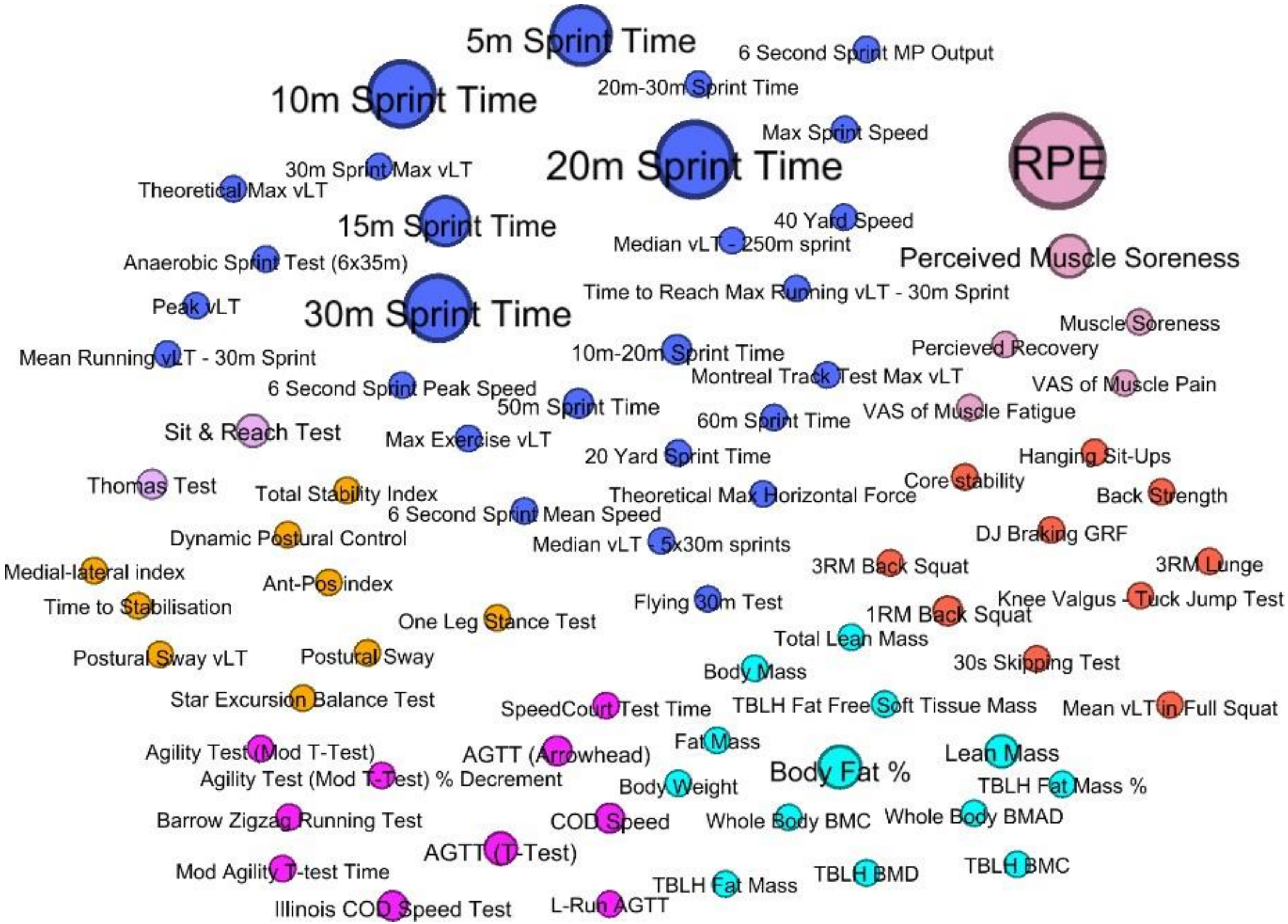


Figure 3.14 Agility, speed, strength, anatomical, ROM, balance and subjective measures at the organism level using football as a stimulus.

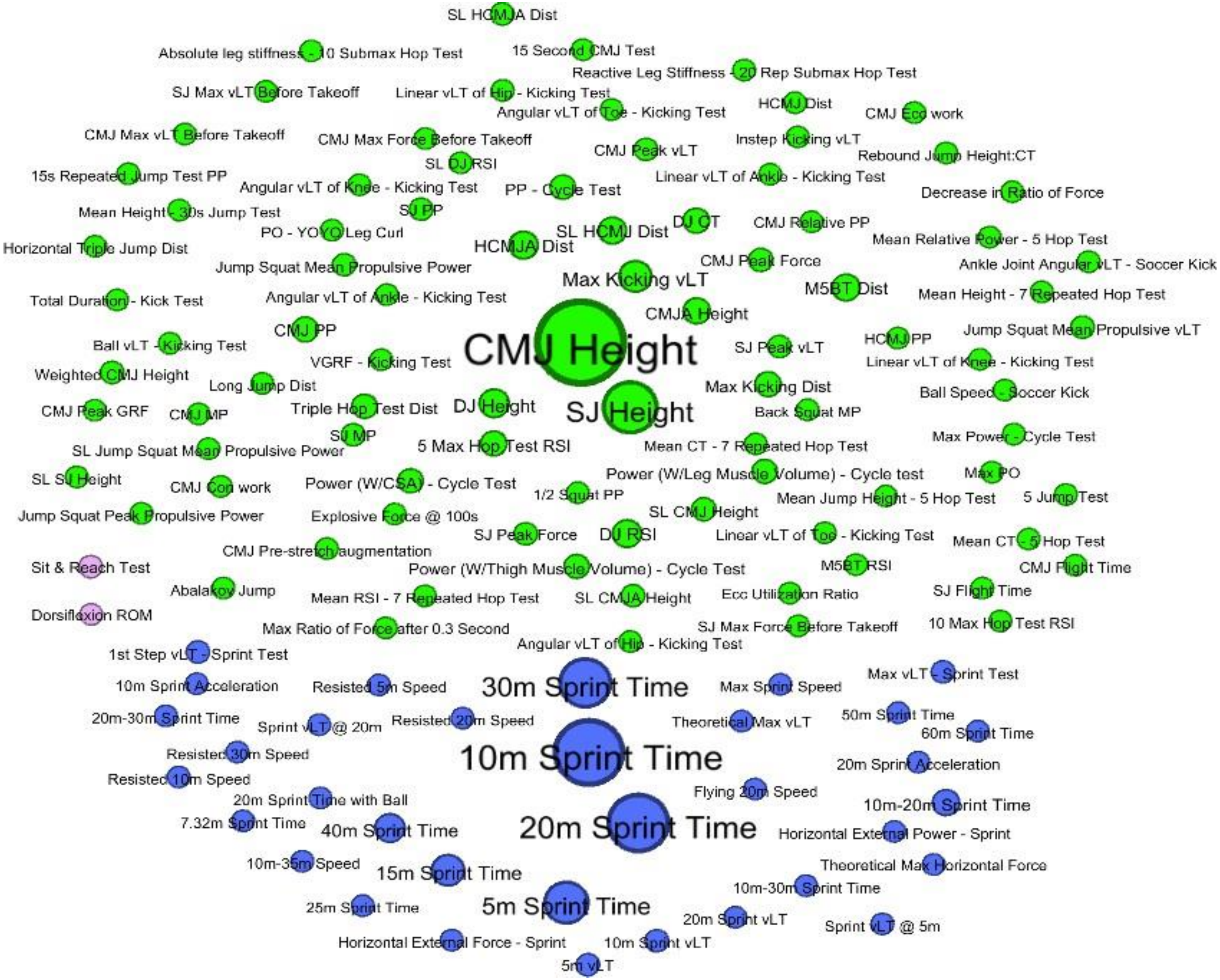


Figure 3.15 Power, speed and ROM measures at the organism level using strength as a stimulus.

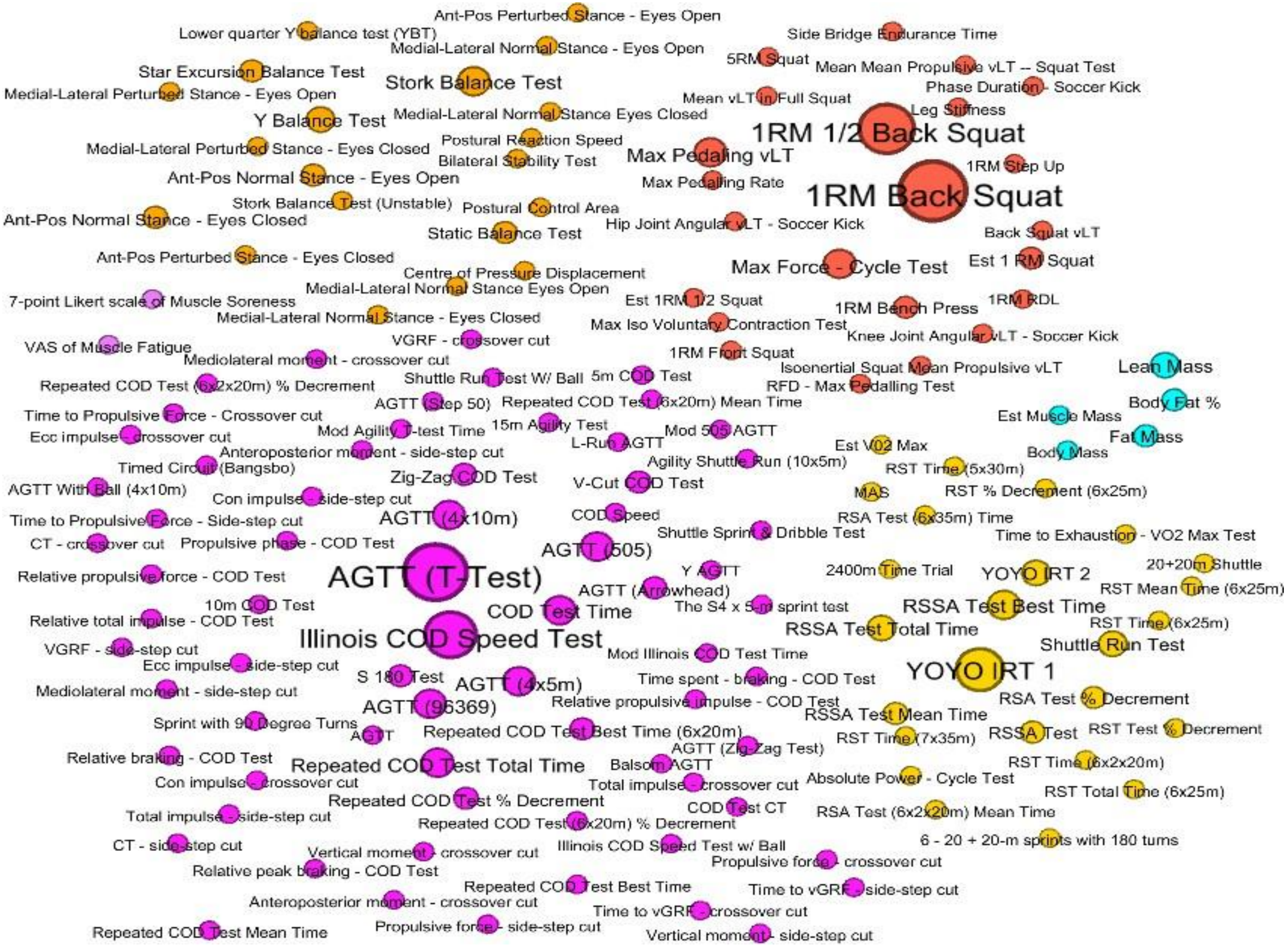


Figure 3.16 Agility, fitness, strength, anatomical and balance measures at the organism level using strength as a stimulus.

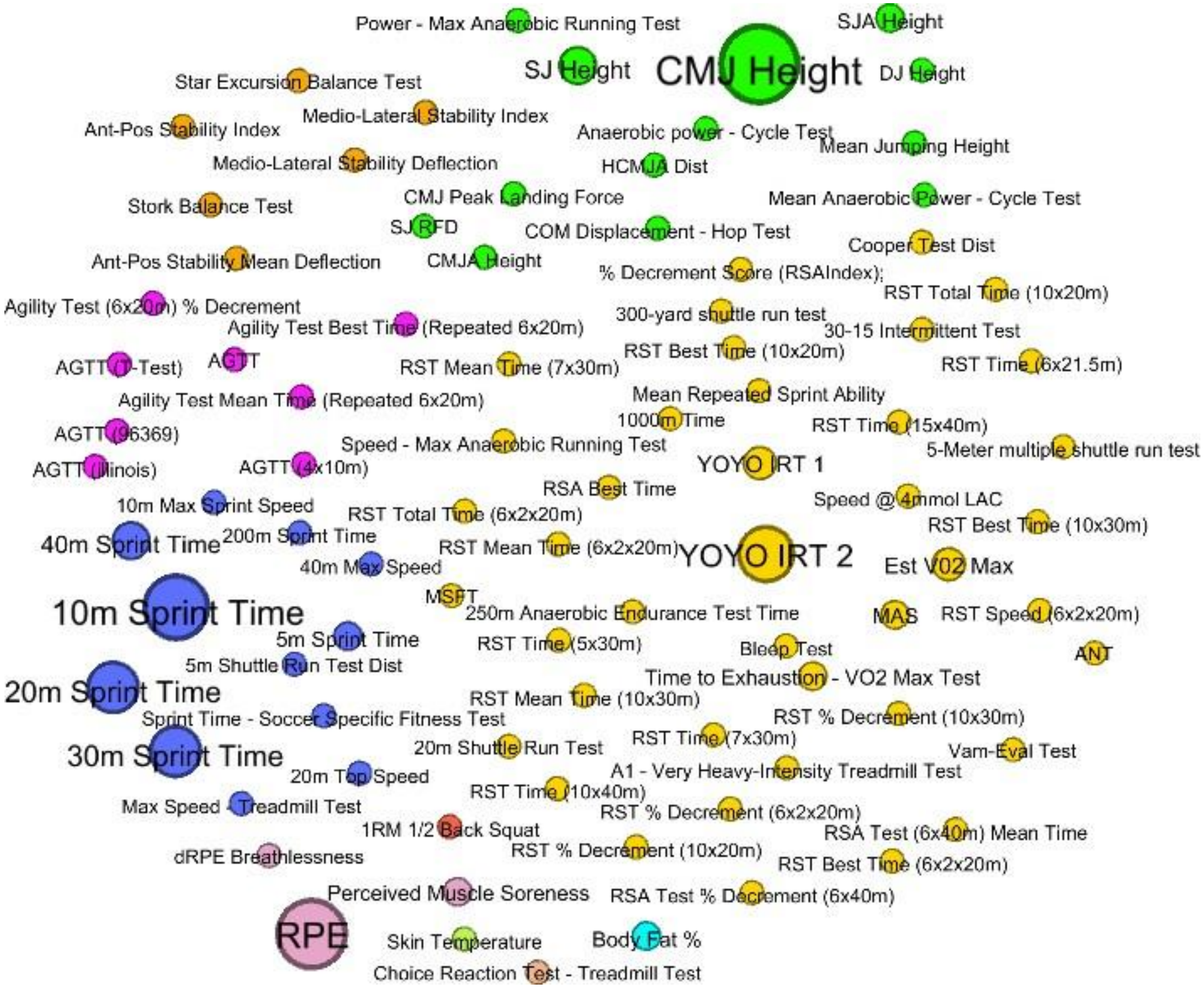


Figure 3.17 Speed, agility, fitness, strength, anatomical, temperature, reactions and balance measures at the organism level using metabolic conditioning as a stimulus

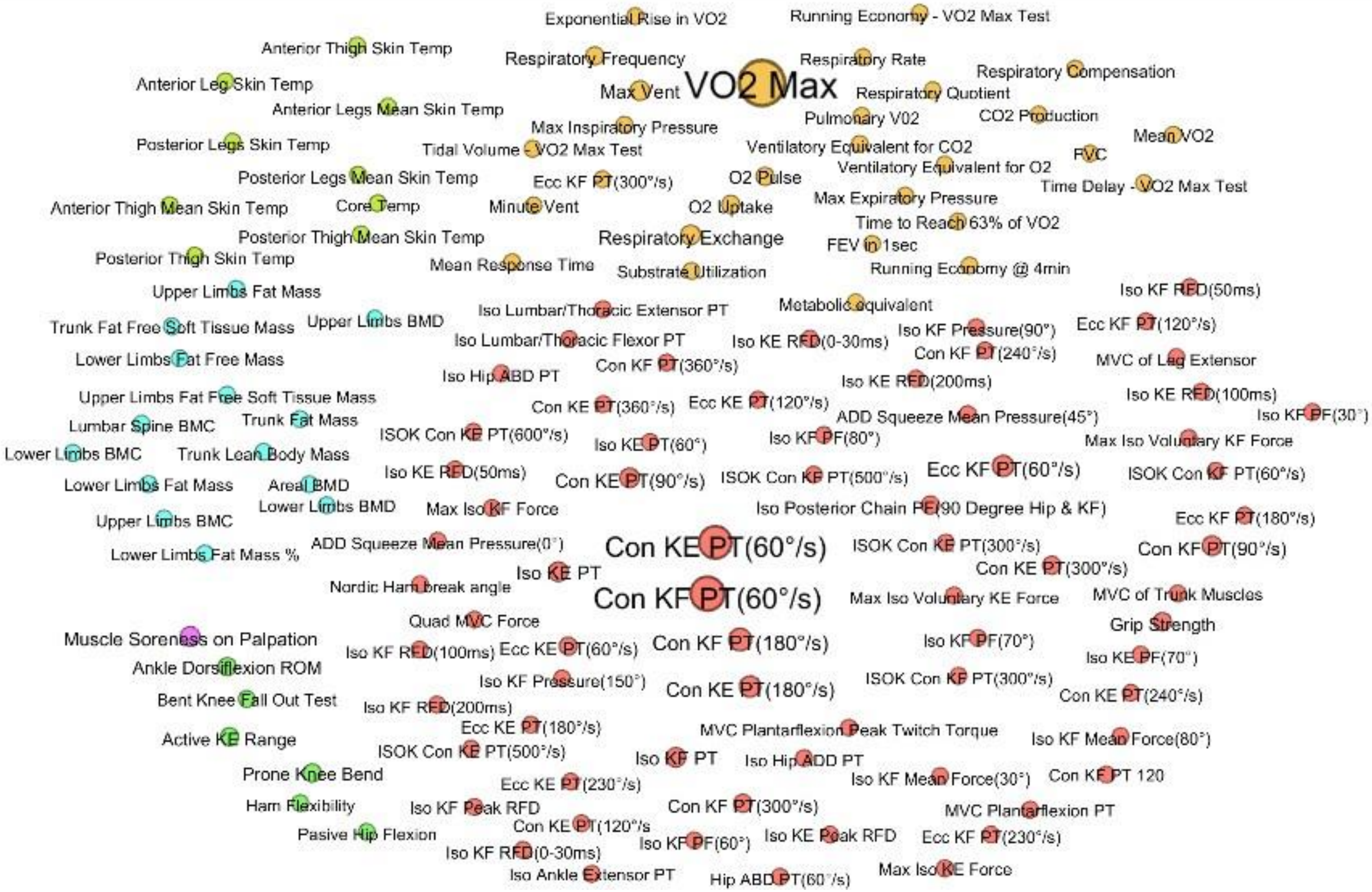


Figure 3.18 Strength, fitness, ROM, anatomical, contractile properties, subjective and temperature measures at the organ system level using football as a stimulus

Figure 3.19 Strength, fitness, ROM, anatomical and contractile property measures at the organ system level using strength as a stimulus

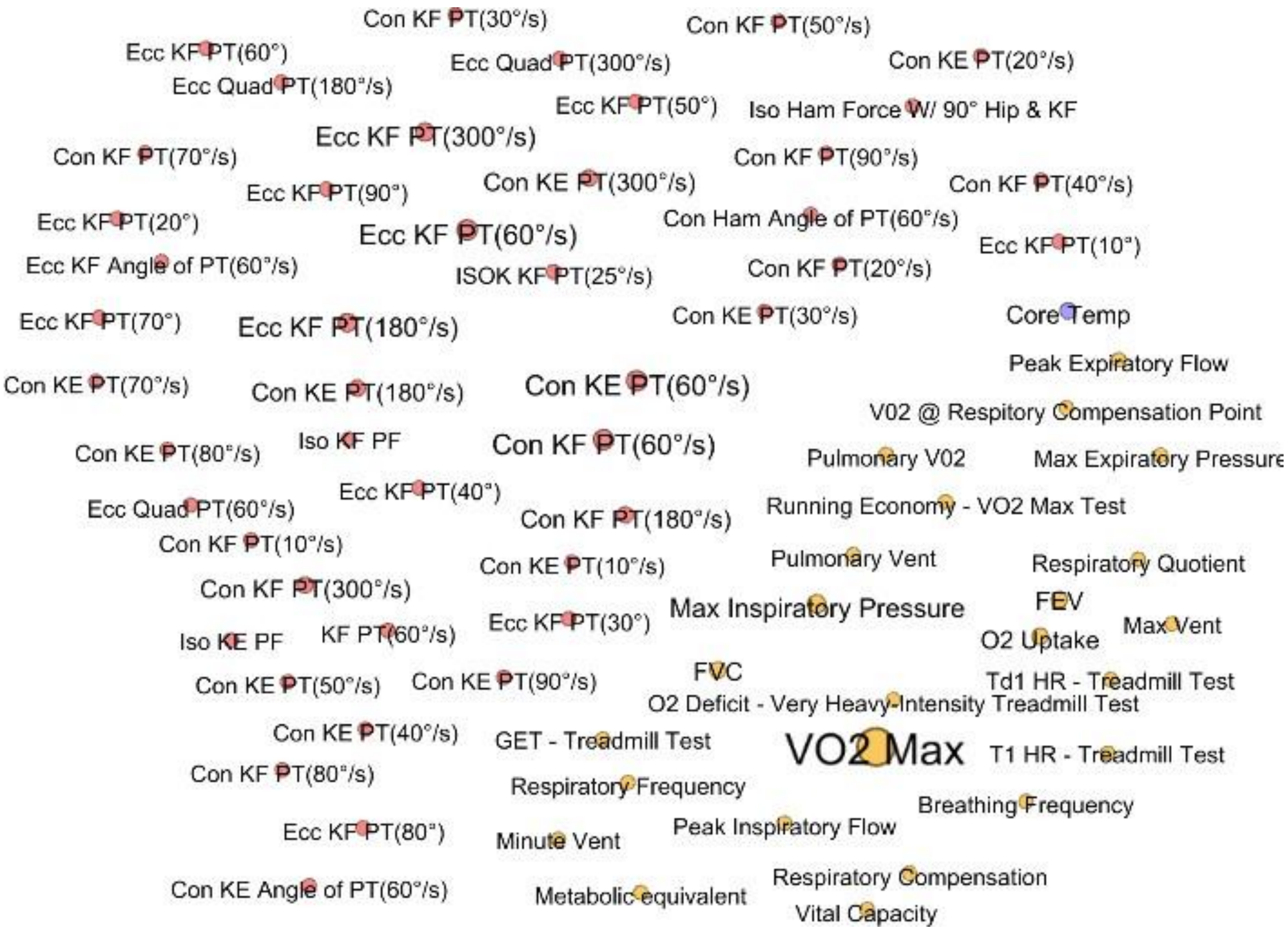


Figure 3.20 Strength, fitness, and temperature measures at the organ system level using metabolic conditioning as a stimulus

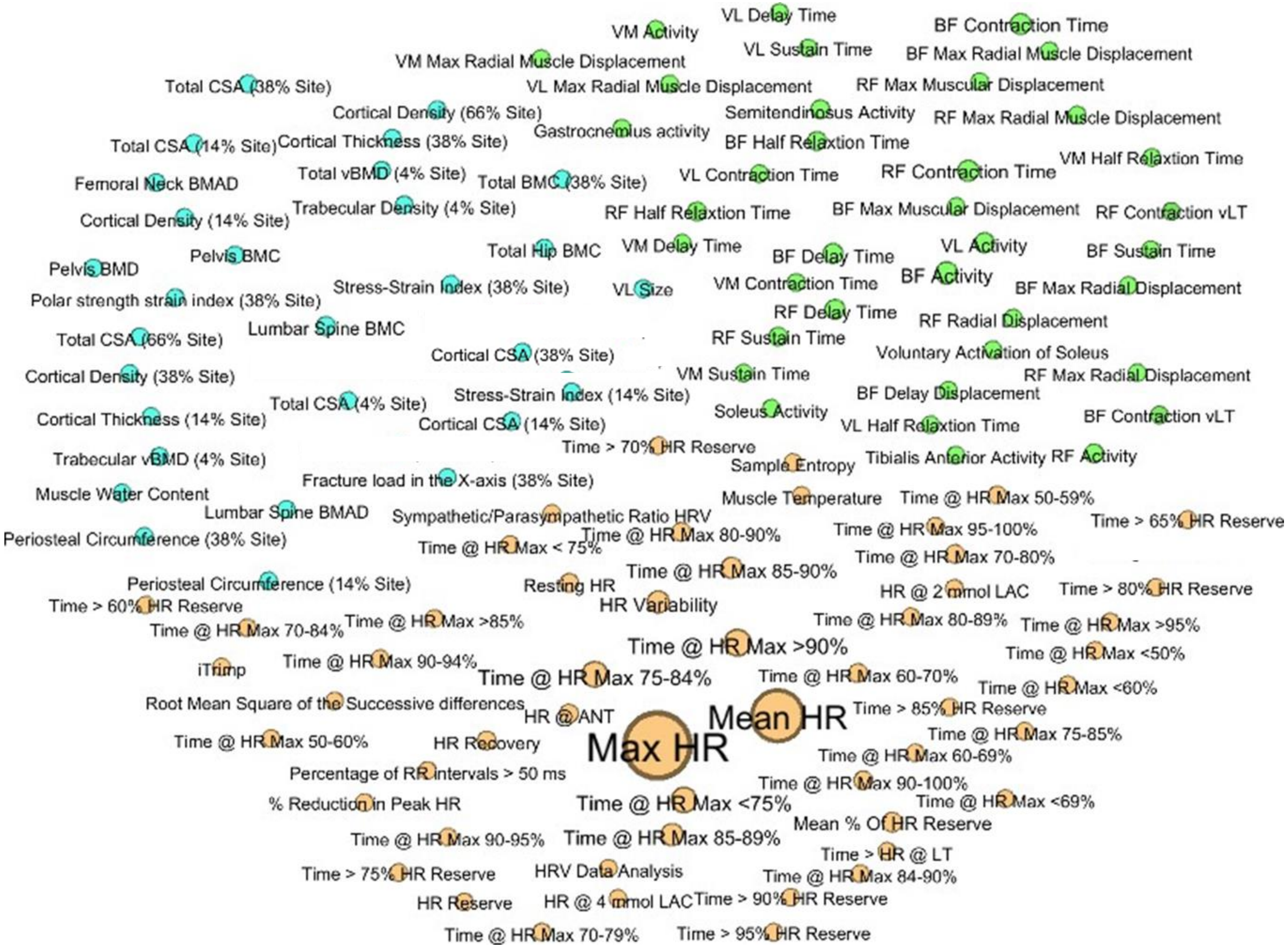


Figure 3.21 HR/Cardiovascular, anatomical, and contractile property measures at the tissue/organ level using football as a stimulus.

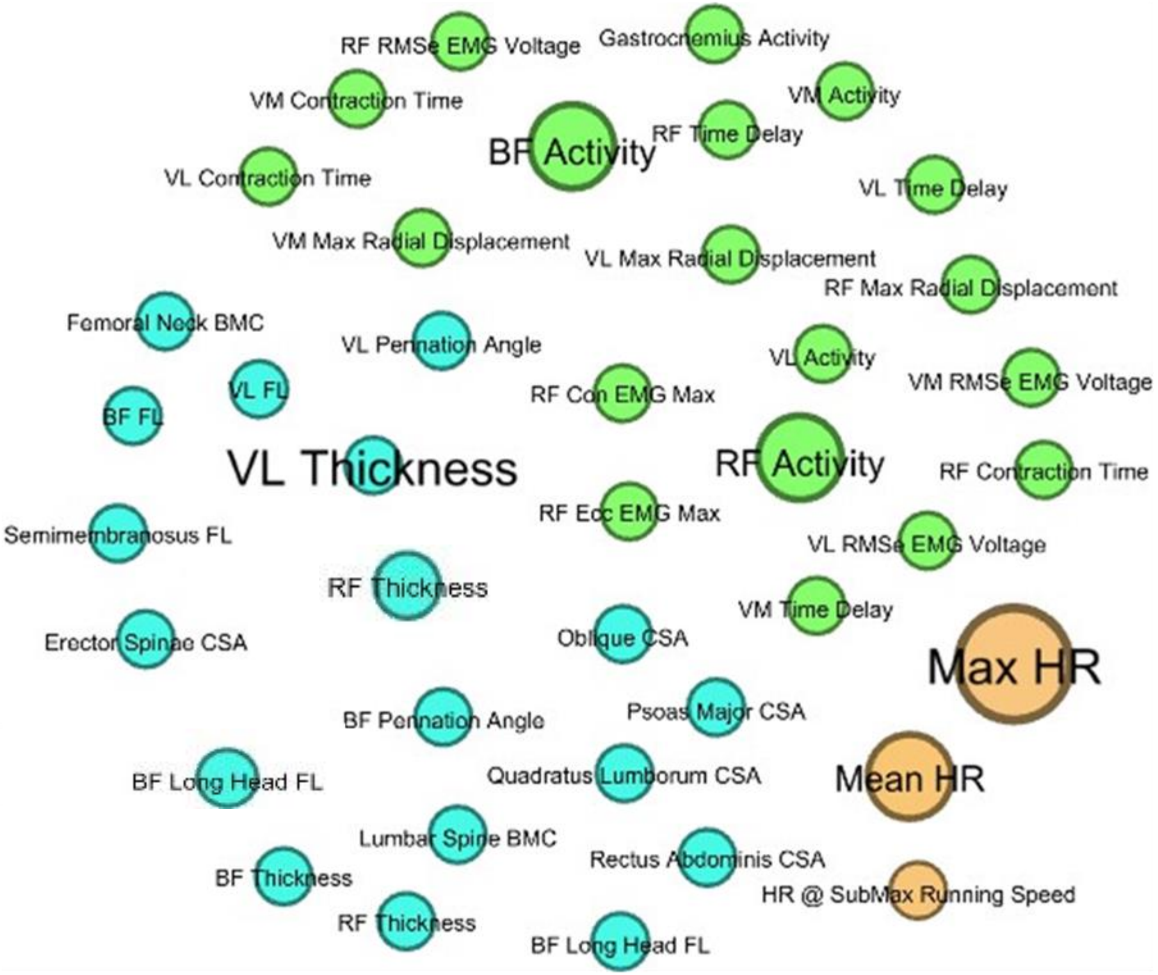


Figure 3.22 HR/Cardiovascular, anatomical, and contractile property measures at the tissue/organ level using strength as a stimulus

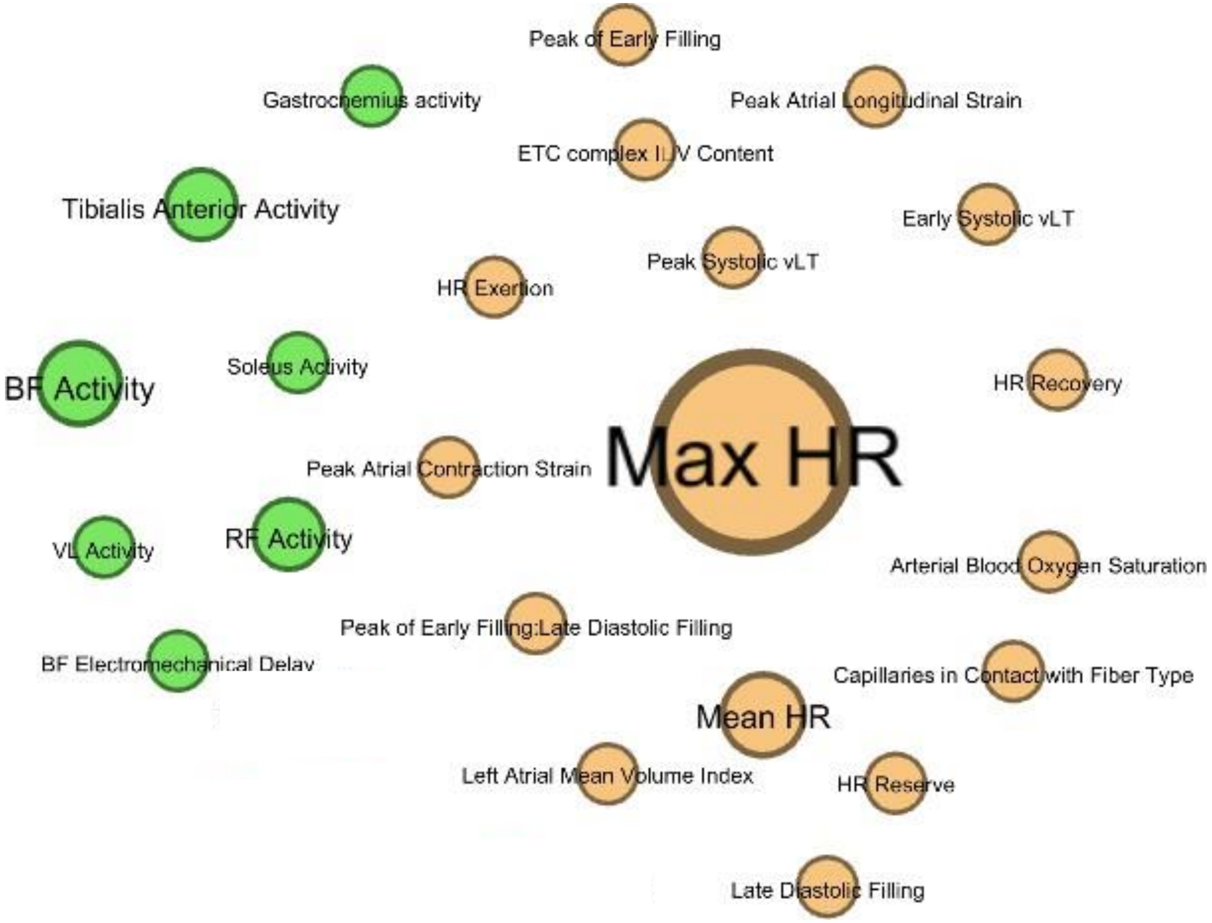


Figure 3.23 HR/Cardiovascular and contractile property measures at the tissue/organ level using metabolic conditioning as a stimulus

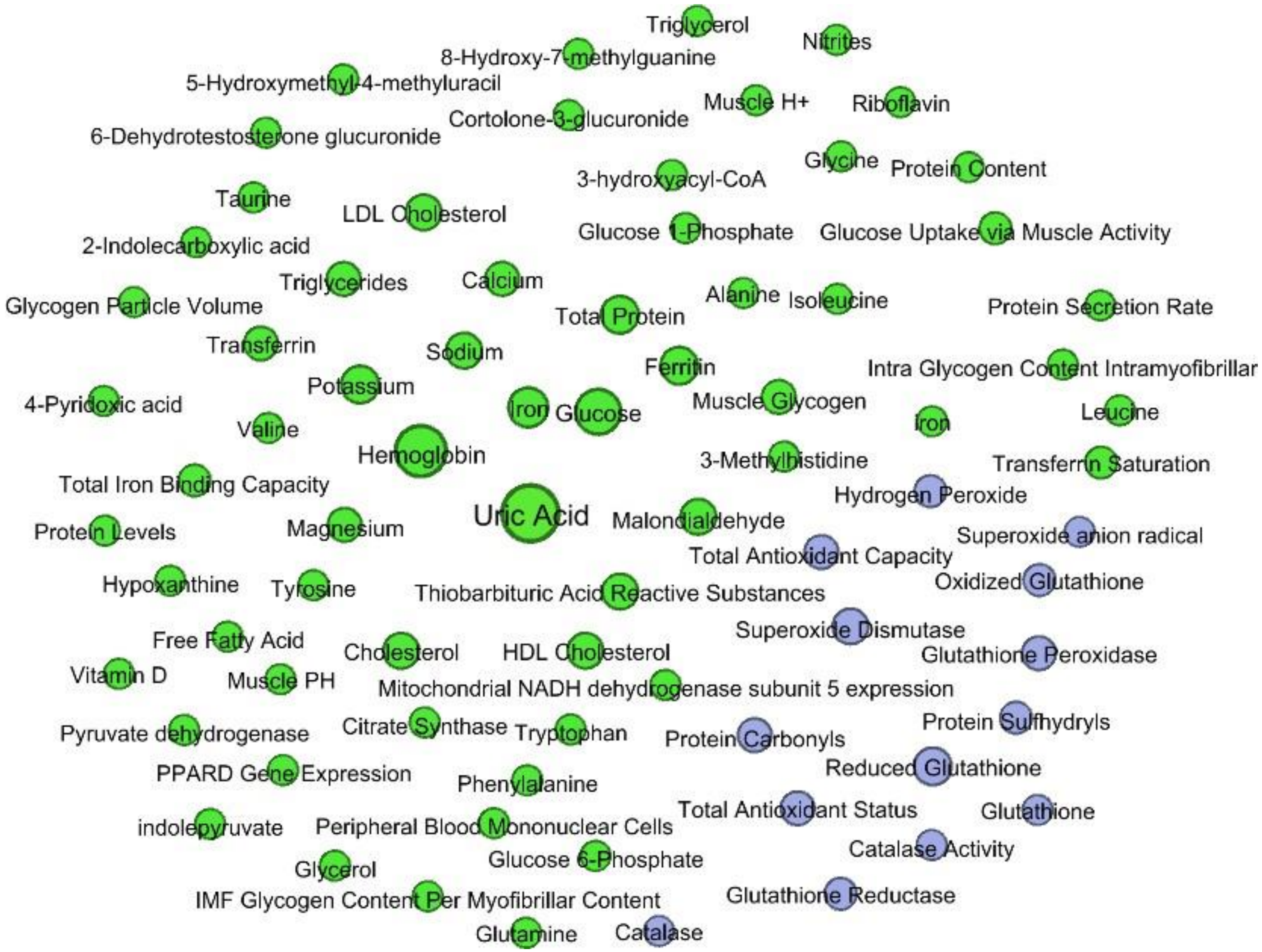


Figure 3.24 Antioxidant status and nutrient metabolism outcome measures at the cellular level using football as a stimulus

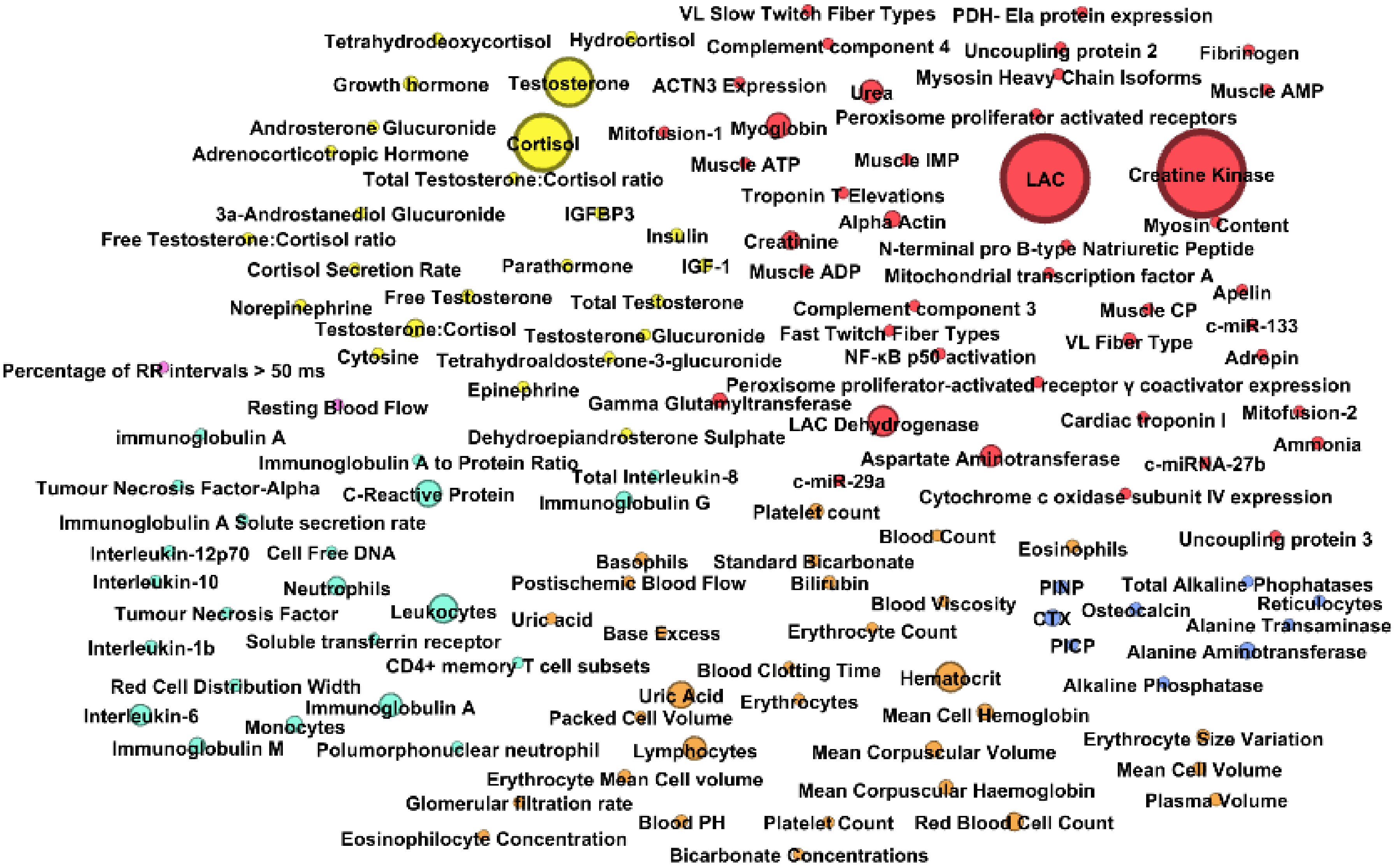


Figure 3.25 Muscle status, cardiovascular, endocrine response, bone and collagen tissue, immune response outcome measures at the cellular level using football as a stimulus

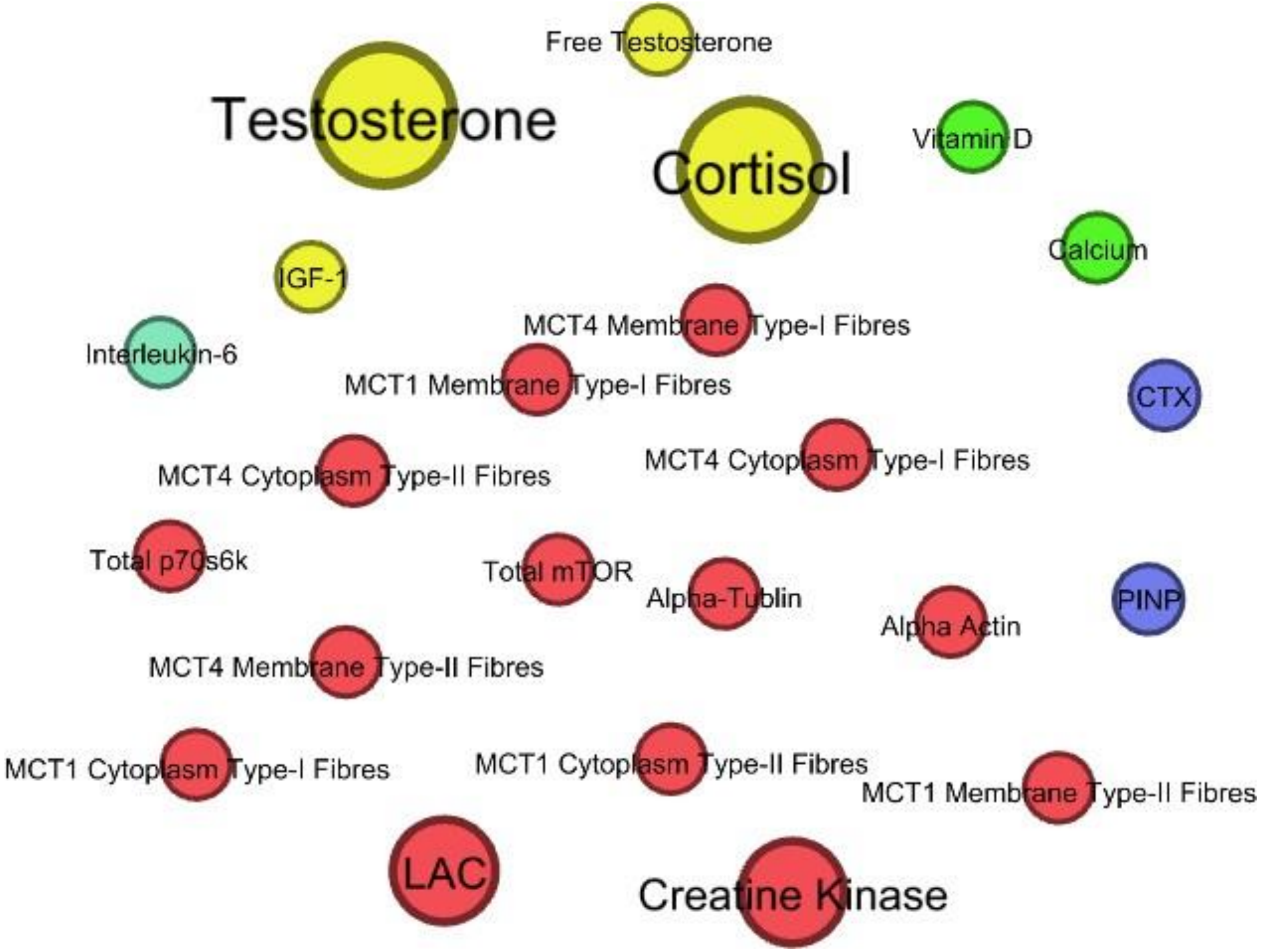


Figure 3.26 Muscle status, endocrine response, bone and collagen tissue, immune response, blood status, antioxidant status and nutrient metabolism outcome measures at the cellular level using strength as a stimulus

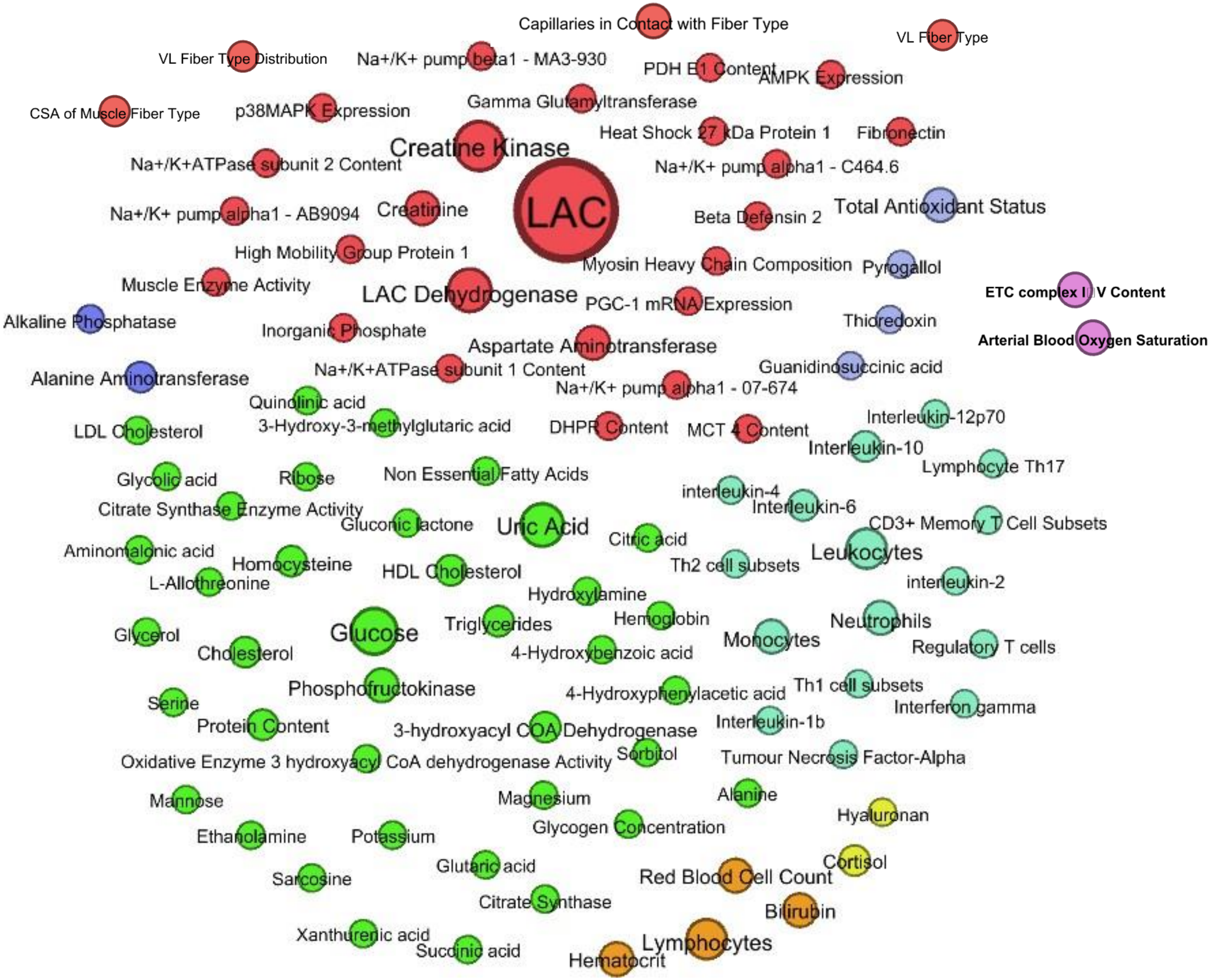


Figure 3.27 Muscle status, cardiovascular, endocrine response, bone and collagen tissue, immune response and nutrient metabolism outcome measures at the cellular level using metabolic conditioning as a stimulus

3.3.2.1 Summary of Outcome Measures

The outcome measures used to assess responses to all stimuli resulted in 1170 unique measures. When categorised by the level they are measured at (Figure 3.12), the organism level commands with 480 unique outcome measures. The cellular level follows with 282 outcome measures, followed by the organ system level with 217 outcome measures, finally, the tissue organ level 191 outcome measures.

When the organism level is segmented by stimulus, the most common outcome measures taken to football stimuli were categorised as measures of power, speed, and fitness used in 74, 59 and 55 studies, respectively. Furthermore, these categories contain the largest outcome measures, with 58, 56, and 28 unique outcome measures falling under each category. Similarly, the dominant outcome measures taken at the organism level to strength stimuli were classified as measures of power, speed, agility, and strength, used in 90, 86, 58, and 44 studies, respectively. The categories power, agility, speed, and strength contain the largest amount of outcome measures, with 84, 75, 44, and 37 unique outcome measures falling under each category, respectively. Finally, the dominant outcome measures taken at the organism level to metabolic conditioning stimuli were classified as speed, fitness, and power, which were used in 23, 23 and 19 studies. Of these categories, speed contained 34 unique outcome measures, with 19 measures under fitness and 13 under power.

Regarding the organ system level, the most common measures taken to assess the response to football stimuli were classified as strength and fitness measures, which were measured in 34 and 26 studies and contained 40 and 30 unique measures, respectively. Similarly, the most common measurements taken at the organ system level to strength stimuli were classified as strength, which was measured in 51 studies, and contained 67 unique measures. Lastly, the most common measurements taken at the organ system level to metabolic conditioning stimuli were classified as fitness measures, measured in 18 studies, with 24 unique measures falling under this category.

Concerning measures taken at the tissue/organ level, measures categorised as HR/cardiovascular dominate at the tissue/organ level in response to football stimuli, which were taken in 76 studies and contained 54 unique measures. As demonstrated previously, a small number of studies measured responses to studies using strength as a stimulus at the tissue/organ level. Nevertheless, measures categorised under contractile properties, anatomical and HR/cardiovascular were taken in 7, 7 and 5 studies, respectively. 20 measures fall under contractile properties, with 19 falling under anatomical and 3 under HE/cardiovascular. In response to metabolic conditioning, measures categorised as HR/cardiovascular were most common, used in 20 studies, with 16 unique measures falling under this category.

Lastly, measures of muscle status and nutrient metabolism were most commonly measured at the cellular level in response to football, which was measured in 94 and 41 studies, respectively. However, 61 unique measures fall under nutrient metabolism, with 39 under muscle status. As mentioned previously, there is also a relatively low number of studies measuring responses to studies using strength as a stimulus on the cellular level, with 6 studies measuring responses under muscle status and 4 under endocrine responses. 14 measures of muscle status and 4 endocrine responses were used to measure responses to studies using strength as a stimulus. Finally, nutrient metabolism and muscle status measures were most commonly used at the cellular level in response to metabolic conditioning stimuli. 38

unique measures were used as indicators of nutrient metabolism, with 25 measures relating to muscle status used in response to metabolic conditioning stimuli. Figures 3.28, 3.29 and 3.30 below bring all the information together regarding the most common level, duration, outcome measurement category and outcome measure for under each stimulus.

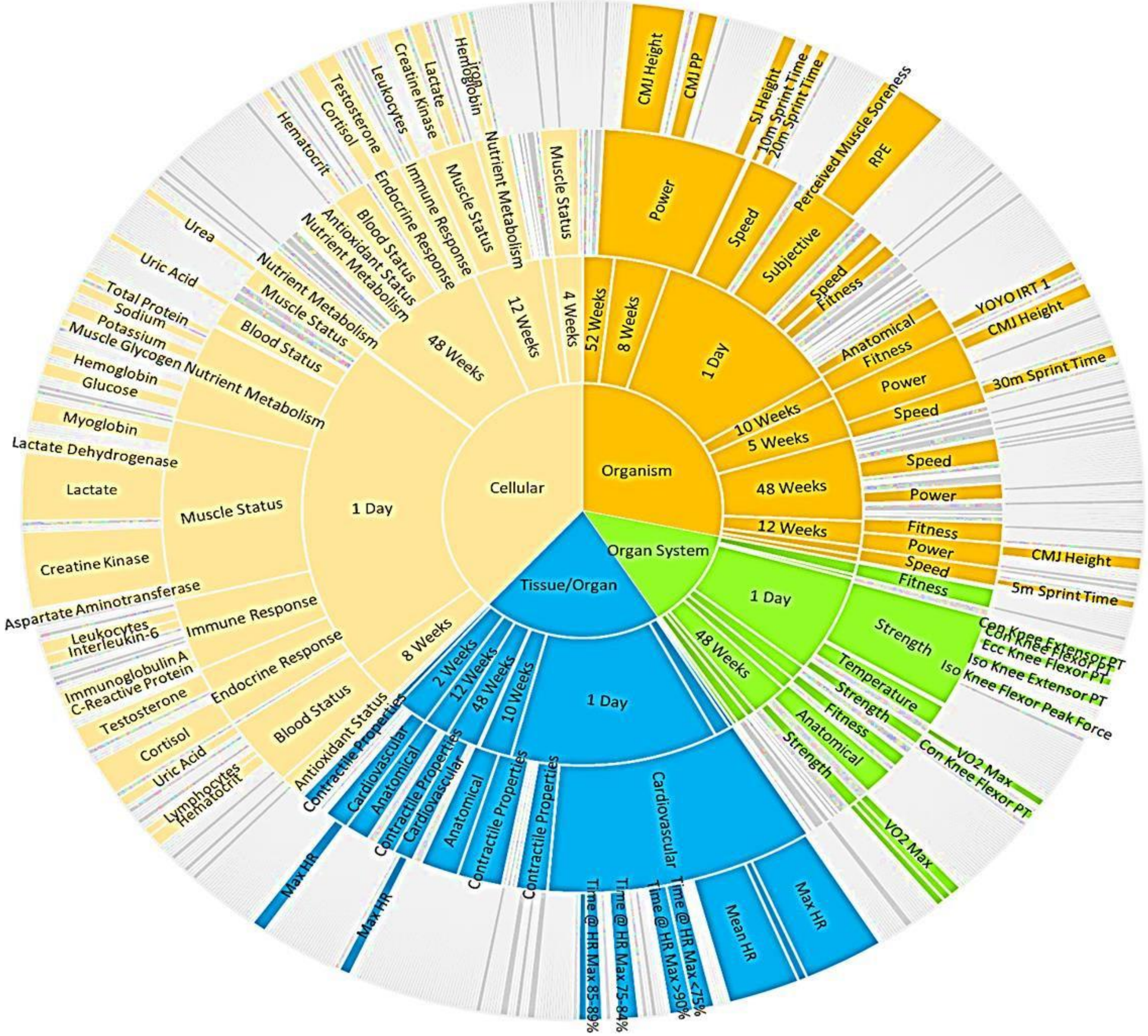


Figure 3.28 Illustration of the most common level, duration, outcome measurement category and outcome measure used to measure responses to football stimuli. Measurement categories and outcome measures in grey illustrate a large number of different measures used by a low number of studies. For example, many power measures have been used at the organism level in response to football over 8 weeks, but only CMJ heigh and CMJ PP were used by numerous studies.

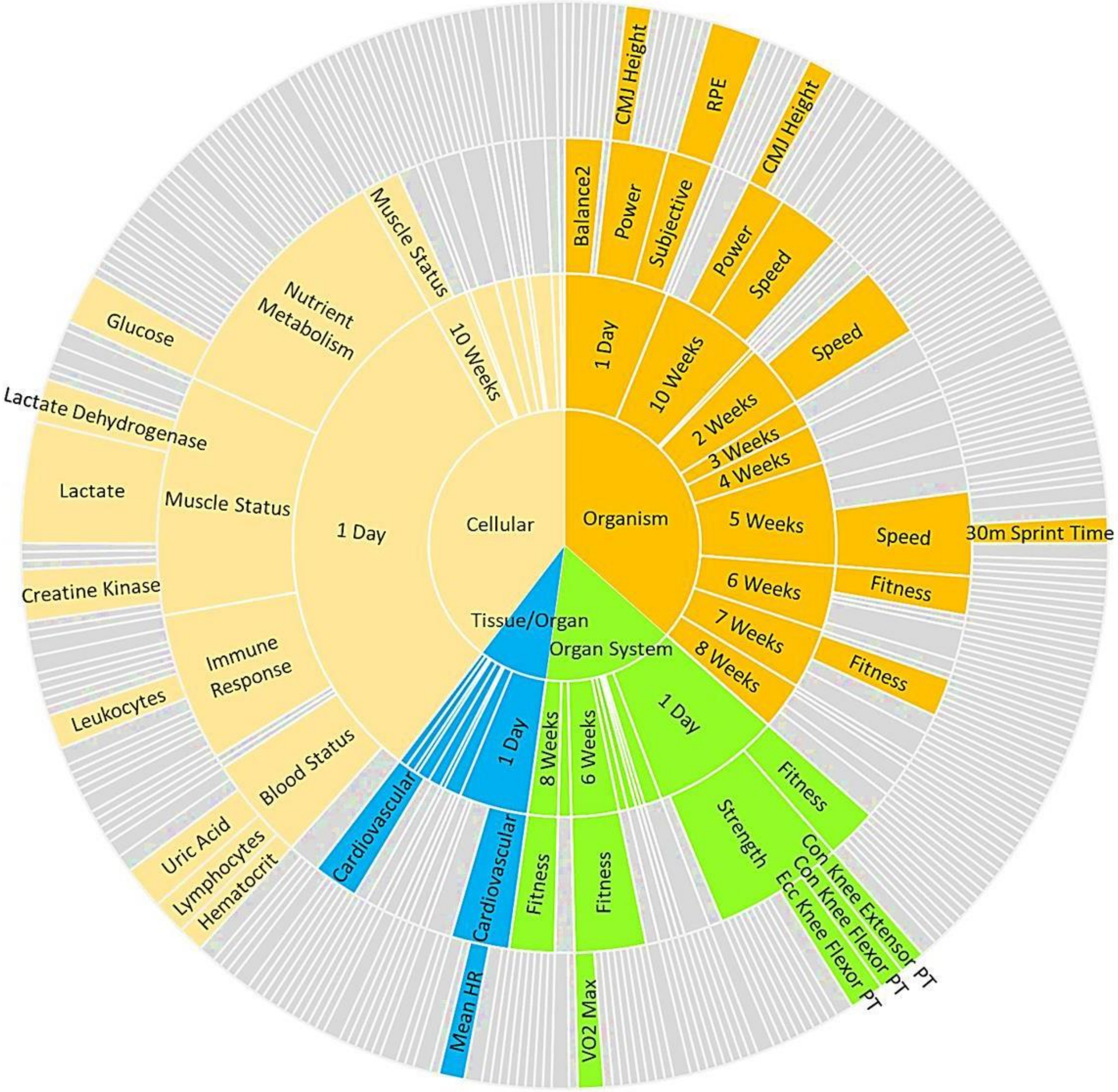


Figure 3.29 Illustration of the most common level, duration, outcome measurement category and outcome measure used to measure responses to metabolic conditioning stimuli.

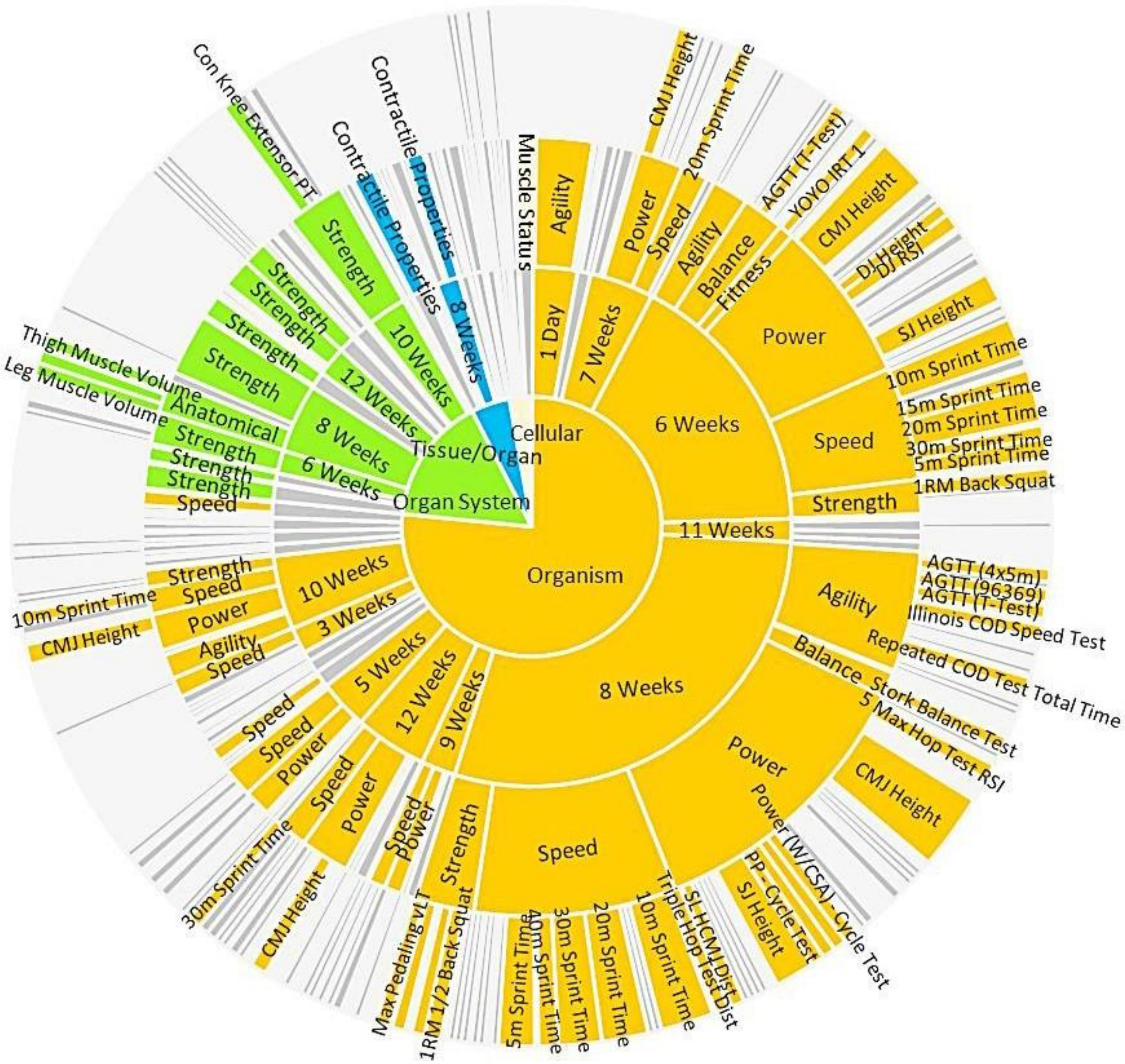


Figure 3.30 Illustration of the most common level, duration, outcome measurement category and outcome measure used to measure responses to strength stimuli.

3.4 Discussion

This review aimed to demonstrate how adaptation has been measured in football. This paper begins to fill a knowledge gap by providing a novel approach to mapping and examining the methods used to assess adaptation in football. Firstly based on the results of this study, it is clear that the problem of mapping the response to multiple stimuli has been approached in many different ways. The difference in approach is reflected in the varied stimuli and approaches to intervention design. This is evidenced by:

1. The varied stimuli that have been used in studies
2. The numerous outcome measures that have been taken at various levels of organisation in the body over many different time courses.

The opportunity for different approaches is a consequence of the numerous levels that adaptation can be measured on. This provides opportunities to map different aspects of the adaptive process using different study designs. Consequently, many different approaches have been taken to measure adaptation in football. These approaches may be influenced by pragmatic or conceptual views of adaptation. These views may dictate the level adaptation is measured on, the measurement techniques used, and the durations used in studies.

Secondly, based on the nature of studies used in this review all players in studies who were subjected to strength training or metabolic conditioning, would also have been subjected to football stimuli, which could influence the responses observed in these studies. However, the majority of strength and metabolic conditioning studies failed to quantify the football stimulus in enough detail that would demonstrate this has been considered or controlled for. This demonstrates that the effect multiple stimuli can have on adaptive responses has not been considered in research design.

Consequently, it is difficult to draw conclusions regarding the dose-response relationship between any stimulus and response in scenarios where multiple stimuli have not been considered in a studies design. Together this suggests that research on football adaptation may be limited in its’ ability to accurately reflect the complexity of the adaptive process. Future studies may need to consider how multiple stimuli influence adaptive responses. Moreover, consideration needs to be given to the time courses over which responses are measured, and the methods used to measure a response may have on any adaptation observed. This may be achieved by mapping the adaptive process using more comprehensive measures across multiple time courses. Eventually, this could result in “football-specific” study designs that consider the multiple stimuli footballers face. This may help the development of football-specific adaptive time courses.

3.4.1 **Response**

This review observed that 1170 outcome measures have been used to assess the relationship between stimulus and response in football across various levels in the body. These measures broadly represent two phenomena: transient changes in process or adaptive changes in outcomes. The number of different outcome measures taken on various levels of organisation demonstrates that there are multiple approaches to measuring adaptation in football. The differences in approaches used may be a consequence of pragmatic and conceptual views of adaptation. The pragmatic approach may centre on an interest in observing the changes in outcome at the organism level. Moreover, this approach may be concerned with the ease at which a measurement can be taken. On the other hand, the conceptual approach may focus on describing complex adaptive processes in more detail. As a consequence, these approaches may require more comprehensive, costly, time-consuming, and invasive measures on lower levels of organisation. An example of this might be a comprehensive blood profile that provides information on the response to football activities at the cellular level.

With regards to a pragmatic approach, tests that are can be obtained in the field, are easy to perform, time-efficient, have a low injury risk and provide outcome measures related to performance, may be favoured by those who adopt this approach. An example of this approach might be using a countermovement jump test to assess changes in power at the organism level. These types of tests are time efficient and although they do not provide detailed insights into the mechanisms behind a change in performance, they indicate that a change in performance may have occurred. Additionally, some tests that are time-consuming and detailed may be restricted by an inherent injury risk. This may explain the speeds most commonly used during isokinetic dynamometer testing, which do not reflect the limb velocities observed during sprinting and high velocity kicking actions (Brown and Greig, 2022). Nevertheless, this pragmatic approach may be favoured in the field by sports practitioners. As noted by Bok and Foster, (2021, p1), “there is a desire to make fitness testing cheaper and easier to conduct in a team-sport setting which has led to the development of numerous tests”. This approach, however, may come at a sacrifice to reliability and validity if the cost and ease of use of any test are considered to be most important.

Evidence that a more pragmatic approach is favoured in this review is demonstrated by the most common outcome measures that were used at various levels and the ease at which they can be gathered in the field. For example, running tests, agility tests, jump tests, subjective tests, various strength tests, measures of heart rate and even levels of capillary blood lactate and creatine kinase, can be taken obtained relatively quickly in the field (Bok and Foster, 2021). In contrast, more conceptual approaches may involve the use of measures of bone quality using a DXA scanner, or measures of peak torque at various speeds using an isokinetic dynamometer to answer more complex questions related to adaptation. These measures may provide more comprehensive and reliable explanations behind any change in performance (Paul and Nassis, 2015). However, these measures may be more costly, time-consuming, and invasive than the field-based measures mentioned previously. This may explain why measures that are time-consuming, invasive, and measured on levels of organisation below the organism level, are not as common as some of the more pragmatic measures mentioned previously. Nevertheless, these more complex approaches to mapping adaptive responses are necessary due to the interactions that occur on and across the levels of organisation in the body (Balagué et al., 2020; Gershenson & Fernández, 2012). Consequently, these conceptual approaches, whilst requiring a lot of time and cost, can provide useful insights into the adaptive process.

Therefore, it would seem pertinent to find ways of integrating both approaches in football (Figure 3.31). This may provide opportunities to map the responses that occur across multiple levels, allowing for greater insights into the adaptive process. This could entail mapping responses at the cellular level daily over a 12-week study period for example. This could provide insights into the causal mechanisms behind the longer-term responses when combined with measurements at the tissue/organ, organ system and organism level. However, the methods used would need to be time-efficient, comprehensive as well as reliable and valid. In time these approaches could lead to more complex methods being developed that seamlessly coordinate responses across multiple levels. This may help guide the monitoring of a dose-response relationship through constant triangulation (Kiely, 2012).

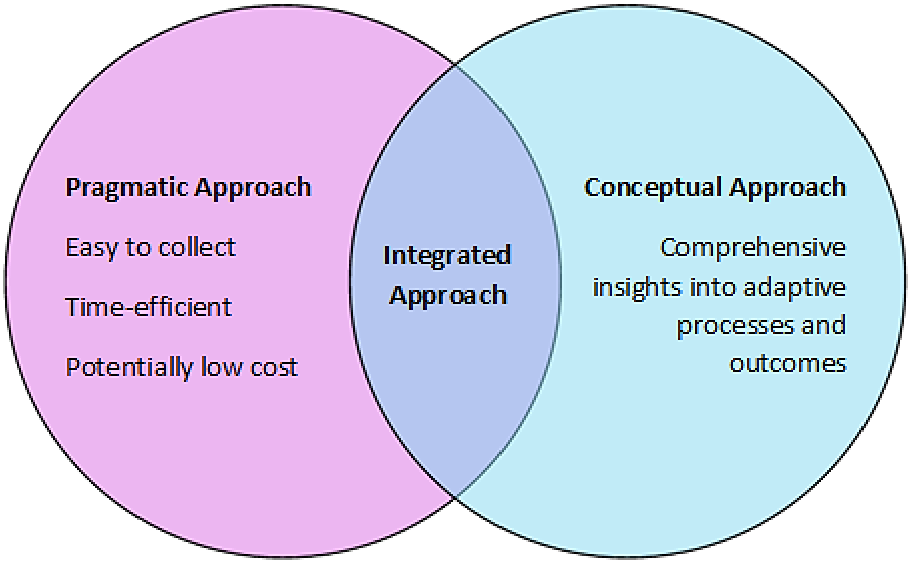


Figure 3.31 Advantages of the pragmatic approach vs the conceptual approach. Developing methods that integrate the benefits of approaches may provide opportunities to comprehensively map responses across numerous levels.

3.4.2 Stimulus

Football players are subjected to numerous different stimuli. These stimuli have been classified in this review as football, strength, and metabolic conditioning. However, there are a low number of studies that used a combination of stimuli (figure 6). This is a consequence of a lack of in-depth definitions and methods used to quantify the stimulus. This suggests that the potential blunting of the training response due to multiple stimuli has not been considered or controlled for in many studies. There exists the potential for different stimuli, with different implications for adaptation, to blunt training responses when administered in close proximity (Enright et al., 2016; Hickson, 1980, Hickson et al., 1980).

For example, it has been demonstrated that aerobic training, when placed in close proximity to strength training, blunts any strength adaptations, which can be related to acute or chronic signalling (Hickson et al., 1980). Furthermore, it is possible that the nutritional demands of both sessions have not been considered, which could have knock-on effects for the performance and therefore, adaptations in the latter session. For example, a demanding strength training session placed before a high-intensity running session, could potentially use up the ‘fuel’ needed to support the performance in the high-intensity running session. This could have implications on the responses to the high-intensity running session. Furthermore, when stimuli of a similar nature are placed in close proximity, such as football and metabolic conditioning, it is difficult to ascertain the true reason by any response observed. Consequently, it is difficult to establish true dose-response relationships between a strength or metabolic conditioning stimulus and any response. Thus, it would seem imperative to quantify multiple stimuli in enough detail to provide greater insights into the potential causes for an observed response. Furthermore, comprehensively detailing any stimulus may allow for standardization and replication in future studies.

3.4.3 Study Durations

For stimuli to lead to adaptation, they must be delivered repeatedly over time. Based on the analysis of the study durations and measurement frequencies used (figures 3.9 and 3.10) it would appear that there is a lack of studies that have attempted to capture the full spectrum of an adaptive process by measuring responses frequently over both short and long durations. This is evidenced by the fact that football and metabolic conditioning study lengths predominantly focused on mapping responses over short durations, primarily 1 day, whilst strength-based studies focused predominantly on mapping responses over study durations lasting 6-12 weeks (Figure 3.9). It would therefore seem that football and metabolic conditioning studies tend to focus on mapping short term changes in processes, whilst strength studies are focused on longer-term changes in outcome. Future study designs may need to map responses over short and long durations simultaneously to capture the full spectrum of an adaptive process.

The short study durations predominantly used by football and metabolic conditioning studies would suggest that there is a focus on describing how these stimuli affect the body acutely. Short study durations may be used to assess the intensity of a stimulus or the magnitude of an acute fatigue response. The fatigue response may lead to favourable long-term adaptations when repeated over time (Stølen et al., 2005; Ascensao et al., 2008; Christensen et al., 2011; Ali and Farrally, 1991; Bangsbo, 1994; Impellizzeri et al., 2019). However, this is difficult to establish if these responses are not mapped over various time courses to determine how these acute responses manifest over time. In contrast, acute responses have not been considered by studies using strength as a stimulus, as evidenced by the lack of short study duration adopted by these studies.

This would suggest that the specific research questions posed by these studies did not require the consideration of the acute responses to strength training. This may be due to pragmatic approaches, as discussed previously. Alternatively, there may an assumption that positive adaptations to strength training would manifest over the study durations employed. This assumption could be the result of existing research detailing the principles and time courses of adaptation, specifically muscular responses to strength training (Wisdom et al., 2015; Schoenfeld et al., 2015; Folland and Williams, 2007; Hughes et al., 2018). However, as mentioned previously, this research and its findings may not apply to football players due to the interference effect that can occur if football and strength training is performed in close proximity (Hickson, 1980).

Future research may help establish best-practice protocols to ensure these stimuli do not interfere with acute responses. However, due to training schedules, it may be difficult to mitigate this interference effect in practice. In this case, it is then important to establish ‘football-specific’ time-courses for strength adaptations by considering the ‘interference-effect.’ It is important to note that study durations observed in this review may be influenced by fixture schedules, the durations of different phases in the season such as pre-season, or the time windows available for researchers to access players. These durations may vary dramatically across teams and influence the durations observed in this review. Nevertheless, it is clear that the adaptive process needs to be mapped across various time courses to football, metabolic conditioning and strength stimuli.

3.5 Limitations

Whilst this review is comprehensive, there are some notable limitations. There was no assessment of quality or bias in the studies included. Furthermore, the screening process did not acknowledge the number of studies using control groups to assess the efficacy of any adaptive response. However, no recommendations have been made in this review that promotes any of the specific methods and techniques used in the screened studies. Due to the volume of articles in the initial screening process, it is plausible that some articles were excluded by their title that may have been eligible based on full-text review. Moreover, studies in English using only uninjured male players were selected. As a result, some information may have been overlooked. However, these limitations are due to the native language of the researcher as well as the profile of players that this thesis is investigating. The focus on levels from the cellular upward means some critical details relating to sub-cellular responses may have been overlooked. Additionally, the classification of outcome measures, specifically at the cellular level, is inherently simplistic due to the number of influences these measures can have outside the categories they have been placed in. Nevertheless, based on the extensive nature of this review, we feel no significant methods or trends have been neglected.

3.6 Conclusion

To conclude, to the author’s knowledge this is the first study of its nature to comprehensively map how adaptation has been measured in football. This review highlights the challenges associated with measuring the relationship between stimulus and response in football players. The results of this scoping review demonstrate that adaptive responses have been measured using a variety of approaches. This is a consequence of the complex array of processes that occur in the body across numerous levels of organisation. The numerous levels adaptation can be measured on, provide many different opportunities to observe the components of an adaptive process. The approaches used to design studies to measure adaptation could be the result of different pragmatic and conceptual views of adaptation. New insights may be gained in future research by merging conceptual and pragmatic approaches to mapping adaptation. Further insights into the complex nature of adaptation in football players could also be developed by considering the interference effect of multiple stimuli on adaptive responses. By mapping these responses more frequently across multiple time courses more detailed ‘football-specific’ insights may be captured.

CHAPTER FOUR

EXPLORATORY MAPPING OF THE PHYSIOLOGICAL AND MECHANICAL STIMULUS DURING THE IN-SEASON USING GPS-DERIVED EXTERNAL LOADS

4.1 Introduction

Chapter 2 demonstrates that football players are exposed to multiple physical stimuli in their training and competitive schedules. However, the most frequently experienced physical stimulus is on-pitch football activities. This makes these activities an important area of focus for research in this area. Chapter 1 illustrates that such football practices are likely to place demands on both the physiological and biomechanical load-adaptation pathways. This could lead to adaptations that have the potential to facilitate improvements in a players' capacity to tolerate the demands of training and match play by improving their physical performance (Stølen et al., 2005; Ali and Farrally, 1991; Bangsbo, 1994; Nicholas et al., 2000; Wragg et al., 2000; Reilly, 1997). Such adaptations are however only a function of repeated exposures to stimuli that stress these physiological and biomechanical systems across the season. It would therefore seem pertinent to map the training stimuli across a season to provide insight into the potential for football training to elicit adaptive responses in the physiological and biomechanical load-adaptation pathways.

In football, the annual cycle is divided into three main phases: the pre-season, competitive in-season, and off-season. Each of these periods is traditionally associated with a specific training stimulus (Walker and Hawkins, 2018; Reilly, 2007; Gamble, 2006). Of these phases, the pre-season phase is usually associated with the period in which the largest adaptations occur (Reilly, 2007; Bangsbo, 1994; Bompa, 2009; Algroy et al., 2011). Consequently, the pre-season phase is a key part of the player's preparation for high performance. Whilst this information is interesting, it is also important to understand the potential for physiological and biomechanical changes to occur in the in-season competitive phase. Adaptations that occur during this phase may be more important as they are likely to be more directly influence competitive game performance. The potential for individuals to adapt to a given training stimulus is less frequently researched, primarily as a consequence of the challenge of scheduling evaluations that describe training related outcome measures during the in-season phase.

The potential for adaptations to occur during the in-season phase will be compounded by the variability frequently in the training response. This variability is largely a function of external and internal factors such as an individual's playing position and their inherent personal characteristics. For example, it has been demonstrated that significant differences in positional outputs exist in football match play (Bloomfield, 2007; Baptista et al., 2019). This will inherently change the external load experienced by players which has the potential to stimulate differences in the adaptive responses of those individuals. For example, players who play in positions that require a high density of changes of direction (e.g. central midfielders) training sessions may require a higher density of these actions to stimulate an adaptive response. Other positions not frequently exposed to such a high density of changes of direction (e.g. wide-midfielders) may find the training session to be intense enough to trigger an adaptive response. Therefore, characterising the stimulus during the in-season phase by playing position would seem pertinent.

If there is potential for adaptation to occur in the in-season phase it is likely these would occur due to specific stimuli and intensities. The intensity of a session exists on a scale (figure 4.1) and can be reflected by the external and internal loads measured via GPS and HR. The key drivers for adaptation to a running-based training stimulus have long been regarded as the volume (how much), intensity (how hard) and frequency (how often) of the training stimulus (Hawley, 2008). However, evidence would suggest that there seems to be a threshold for the magnitude of response to specific combinations of these drivers, such as low intensity, high volume, and frequency training.

This threshold is highly individual, yet from a mechanical perspective research demonstrates that long distance runners demonstrate lower bone mineral densities when compared with athletes from ball sports (Scofield and Hecht, 2012). This is due to the high intensity intermittent stresses placed on bone which have greater osteogenic potential compared to low intensity high volume stress (Klein-Nulend et al., 2012; Bakker and Klein- Nulend, 2009). Furthermore, from a physiological perspective, research demonstrates that low volume, high intensity interval training (HIIT), leads to similar central and peripheral adaptations when compared to high volume training (Ross & Leveritt, 2001; Gibala et al., 2006; Little et al., 2010; Gibala et al., 2014). In addition, it has been demonstrated that endurance trained athletes can improve performance and work capacity by augmenting their current training with high intensity activities at V̇ O2 max, despite reductions in training volume (Esfarjani and Laursen 2007; Iaia et al. 2008; Iaia et al. 2010)

Considering the highest volume of training players will face is during the pre-season phase, it would therefore seem plausible that based on the evidence presented previously, if adaptations were to occur in the in-season phase, it would be a product of intensity as opposed to volume. Indeed, Krustrup et al (2009), suggested that intense nature of soccer training, rather than the duration of training, was a key factor in performance enhancements seen in a study which compared the effects of a 12-week football training intervention with moderate intensity running. It is important to acknowledge that adaptations can occur due to submaximal stimuli during training. However, it is conceivable that the volume needed to elicit adaptations, may not be possible to achieve in the in-season phase due to time constraints on training. particularly following a pre-season phase where the volume of work would be highest (Impellizzeri et al., 2006).

Adaptation is likely to occur based on the intensity of stress caused by a stimulus (Sands and Stone, 2005). A stimulus could be considered relatively light or moderate based on previous exposures, in this case, the stimulus may have no effect on adaptation. However, a stimulus that is considered intense relative to previous exposure could have the potential to stimulate an adaptive response (Viru, 2000). Positive adaptations in the in-season phase as a result of an additional intense stimuli have been demonstrated by Nyberg et al. (2016), who demonstrated that low volume, high intensity speed endurance training performed once a week over 9 weeks in trained soccer players during an in-season phase, was enough of a stimulus to lead to improved intense intermittent exercise performance and faster phase II pulmonary V˙O2 kinetics. Moreover, Jensen et al. (2009) showed that a single 30-min aerobic high intensity session completed once a week was enough of a stimulus to improve intermittent exercise capacity in experienced football players during the season. Whilst these adaptations are related to an extra stimulus during the season on top of normal training, what this demonstrates is that a single intense stimulus is enough of a deviation away from normal training to stimulate an adaptive response.

If we consider the volume of intense activity in a session as a proxy for adaptation, then it would seem logical that the more sprint distance or intense accelerations and decelerations accumulated, then the greater the potential there is for adaptation. On the contrary, a lower volume of sprint distance or a number of intense accelerations and decelerations could potentially have no effect on adaptation. Whilst the volume of one specific external marker may not directly link to adaptation, it could point the general nature of the session (i.e highly mechanical with large amounts of accelerations and decelerations or highly physiological with a large amount of high intensity running and sprinting).Therefore, identifying the highest volume of intense activities for each position may reflect a simple way to estimate what stimulus could be important for triggering individual adaptive responses. As mentioned previously, intense stimuli would need to be repeated over time to elicit favourable adaptations. Thus, it would seem important to describe the training stimulus during the in-season phase to understand if the intensity of the stimulus, and where it occurs during this phase. Thus, the frequency of these exposures also needs to be established.

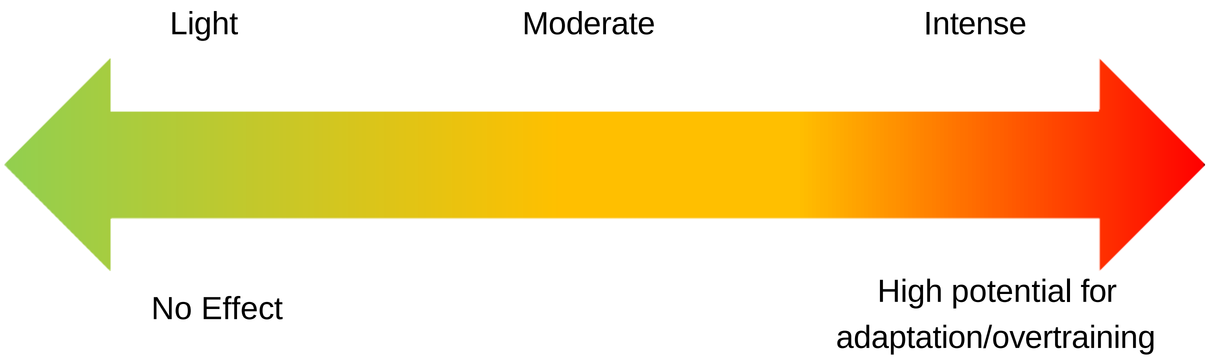


Figure 4.1 the potential for a stimulus to lead to adaptation exists on a scale. Training sessions considered light/moderate may theoretically have no effect on adaptive responses. In contrast, intense training sessions will have a higher potential to stimulate adaptive responses/overtraining when compared to light/moderate sessions.

Any adaptations related to the physiological and biomechanical load-adaptation pathways may be triggered by different physical demands. As alluded to in Chapter 2 metabolically demanding activities such as sprinting may lead to favourable physiological adaptations. Alternatively, high-intensity changes of direction may lead to favourable biomechanical adaptations. It would therefore seem pertinent to identify intense training sessions with the potential to stimulate responses in these two load-adaptation pathways. Furthermore, it would also seem appropriate to examine how often these types of sessions occur during the in-season phase.

Consequently, this study aims to retrospectively map where intense sessions occurred across a single season for each playing position. This will be achieved by retrospectively analysing data from a single season in an u23s elite football squad and Identifying football-based sessions with the highest external demands across the season using an exploratory method. These intense sessions will then be mapped across the season to demonstrate how the physiological and biomechanical load-adaptation pathways could be stimulated during the in-season phase for different positions. From a practical standpoint, this information could be used to demonstrate to coaches where sessions with the highest external demands occurred across the season, for both the team and individual playing positions. This could then be compared with training plans to help guide future processes. Furthermore, this study could encourage fitness coaches and sports scientists to think about the potential implications of the training on the physiological and biomechanical load-adaptation pathways and how best to monitor those implications.

4.2 Methods

4.2.1 Participants

Twenty-one elite male football players that represented an English Premier League u23s team. were used in this study (mean ± SD; age: 19 ± 1.64 yrs, height: 1.81 ± 0.07m and body mass: 69 ± 16.1kg). This sample included players from various playing positions (centre backs (CB) n=6, fullbacks (FB) n=7, midfielders (MID) n=7 and forwards (FOR) n=4).

4.2.2 Experimental Design

Data collection for the study was carried out over a 36-week competitive in-season phase. The content of each training session was determined by the team’s technical football coaches and fitness coach in line with the physical, tactical, and technical objectives for each given session. The content for each session was not influenced by this study. Data collection for this study was carried out at the football club’s training pitches. Approval for the study was obtained from the professional club and the appropriate university research ethics committee.

Football-based training sessions were retrospectively classified as intense using a multi- stage process based on the average positional sprint distance (m) (>7 m/s) and positional high-intensity accelerations & decelerations (>3.0 $m \cdot s^{-2}$). These thresholds are defined by the manufacturer (Statsports) and were chosen based on the fact that they have been adopted by the club in question for many seasons. Thus, technical and fitness coaches were familiar with these thresholds and understood how certain drills/session designs affected these GPS markers. Football-based sessions where average positional sprint distance exceeded the threshold for an intense session for that position, were classified as physiological sessions.

When a positional average for high-intensity accelerations & decelerations exceeded the threshold for an intense session for that position, the session was classified as a mechanical session. When both positional sprint distance and High-intensity accelerations & decelerations exceeded positional thresholds in the same session, this session was classified as a mechanical & physiological session. The methods used to set positional thresholds will be expanded upon further in the methods section. Football-based sessions that did not exceed positional thresholds for sprint distance or High-intensity accelerations & decelerations were classed as light/moderate sessions.

4.2.3 Characteristics of on-pitch exposures

The on-pitch exposures were separated into three distinct categories: matches, football training sessions or metabolic conditioning sessions. These stimuli were broadly categorised based on the primary content of the session. Thus, a football session that may have had an isolated metabolic conditioning drill within the session would be classed as a football session since the bulk of the session was football-based. Whilst this paper is focusing on the potential for adaptation through the football training stimulus, matches and metabolic conditioning sessions have been included for illustrative purposes. This is to allow for physical comparisons that may provide interesting insights. 90-minute match data was used in this instance. The data for matches and metabolic conditioning sessions were calculated in the same manner as football-based training sessions. Contextual factors for matches such as opposition, tactics, formation, and venue were excluded as match data is presented purely to compare training sessions against matches. Furthermore, training in-season is dictated by the match schedule and as such, it is important to map where matches occur to provide further insights into training prescription.

4.2.4 Quantifying external and internal loads

External loads were quantified using GPS, with data collected from all football training sessions, metabolic conditioning sessions and matches. The GPS units (Apex, StatSports, Ireland) were placed between the scapulae of the players using bespoke vests. These GPS units sampled at 18 Hz and the accelerometers at 100 Hz. Recent research has demonstrated these units show good levels of accuracy (bias>5%) for distance travelled at various speeds as well as peak velocity (Beato et al., 2018). Each GPS unit was switched on before the warm-up for each session to ensure that a satellite link was established before the beginning of the activity. To ensure consistency and reduce the effect of interunit reliability issues, each player was assigned a GPS unit that they wore throughout the entire season. Variables selected for analysis were total distance (m), high-speed running distance (m) (>5.5 m/s) sprint distance (m) (>7 m/s) and high-intensity accelerations & decelerations (>3.0 $m \cdot s^{-2}$). The thresholds used were based on the manufacturers set thresholds which were adopted and used by the club as part of daily practice. Following each session, the data was downloaded using the manufacturer's software package (Apex Sonra).

From there the data was cut from the session start time to the session end time, to cut out any data associated with players walking in or off the training pitch, for example. Data was then exported into a custom Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA). Only data derived from team-based pitch training sessions and matches were analysed. Off-feet conditioning sessions, such as bike sessions, were solely utilised for players in rehab and as such were excluded from the analysis. Furthermore, international data from one international break for two players were excluded from the analysis. Goalkeepers were not included in the analysis due to the difference in the nature of their demands. Heart rate (HR) based measures of internal load were also collected during training sessions only in this study using HR belts (Polar H1 system). HR traces were checked for accurate and reliable traces for each session upon completion using Apex Sonra software. The players' maximum heart rate was determined by an incremental maximal field-based test during pre-season and was repeated at the mid-point of the season, with maximum HR updated if a higher value was achieved.

Players did not wear HR belts during matches.

4.2.5 Identifying intense sessions

To identify the potential implications of football-based training across this season, a multi-step process was used to categorise and identify football training sessions that could be considered intense from a physiological and mechanical standpoint. It is important to note that this process was conducted retrospectively once the season was completed. The process for establishing intense sessions is outlined below.

1. Sprint distance was selected as an indicator of ‘physiological’ load whilst the number of high-intensity accelerations and decelerations was selected as the indicator of ‘mechanical’ load. Following the principle that the most intense stimulus is most likely to lead to adaptation, sprinting was chosen as an indicator of physiological external load. High speed distance was not chosen as it is possible to have a large amount of high-speed distance with little sprint distance, hence for the purposes of this study only one metric was used. From a metabolic standpoint sprinting is highly intense and can lead to adaptation in enzymes related to all energy systems (Ross and Leveritt, 2001). Based on estimated metabolic demands (Gaudino et al., 2014), concluded that the energy expenditure and the distances run at high power increase when the pitch sizes increase, allowing for greater high speed to be achieved, and consequently, higher metabolic values.

   By the same token, High-intensity accelerations & decelerations (>3.0 $m\cdot s^{-2}$) are highly intense from a mechanical standpoint, mainly due to the high impact and ground reaction forces (Verheul et al., 2019). Thus, the higher the intensity of these actions, the higher demands placed on the mechanical load-adaptation pathways, thus the higher potential for adaptation. Furthermore, high-intensity accelerations and decelerations have been previously defined in the literature as being >2.5 $m \cdot s^{-2}$ (Harper et al., 2019).

2. 148 training sessions in total across the season were retrospectively analysed. The average outputs for every training session was calculated for sprint distance and high-intensity accelerations & decelerations. To act as the threshold for intense sessions, the 75th percentile was applied across all 148 training sessions. The advantage of using percentiles is it creates an unbiased baseline that ignores what may seem to the practitioner as ‘intense’, based on their own experience. The 75th percentile created a cut-off point for sprint distance and high-intensity accelerations & decelerations. Therefore, any training session with an average sprint distance above the 75th percentile was considered a ‘physiological’ session. On the other hand, any training session where the average no. of high-intensity accelerations & decelerations was above the 75th percentile was considered a ‘mechanical’ session. Furthermore, training sessions where the 75th percentile was exceeded for both high-intensity accelerations & decelerations and sprint distance were considered a ‘mechanical & physiological’ session. This was considered as this type of session demonstrates the possibility that two distinct adaptive processes could be triggered.

4.2.6 Mapping intense sessions

Once this process was completed the stimulus was mapped across the season. Mapping the stimulus across the season aimed to identify where the most intense football-based training sessions occur during the competitive calendar. Whilst total distance and high-speed distance was not used as a physiological or biomechanical marker, it was included as a common indicator of the training stimulus to provide extra context for the reader. Furthermore, the time spent in minutes above 85% of individual max HR was also included as an indicator of the internal training load. In summary, this process has been adopted as an attempt to identify sessions with the highest sprint and/or intense acceleration and deceleration stimulus for each position. Thus highlighting sessions with the greatest potential to elicit adaptive responses.

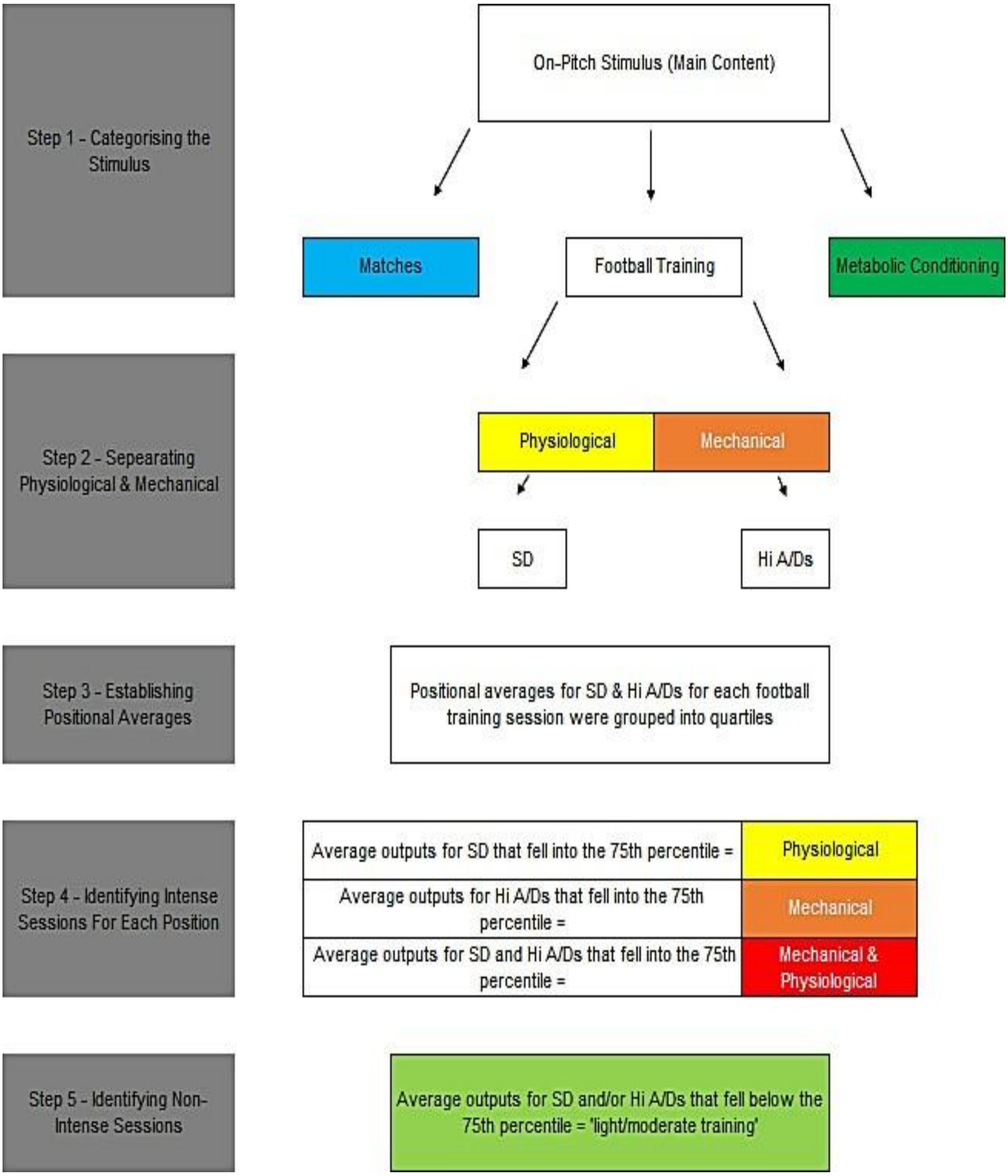


Figure 4.2 The multi-step process used to identify intense sessions. Abbreviations: SD – Sprint distance, Hi A/Ds – High-intensity accelerations & decelerations.

Table 4.1 demonstrates how many sessions were intense from a mechanical or physiological standpoint following the calculation of the 75th percentile for sprint distance and high-intensity accelerations & decelerations for each position. It is clear from this table how varied the thresholds for each position are, demonstrating the importance of categorising these sessions by position.

Table 4.1 Threshold established for intense sessions by position using the 75th percentile. The values for SD and Hi A/Ds below the threshold were separated into five different bins to illustrate how many sessions took place that fell within specific ranges. To illustrate using FB as an example; 15 sessions took place where the average HI A/Ds for FB fell between 29 and 69 Hi A/Ds. 66 sessions took place where the average SD was between 0 and 15m. This table does not demonstrate how many sessions were classed as mechanical & physiological as the process used for this table was focused on establishing the thresholds for intense mechanical or physiological sessions for each position. Centre backs (CB) n=6, fullbacks (FB) n=7, midfielders (MID) n=7 and forwards (FOR) n=4.

		Light/Moderate Training Session			**Intense Training Session Threshold**
FB	**Avg No. of Hi A/Ds per session**	**36 to 77**	**77 to 119**	**119 to 160**	**>160 (75th Percentile)**
	No. of sessions	27	37	46	38
	Avg SD (m) per session	**0 to 20m**	**20 to 41m**	**41 to 61m**	**> 61m (75th Percentile)**
	No. of sessions	74	25	11	38
CB	**Avg No. of Hi A/Ds per session**	**15 to 57**	**57 to 98**	**98 to 140**	**>140 (75th Percentile)**
	No. of sessions	8	45	58	37
	Avg SD (m) per session	**0 to 10m**	**10 to 19m**	**19 to 29m**	**>29m (75th Percentile)**
	No. of sessions	87	14	8	39
MID	**Avg No. of Hi A/Ds per session**	**37 to 77**	**77 to 116**	**116 to 156**	**>156 (75th Percentile)**
	No. of sessions	24	36	49	39
	Avg SD (m) per session	**0 to 21m**	**21 to 43m**	**43 to 64m**	**>64m (75th Percentile)**
	No. of sessions	83	20	7	38
FOR	**Avg No. of Hi A/Ds per session**	**28 to 71**	**71 to 115**	**115 to 158**	**>158 (75th Percentile)**
	No. of sessions	21	38	48	41
	Avg SD (m) per session	**0 to 21m**	**21 to 41m**	**41 to 61m**	**> 61m (75th Percentile)**
	No. of sessions	82	16	14	36

4.3 Statistical Analysis

All analyses were conducted using statistical software (SPSS, Chicago, USA). Since it is postulated that adaptation will come from the highest stimuli it is important to determine if the external and internal loads are significantly higher in intense sessions (sessions that exceed the positional 75th percentiles) when compared to light/moderate sessions (sessions that fall below the positional 75th percentiles). Therefore, to analyse the difference between the mean positional GPS outputs for different sessions, one-way repeated measures ANOVA and Tukey post hoc tests were utilised. Initially a Shapiro-Wilk test was conducted to assess normality of data, followed by a Levene's test for homogeneity of variance. Statistical significance was set at $P<0.05$. Only significant differences are reported in the results section.

4.4 Results

The number of on-pitch exposures completed by the squad is outlined in table 4.2. Overall light/moderate sessions (highlighted in green) occurred more times (85) than intense sessions (63) (highlighted in pink). When considered as a percentage, 43% of all football training sessions were intense, suggesting that throughout the season players were regularly exposed to intense stimuli. On observation, the majority of these sessions were either intense solely from a mechanical or physiological perspective, not a combination of mechanical & physiological. Thus, whilst there would seem to be a regular exposure to intense training, these sessions may impact two distinct load-adaptation pathways. When the number of exposures to intense sessions are observed according to position as in table 4.3 there would seem to be little variation in the exposures between positions. However, It is important to next identify the pattern of these exposures during the in-season phase in terms of where they occur.

Table 4.2 No of exposures throughout the season for all stimuli. Intense sessions are highlighted in pink whilst light/moderation training sessions are highlighted in green.

Pitch-Based Sessions					
Matches	**Light/Moderate Training**	**Mechanical**	**Physiological**	**Mechanical & Physiological**	**Metabolic Conditioning**
34	85	26	24	13	7

Table 4.3 No of exposures throughout the season for all stimuli by position. Centre backs (CB) n=6, fullbacks (FB) n=7, midfielders (MID) n=7 and forwards (FOR) n=4.

Pitch-Based Sessions						
	Matches	**Light/Moderate Training**	**Mechanical**	**Physiological**	**Physiological & Mechanical**	**Metabolic Conditioning**
FB	34	87	24	24	14	7
CB	34	85	24	26	13	7
MID	34	82	28	27	11	7
FOR	34	83	29	24	12	7

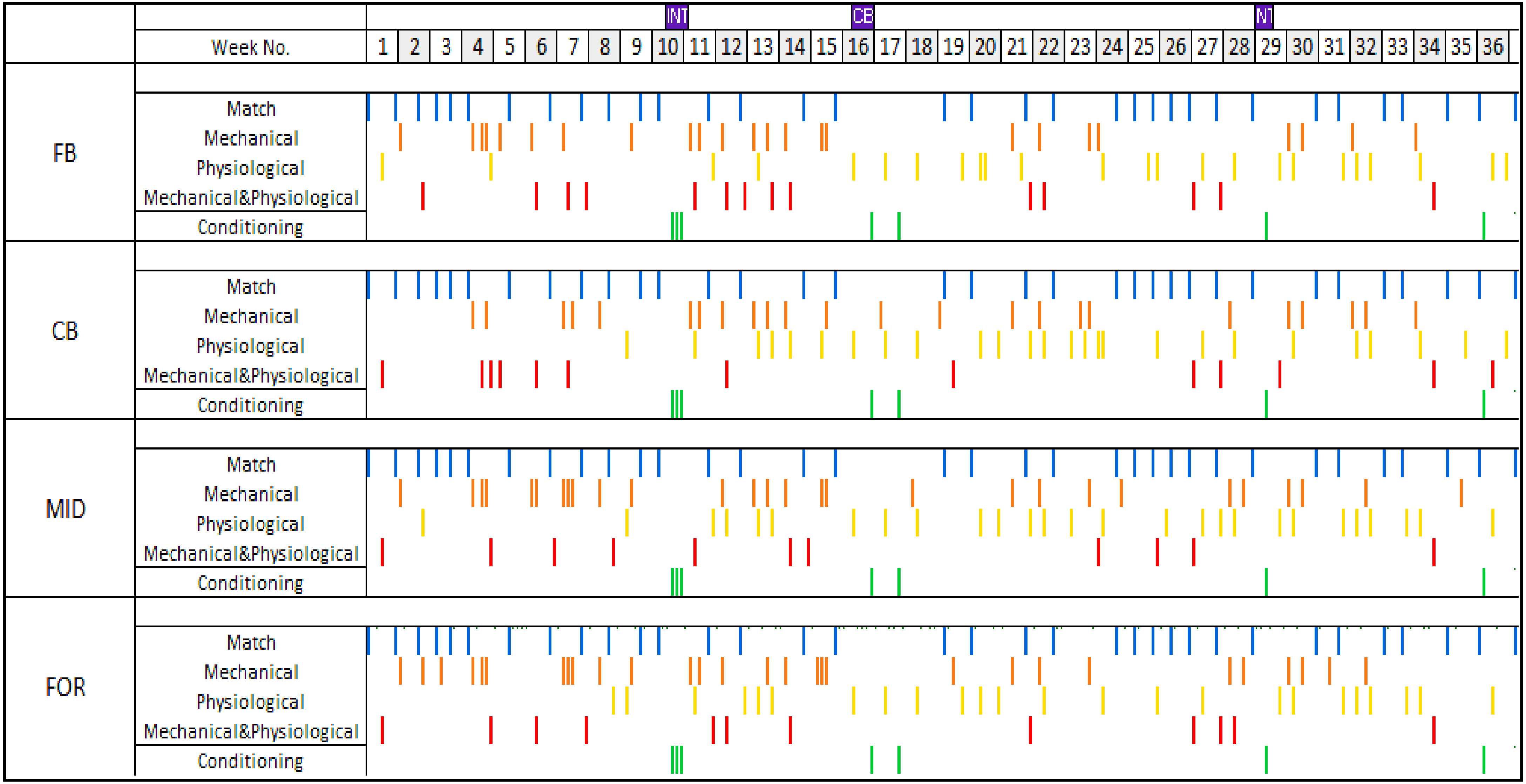


Figure 4.3 Full season snapshot of where matches, intense sessions and metabolic conditioning sessions occur during the in-season phase for each position. Sessions can be identified by coloured blocks. To understand the spacing of these blocks with respect to time, the reader's attention is directed to the metabolic conditioning sessions in week 10, which occurred over 3 consecutive days. For further clarity Week 9 for MID demonstrates a physiological session on a MD-3, a mechanical session on a MD-2, a gap where a light/moderate session occurred on a MD-1 and then a game. Centre backs (CB) n=6, fullbacks (FB) n=7, midfielders (MID) n=7 and forwards (FOR) n=4.

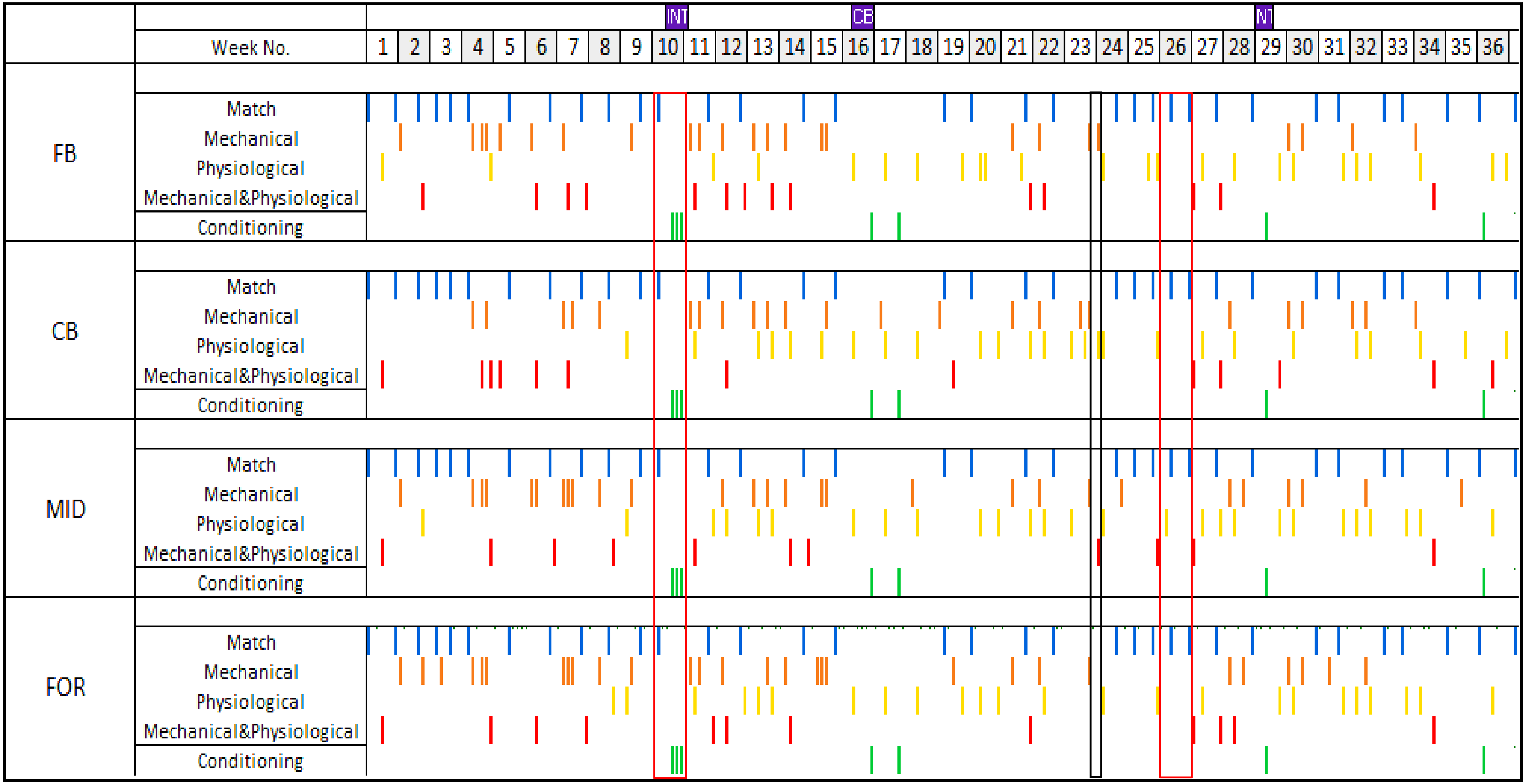


Figure 4.4 Full season snapshot with areas highlighted to support results. The black outline highlights a single training session in week 24 where the same session qualifies as an intense mechanical session for FB, an intense physiological session for CB, an intense mechanical & physiological session for MID and a light/moderate session for FOR. Week 10 is highlighted in red to show that no intense sessions occurred during an international break. Week 26 is highlighted in red to show that no intense sessions occurred for FB.CB and FOR. Centre backs (CB) n=6, fullbacks (FB) n=7, midfielders (MID) n=7 and forwards (FOR) n=4.

When these sessions are mapped across the season according to position as in Figure 4.2, some key patterns emerge.

1. **There is a low number of weeks in the season when intense training sessions do not take place for each position.**

It has already been established that players in each position are exposed to intense sessions frequently during the in-season phase. However, when mapped across the season, figure 4.4 demonstrates that players in each position are subjected to intense sessions consistently throughout the in-season phase. Weeks 3, 8, 10, 26, 33 and 35 contain no intense sessions for FB. Weeks 2, 10, 26, 31 and 33 contain no intense sessions for CB, Weeks 3, 5, 10, 19 and 24 contain no intense sessions for MID. Weeks 5, 10, 26 and 35 contain no intense sessions for FOR. Week 10 was an international break, as a result, players took part in self-led conditioning sessions away from training. Week 26 contained two games within the week, only MID were exposed to a physiological session in this week. Overall, this demonstrates that players were consistently exposed to intense sessions throughout the season.

2. **The times when players are exposed to intense sessions during the season seem to be a function of playing position.**

Previously, it was established that the thresholds for intense mechanical or physiological sessions differ between positions. Furthermore, it would seem that playing position could determine the amount of intense mechanical, physiological, or mechanical & physiological sessions players are exposed to. This is further evidenced by figures 2 and 3, which demonstrate that at times throughout the season the same training session qualifies as intense or light/moderate depending on the playing position. This is most evident at the beginning of week 24 as demonstrated in figure 4.3. The same training session qualified as a mechanical session for FB, a physiological session for CB, and mechanical & physiological session for MID and a light/moderate session for FOR. For context, this session was a team-based MD-4 training session which would typically include technical and tactical based work in small spaces as well as large spaces. Further evidence of this can be observed in week 1 where FBs were exposed to a physiological session, whilst CB, MID and FOR were exposed to a mechanical & physiological session. This pattern is also clearly evident in week 4.

3. **The mechanical load-adaptation pathway may be stimulated more frequently in the first 18 weeks of the in-season phase compared to the remaining 18 weeks for FB, MID and FOR. The opposite trend is apparent for potential adaptations in the physiological load-adaptation pathway.**

Figure 4.2 demonstrates that when mechanical and mechanical & physiological sessions are considered together, then players were exposed to more intense sessions from a mechanical standpoint in the first 18 weeks of the in-season phase when compared to the second 18 weeks. FB, CB, MID and FOR were exposed to 25, 21, 25 and 26 sessions in the first 18 weeks of the season that were considered mechanically intense. In contrast, FB, CB, MID and FOR were exposed to just 13, 16, 14 and 15 sessions in the remaining 18 weeks of the season that were considered mechanically intense. In contrast, from a physiological perspective, when physiological and mechanical & physiological sessions are considered together FB, CB, MID and FOR were exposed to 22, 23, 22 and 20 physiologically intense sessions. Whereas in the first 18 weeks of the season FB, CB, MID and FOR were exposed to 16, 16, 16 and 16 sessions that were considered physiologically intense. This would suggest that the potential for training adaptations in the mechanical & physiological load-adaptation pathways, based on the number of exposures were at their greatest at different points throughout the season.

It should be noted that FB, CB, MID and FOR were exposed to 5, 1, 4 and 5 mechanically intense sessions respectively in the first 18 weeks of the season as part of an MD+1 session. This was due to not playing more than 30 minutes in the previous day's match. For physiological sessions in the first 18 weeks FB, CB, MID and FOR were exposed to 3, 0, 4 and 4 sessions respectively as part of an MD+1 session. In the second 18 weeks of the season FB, CB, MID and FOR were exposed to 2, 2, 3 and 3 mechanically intense sessions respectively as part of an MD+1 session. For physiological sessions, FB, CB, MID and FOR were exposed to 5, 4, 6, and 6 sessions respectively as part of an MD+1 in the second 18 weeks of the in- season period.

Thus far, it has been demonstrated that intense sessions occur frequently and regularly throughout the season for each position. However, what qualifies as intense and where those intense sessions occur throughout the season is dependent upon position and in some cases, match exposures. If these trends are to be linked to any potential adaptations, it seems logical to establish if significant differences exist in the external and internal loads between different intense sessions and light/moderate sessions. Initially this was analysed by pooling all positions together (n = 24) to look at the squad average outputs for each session and comparing between sessions. Overall the average outputs for 189 sessions were analysed. Table 4.4 demonstrates that significant differences were found in external loads between intense sessions and light/moderate sessions, suggesting the methods used to classify intense sessions are justified.

This is evidenced by the fact that mechanical sessions had significantly higher high-intensity accelerations & decelerations than light/moderate sessions. Moreover, physiological sessions had significantly higher sprint distances than light/moderate sessions, whilst mechanical & physiological sessions had significantly higher sprint distances and high-intensity accelerations & decelerations than light/moderate sessions. To further demonstrate the intense nature of these sessions the results show that matches did not have significantly higher high-intensity accelerations & decelerations than mechanical or mechanical & physiological sessions. Furthermore, matches did not have significantly higher sprint distances than physiological sessions.

Table 4.4 also demonstrates that significant differences were found between mechanical sessions and physiological sessions. These differences were primarily found in the metrics that defined these sessions, demonstrating distinct external load profiles between these sessions. It was found that mechanical sessions had significantly higher High-intensity accelerations & decelerations than physiological sessions, but significantly lower sprint distance. furthermore, mechanical sessions also demonstrated significantly lower high-speed distance when compared to physiological sessions. Whilst this may seem an obvious finding based on the methods used to define these sessions, this demonstrates that these sessions have significantly different external load profiles.

When internal loads are examined in table 4.5, players spent significantly less time above 85% of their maximum HR when light/moderate sessions are compared with mechanical, physiological and mechanical & physiological sessions.

Table 4.4 Average external load data for on-pitch exposures presented in grey. Values presented as means ± standard deviations in grey. Results of one-way ANOVA presented alongside in white. Abbreviations :TD – Total Distance, HSR – High Speed Running, SD – Sprint Distance, Hi A/Ds – High Intensity Accelerations & Decelerations. (N=24)

Average Session Outputs (Mean ± SD)					**Sig. Differences (Alpha set to 0.05)**													
					Session Type		**TD**			**HSD**			**SD**			**Hi A/Ds**		
Stimulus	**TD**	**HSR**	**SD**	**Hi A/Ds**			Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Match (N=34)	10654 ± 1336	778 ± 138	168 ± 49	168 ± 15	Match	Mechanical	<.001	3891.4	5296.5	<.001	531.7	738.1	<.001	131.0	178.9	0.77	-27.6	10.1
						Physiological	<.001	4280.1	5583.3	<.001	233.0	424.4	0.01	6.1	51.8	<.001	22.4	57.5
						Mechanical & Physiological	<.001	3649.3	5188.8	<.001	188.7	414.9	<.001	51.4	103.9	1.00	-23.1	18.3
						Light/Moderate	<.001	5717.3	6814.5	<.001	576.8	738.0	<.001	135.8	173.2	<.001	43.3	72.9
						Metabolic Conditioning	<.001	3857.4	5603.3	<.001	465.0	728.9	<.001	119.8	181.0	<.001	123.9	170.8
Mechanical (N=26)	6060 ± 616	143 ± 67	13 ± 12	176 ± 16	Mechanical	Match	<.001	-5296.5	-3891.4	<.001	-738.1	-531.7	<.001	-178.9	-131.0	0.77	-10.1	27.6
						Physiological	0.72	-346.2	1021.8	<.001	-406.7	-205.7	<.001	-149.9	-102.0	<.001	30.3	67.1
						Mechanical & Physiological	0.99	-972.3	622.4	<.001	-450.2	-216.0	<.001	-104.4	-50.1	0.96	-15.1	27.8
						Light/Moderate	<.001	1085.2	2258.7	0.98	-63.7	108.7	1.00	-20.4	19.6	<.001	51.1	82.6
						Metabolic Conditioning	1.00	-761.0	1033.8	0.97	-173.4	97.5	1.00	-35.9	26.9	<.001	132.0	180.2
Physiological (N-24)	6046 ± 1055	507 ± 122	139 ± 37	134 ± 25	Physiological	Match	<.001	-5583.3	-4280.1	<.001	-424.4	-233.0	0.01	-51.8	-6.1	<.001	-57.5	-22.4
						Mechanical	0.72	-1021.8	346.2	<.001	205.7	406.7	<.001	102.0	149.9	<.001	-67.1	-30.3
						Mechanical & Physiological	0.37	-1265.5	240.1	0.98	-137.5	83.7	<.001	22.5	75.0	<.001	-62.6	-22.1
						Light/Moderate	<.001	809.5	1858.8	<.001	251.6	405.8	<.001	106.8	144.3	0.00	4.0	32.2
						Metabolic Conditioning	0.99	-1059.4	656.7	<.001	138.4	398.1	<.001	90.9	152.1	<.001	84.3	130.5

Table 4.4 continued

Average Session Outputs (Mean ± SD)					Sig. Differences (Alpha set to 0.05)													
					Session Type		TD			HSD			SD			Hi A/Ds		
Stimulus	**TD**	**HSR**	**SD**	**Hi A/Ds**			Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Mechanical & Physiological (N=13)	6235 ± 864	476 ± 193	91 ± 63	170 ± 20	Mechanical & Physiological	Mechanical	<.001	-5188.8	-3649.3	<.001	-414.9	-188.7	<.001	-103.92	-51.44	0.999	-18.32	23.07
						Physiological	0.989	-622.4	972.3	<.001	216.0	450.2	<.001	50.05	104.41	0.957	-27.81	15.07
						Mechanical & Physiological	0.37	-240.1	1265.5	0.982	-83.7	137.5	<.001	-74.98	-22.51	<.001	22.09	62.58
						Light/Moderate	<.001	1181.1	2512.6	<.001	257.8	453.4	<.001	54.11	99.50	<.001	42.57	78.38
						Metabolic Conditioning	0.935	-639.6	1262.2	<.001	152.1	438.3	<.001	39.54	105.95	<.001	124.18	175.31
Training (N=85)	4388 ± 1096	120 ± 80	14 ± 17	110 ± 36	Training	Match	<.001	-6814.5	-5717.3	<.001	-738.0	-576.8	<.001	-173.19	-135.78	<.001	-72.85	-43.35
						Physiological	<.001	-2258.7	-1085.2	0.975	-108.7	63.7	1	-19.58	20.43	<.001	-82.63	-51.07
						Mechanical & Physiological	<.001	-1858.8	-809.5	<.001	-405.8	-251.6	<.001	-144.25	-106.85	0.004	-32.25	-4.03
						Light/Moderate	<.001	-2512.6	-1181.1	<.001	-453.4	-257.8	<.001	-99.50	-54.11	<.001	-78.38	-42.57
						Metabolic Conditioning	<.001	-2318.3	-752.8	0.69	-179.6	58.7	0.998	-31.70	23.58	<.001	68.22	110.32
Metabolic Conditioning (n-7)	6043 ± 732	181 ± 437	18 ± 48	14 ± 35	Metabolic Conditioning	Match	<.001	-5603.3	-3857.4	<.001	-728.9	-465.0	<.001	-181.04	-119.81	<.001	-170.84	-123.90
						Mechanical	0.998	-1033.8	761.0	0.966	-97.5	173.4	0.998	-26.94	35.92	<.001	-180.25	-131.98
						Mechanical & Physiological	0.985	-656.7	1059.4	<.001	-398.1	-138.4	<.001	-152.11	-90.87	<.001	-130.48	-84.33
						Light/Moderate	0.935	-1262.2	639.6	<.001	-438.3	-152.1	<.001	-105.95	-39.54	<.001	-175.31	-124.18
						Metabolic Conditioning	<.001	752.8	2318.3	0.69	-58.7	179.6	0.998	-23.58	31.70	<.001	-110.32	-68.22

Table 4.5 Average internal load data for on-pitch exposures expressed as time spent above 85% of individual HR max. Values presented as means ± standard deviations in grey. Results of one-way ANOVA presented alongside in white. (N=24)

Average Session Outputs (Mean ± SD)		**Sig. Differences (Alpha set to 0.05)**				
		Session Type		**Mins Spent >85% Max HR**		
Stimulus	**Mins Spent >85% Max HR**	Session Type		Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mechanical	16 ± 4	Mechanical	Physiological	1.00	-3.94	4.77
			Mechanical & Physiological	0.83	-6.83	3.04
			Light/Moderate	0.00	3.10	10.40
			Metabolic Conditioning	0.72	-3.11	8.27
Physiological	15 ± 8	Physiological	Mechanical	1.00	-4.77	3.94
			Mechanical & Physiological	0.66	-7.03	2.41
			Light/Moderate	0.00	2.97	9.69
			Metabolic Conditioning	0.82	-3.35	7.67
Mechanical & Physiological	17 ± 6	Mechanical & Physiological	Mechanical	0.83	-3.04	6.83
			Physiological	0.66	-2.41	7.03
			Light/Moderate	0.00	4.56	12.72
			Metabolic Conditioning	0.24	-1.51	10.45
Training	8 ± 5	Light/Moderate	Mechanical	0.00	-10.40	-3.10
			Physiological	0.00	-9.69	-2.97
			Mechanical & Physiological	0.00	-12.72	-4.56
			Metabolic Conditioning	0.15	-9.15	0.80
Metabolic Conditioning	13 ± 6	Metabolic Conditioning	Mechanical	0.72	-8.27	3.11
			Physiological	0.82	-7.67	3.35
			Mechanical & Physiological	0.24	-10.45	1.51
			Light/Moderate	0.15	-0.80	9.15

When analysed by position, table 4.6 demonstrates similar trends to those found in table 4.4, whereby all positions cover significantly more sprint distance in mechanical & physiological sessions and physiological sessions when compared to light/moderate sessions. This trend is also apparent when sprint distance in mechanical & physiological sessions and physiological sessions is compared to mechanical sessions. In terms of high-intensity accelerations & decelerations, FB, CB and MID perform significantly more High-intensity accelerations & decelerations in mechanical and mechanical & physiological sessions compared to light/moderate sessions. This trend is also apparent when mechanical and mechanical & physiological sessions are compared to physiological sessions, However, FOR only perform significantly more high-intensity accelerations & decelerations in mechanical sessions when compared to light/moderate sessions.

Similar to findings from table 4.4, no significant differences were found between positional high-intensity accelerations & decelerations in matches when compared to mechanical or mechanical & physiological sessions. However, matches did not have significantly higher positional sprint distance than physiological sessions for CB and MID. These trends are not evident for FB and FOR, who cover significantly more sprint distance in games compared to any other session. This demonstrates that for each position, intense sessions produce similar outputs to matches for high-intensity accelerations & decelerations, but this is not the case for sprint distance.

When internal loads are examined table 4.7 reveals that CB spent significantly more time above 85% of their HR max when physiological sessions when compared to light/moderate sessions. FB spent significantly more time above 85% of their HR max when mechanical sessions when compared to light/moderate sessions. Interestingly, FB only spent significantly more time above 85% of their HR max when physiological sessions when compared to metabolic conditioning sessions and not light/moderate sessions. MID spent significantly more time above 85% of their HR max when physiological sessions and mechanical sessions when compared to light/moderate sessions and metabolic conditioning sessions. Finally, . FOR spent significantly more time above 85% of their HR max when physiological sessions and mechanical & physiological sessions when compared to light/moderate sessions.

Table 4.6 Average positional external load data for on-pitch exposures. Centre backs (CB) n=6, fullbacks (FB) n=7, midfielders (MID) n=7 and forwards (FOR) n=4. Values presented as means ± standard deviations in gey. Results of one-way ANOVA presented alongside in white. Abbreviations :TD – Total Distance, HSR – High Speed Running, SD – Sprint Distance, Hi A/Ds – High Intensity Accelerations & Decelerations.

Average Session Outputs CB (Mean ± SD)					**Sig. Differences (Alpha set to 0.05)**													
					Session Type		**TD**			**HSD**			**SD**			**Hi A/Ds**		
Stimulus	**TD**	**HSR**	**SD**	**Hi A/Ds**			Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Match	10252 ± 871	650 ± 200	129 ± 64	162 ± 43	Match	Mechanical	<.001	3891.4	5296.5	<.001	422.7	629.9	<.001	88.7	136.4	1	-20.8	19.5
						Physiological	<.001	4280.1	5583.3	<.001	69.2	286.2	0.902	-34.1	15.9	<.001	14.1	56.3
						Mechanical & Physiological	<.001	3649.3	5188.8	<.001	137.6	401.8	0.039	1.0	61.8	0.454	-9.4	41.9
						Light/Moderate	<.001	5717.3	6814.5	<.001	470.8	633.8	<.001	95.4	132.9	<.001	48.1	79.8
						Metabolic Conditioning	<.001	3857.4	5603.3	<.001	317.2	581.3	<.001	72.3	133.1	<.001	120.2	171.6
Mechanical	5657 ± 672	92 ± 56	6 ± 8	158 ± 13	Mechanical	Match	<.001	-5296.5	-3891.4	<.001	-629.9	-422.7	<.001	-136.4	-88.7	1	-19.5	20.8
						Physiological	0.715	-346.2	1021.8	<.001	-460.7	-236.6	<.001	-147.4	-95.8	<.001	14.1	57.6
						Mechanical & Physiological	0.989	-972.3	622.4	<.001	-391.6	-121.7	<.001	-112.3	-50.1	0.433	-9.3	43.2
						Light/Moderate	<.001	1085.2	2258.7	0.954	-60.1	112.0	1	-18.2	21.5	<.001	47.9	81.4
						Metabolic Conditioning	0.998	-761.0	1033.8	0.57	-212.0	57.9	0.944	-40.9	21.3	<.001	120.3	172.8
Physiological	5677 ± 1029	327 ± 126	93 ± 46	114 ± 17	Physiological	Match	<.001	-5583.3	-4280.1	<.001	-286.2	-69.2	0.902	-15.9	34.1	<.001	-56.3	-14.1
						Mechanical	0.715	-1021.8	346.2	<.001	236.6	460.7	<.001	95.8	147.4	<.001	-57.6	-14.1
						Mechanical & Physiological	0.37	-1265.5	240.1	0.4	-46.8	230.8	0.005	8.5	72.4	0.334	-45.9	8.0
						Light/Moderate	<.001	809.5	1858.8	<.001	282.6	466.6	<.001	102.1	144.4	<.001	10.9	46.6
						Metabolic Conditioning	0.985	-1059.4	656.7	<.001	132.8	410.3	<.001	79.8	143.8	<.001	83.7	137.7

Table 4.6 continued

Average Session Outputs CB (Mean ± SD)					**Sig. Differences (Alpha set to 0.05)**													
					Session Type		**TD**			**HSD**			**SD**			**Hi A/Ds**		
Stimulus	**TD**	**HSR**	**SD**	**Hi A/Ds**			Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Mechanical& Physiological	6224 ± 698	386 ± 203	88 ± 55	153 ± 15	Mechanical & Physiological	Match	<.001	-5188.8	-3649.3	<.001	-401.8	-137.6	0.039	-61.8	-1.0	0.454	-41.9	9.4
						Mechanical	0.989	-622.4	972.3	<.001	121.7	391.6	<.001	50.1	112.3	0.433	-43.2	9.3
						Physiological	0.37	-240.1	1265.5	0.4	-230.8	46.8	0.005	-72.4	-8.5	0.334	-8.0	45.9
						Light/Moderate	<.001	1181.1	2512.6	<.001	163.8	401.4	<.001	55.4	110.2	<.001	24.6	70.8
						Metabolic Conditioning	0.935	-639.6	1262.2	0.016	21.7	337.4	<.001	35.0	107.7	<.001	99.0	160.4
Training	4118 ± 1637	81 ± 94	4 ± 7	92 ± 39	Light/Moderate	Match	<.001	-6814.5	-5717.3	<.001	-633.8	-470.8	<.001	-132.9	-95.4	<.001	-79.8	-48.1
						Mechanical	<.001	-2258.7	-1085.2	0.954	-112.0	60.1	1	-21.5	18.2	<.001	-81.4	-47.9
						Physiological	<.001	-1858.8	-809.5	<.001	-466.6	-282.6	<.001	-144.4	-102.1	<.001	-46.6	-10.9
						Mechanical & Physiological	<.001	-2512.6	-1181.1	<.001	-401.4	-163.8	<.001	-110.2	-55.4	<.001	-70.8	-24.6
						Metabolic Conditioning	<.001	-2318.3	-752.8	0.131	-221.8	15.8	0.834	-38.8	15.9	<.001	58.9	105.1
Metabolic Conditioning	5770 ± 694	226 ± 71	34 ± 386	21 ± 37	Metabolic Conditioning	Match	<.001	-5603.3	-3857.4	<.001	-581.3	-317.2	<.001	-133.1	-72.3	<.001	-171.6	-120.2
						Mechanical	0.998	-1033.8	761.0	0.57	-57.9	212.0	0.944	-21.3	40.9	<.001	-172.8	-120.3
						Physiological	0.985	-656.7	1059.4	<.001	-410.3	-132.8	<.001	-143.8	-79.8	<.001	-137.7	-83.7
						Mechanical & Physiological	0.935	-1262.2	639.6	0.016	-337.4	-21.7	<.001	-107.7	-35.0	<.001	-160.4	-99.0
						Light/Moderate	<.001	752.8	2318.3	0.131	-15.8	221.8	0.834	-15.9	38.8	<.001	-105.1	-58.9

Table 4.6 continued

Average Session Outputs FB (Mean ± SD)					Sig. Differences (Alpha set to 0.05)													
Stimulus	TD	HSR	SD	Hi A/Ds	Session Type		TD			HSD			SD			Hi A/Ds		
							Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Match	10473 ± 479	891 ± 162	227 ± 91	169 ± 27	Match	Mechanical	<.001	3739.8	5269.7	<.001	599.5	847.5	<.001	169.0	246.2	0.661	-39.1	12.2
						Physiological	<.001	3752.6	5256.4	<.001	244.7	488.5	<.001	36.4	112.2	0.009	5.1	55.5
						Mechanical & Physiological	<.001	3526.6	5186.7	<.001	284.2	553.3	<.001	81.6	165.3	0.985	-34.3	21.4
						Light/Moderate	<.001	5502.3	6753.5	<.001	648.6	851.5	<.001	176.0	239.1	<.001	34.4	76.4
						Metabolic Conditioning	<.001	3546.8	5470.5	<.001	515.0	826.8	<.001	153.8	250.8	<.001	120.2	184.7
Mechanical	6006 ± 474	197 ± 124	21 ± 17	182 ± 21	Mechanical	Match	<.001	-5269.7	-3739.8	<.001	-847.5	-599.5	<.001	-246.2	-169.0	0.661	-12.2	39.1
						Physiological	1	-717.5	717.0	<.001	-473.2	-240.6	<.001	-169.5	-97.1	<.001	19.6	67.7
						Mechanical & Physiological	0.995	-946.9	650.8	<.001	-434.2	-175.2	<.001	-124.4	-43.9	0.975	-19.8	33.8
						Light/Moderate	<.001	1039.6	2206.7	0.966	-68.0	121.1	1	-29.4	29.4	<.001	49.3	88.4
						Metabolic Conditioning	1	-931.2	938.9	0.917	-204.2	98.9	1	-52.5	41.8	<.001	134.5	197.2
Physiological	5556 ± 1245	481 ± 162	137 ± 71	123 ± 28	Physiological	Match	<.001	-5256.4	-3752.6	<.001	-488.5	-244.7	<.001	-112.2	-36.4	0.009	-55.5	-5.1
						Mechanical	1	-717.0	717.5	<.001	240.6	473.2	<.001	97.1	169.5	<.001	-67.7	-19.6
						Mechanical & Physiological	0.994	-934.2	638.5	0.847	-75.3	179.6	0.006	9.5	88.8	0.001	-63.1	-10.3
						Light/Moderate	<.001	1057.0	2189.7	<.001	291.6	475.2	<.001	104.7	161.8	0.003	6.1	44.1
						Metabolic Conditioning	1	-920.3	928.5	<.001	154.4	454.1	<.001	81.4	174.6	<.001	91.2	153.2

Table 4.6 continued

Average Session Outputs FB (Mean ± SD)					Sig. Differences (Alpha set to 0.05)													
					Session Type		TD			HSD			SD			Hi A/Ds		
Stimulus	**TD**	**HSR**	**SD**	**Hi A/Ds**			Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Mechanical& Physiological	6013 ± 962	437 ± 141	121 ± 54	182 ± 16	Mechanical & Physiological	Match	<.001	-5186.7	-3526.6	<.001	-553.3	-284.2	<.001	-165.3	-81.6	0.985	-21.4	34.3
						Mechanical	0.995	-650.8	946.9	<.001	175.2	434.2	<.001	43.9	124.4	0.975	-33.8	19.8
						Physiological	0.994	-638.5	934.2	0.847	-179.6	75.3	0.006	-88.8	-9.5	0.001	10.3	63.1
						Light/Moderate	<.001	1104.6	2437.9	<.001	223.2	439.3	<.001	50.5	117.7	<.001	39.5	84.2
						Metabolic Conditioning	0.998	-837.1	1141.0	<.001	91.8	412.4	<.001	29.0	128.7	<.001	125.7	192.0
Training	4343 ± 1017	151 ± 131	17 ± 19	112 ± 31	Light/Moderate	Match	<.001	-6753.5	-5502.3	<.001	-851.5	-648.6	<.001	-239.1	-176.0	<.001	-76.4	-34.4
						Mechanical	<.001	-2206.7	-1039.6	0.966	-121.1	68.0	1	-29.4	29.4	<.001	-88.4	-49.3
						Physiological	<.001	-2189.7	-1057.0	<.001	-475.2	-291.6	<.001	-161.8	-104.7	0.003	-44.1	-6.1
						Mechanical & Physiological	<.001	-2437.9	-1104.6	<.001	-439.3	-223.2	<.001	-117.7	-50.5	<.001	-84.2	-39.5
						Metabolic Conditioning	<.001	-2444.2	-794.3	0.53	-212.9	54.5	0.999	-46.9	36.3	<.001	69.4	124.7
Metabolic Conditioning	5791 ± 850	295 ± 111	51 ± 488	20 ± 35	Metabolic Conditioning	Match	<.001	-5470.5	-3546.8	<.001	-826.8	-515.0	<.001	-250.8	-153.8	<.001	-184.7	-120.2
						Mechanical	1	-938.9	931.2	0.917	-98.9	204.2	1	-41.8	52.5	<.001	-197.2	-134.5
						Physiological	1	-928.5	920.3	<.001	-454.1	-154.4	<.001	-174.6	-81.4	<.001	-153.2	-91.2
						Mechanical & Physiological	0.998	-1141.0	837.1	<.001	-412.4	-91.8	<.001	-128.7	-29.0	<.001	-192.0	-125.7
						Light/Moderate	<.001	794.3	2444.2	0.53	-54.5	212.9	0.999	-36.3	46.9	<.001	-124.7	-69.4

Table 4.6 continued

Average Session Outputs MID (Mean ± SD)					Sig. Differences (Alpha set to 0.05)													
Stimulus	TD	HSR	SD	Hi A/Ds	Session Type		TD			HSD			SD			Hi A/Ds		
							Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Match	11416 ± 689	812 ± 197	152 ± 72	172 ± 29	Match	Mechanical	<.001	4469.1	5970.0	<.001	558.4	779.1	<.001	111.5	163.3	0.996	-23.8	16.7
						Physiological	<.001	4469.9	5904.5	<.001	174.9	385.9	0.711	-12.5	37.0	<.001	15.9	54.7
						Mechanical & Physiological	<.001	4232.7	5902.7	<.001	212.6	458.2	<.001	39.6	97.3	0.992	-18.0	27.2
						Light/Moderate	<.001	6235.0	7414.3	<.001	585.0	758.4	<.001	113.0	153.7	<.001	43.6	75.5
						Metabolic Conditioning	<.001	4304.2	6217.5	<.001	489.6	771.0	<.001	98.4	164.5	<.001	128.9	180.6
Mechanical	6155 ± 773	197 ± 163	15 ± 16	175 ± 17	Mechanical	Match	<.001	-5970.0	-4469.1	<.001	-779.1	-558.4	<.001	-163.3	-111.5	0.996	-16.7	23.8
						Physiological	1	-777.2	712.4	<.001	-497.9	-278.8	<.001	-150.8	-99.4	<.001	18.7	59.0
						Mechanical & Physiological	0.996	-1010.6	706.9	<.001	-459.6	-207.1	<.001	-98.6	-39.3	0.915	-15.1	31.4
						Light/Moderate	<.001	982.2	2227.9	1	-88.6	94.6	0.994	-25.6	17.4	<.001	46.2	79.9
						Metabolic Conditioning	1	-936.2	1018.7	0.972	-182.2	105.3	0.996	-39.7	27.8	<.001	131.9	184.8
Physiological	5843 ± 1132	439 ± 144	119 ± 47	128 ± 19	Physiological	Match	<.001	-5904.5	-4469.9	<.001	-385.9	-174.9	0.711	-37.0	12.5	<.001	-54.7	-15.9
						Mechanical	1	-712.4	777.2	<.001	278.8	497.9	<.001	99.4	150.8	<.001	-59.0	-18.7
						Mechanical & Physiological	0.998	-949.4	710.5	0.787	-67.1	177.1	<.001	27.5	84.8	0.002	-53.2	-8.3
						Light/Moderate	<.001	1055.0	2219.9	<.001	305.7	477.0	<.001	100.9	141.2	<.001	8.5	40.0
						Metabolic Conditioning	1	-878.6	1025.9	<.001	209.9	490.0	<.001	86.3	152.0	<.001	93.7	145.2

Table 4.6 continued

Average Session Outputs MID (Mean ± SD)					Sig. Differences (Alpha set to 0.05)													
					Session Type		TD			HSD			SD			Hi A/Ds		
Stimulus	TD	HSR	SD	Hi A/Ds			Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Mechanical& Physiological	6475 ± 847	520 ± 170	118 ± 32	170 ± 13	Mechanical & Physiological	Match	<.001	-5902.7	-4232.7	<.001	-458.2	-212.6	<.001	-97.3	-39.6	0.992	-27.2	18.0
						Mechanical	0.996	-706.9	1010.6	<.001	207.1	459.6	<.001	39.3	98.6	0.915	-31.4	15.1
						Physiological	0.998	-710.5	949.4	0.787	-177.1	67.1	<.001	-84.8	-27.5	0.002	8.3	53.2
						Light/Moderate	<.001	1034.4	2479.4	<.001	230.1	442.6	<.001	39.9	89.8	<.001	35.4	74.5
						Metabolic Conditioning	0.995	-850.7	1236.9	<.001	141.4	448.4	<.001	27.0	99.0	<.001	122.0	178.4
Training	4670 ± 1196	146 ± 115	14 ± 15	109 ± 28	Light/Moderate	Match	<.001	-7414.3	-6235.0	<.001	-758.4	-585.0	<.001	-153.7	-113.0	<.001	-75.5	-43.6
						Mechanical	<.001	-2227.9	-982.2	1	-94.6	88.6	0.994	-17.4	25.6	<.001	-79.9	-46.2
						Physiological	<.001	-2219.9	-1055.0	<.001	-477.0	-305.7	<.001	-141.2	-100.9	<.001	-40.0	-8.5
						Mechanical & Physiological	<.001	-2479.4	-1034.4	<.001	-442.6	-230.1	<.001	-89.8	-39.9	<.001	-74.5	-35.4
						Metabolic Conditioning	<.001	-2424.0	-703.6	0.935	-167.9	85.1	1	-31.6	27.8	<.001	72.0	118.5
Metabolic Conditioning	5998 ± 828	222 ± 64	32 ± 386	22 ± 41	Metabolic Conditioning	Match	<.001	-6217.5	-4304.2	<.001	-771.0	-489.6	<.001	-164.5	-98.4	<.001	-180.6	-128.9
						Mechanical	1	-1018.7	936.2	0.972	-105.3	182.2	0.996	-27.8	39.7	<.001	-184.8	-131.9
						Physiological	1	-1025.9	878.6	<.001	-490.0	-209.9	<.001	-152.0	-86.3	<.001	-145.2	-93.7
						Mechanical & Physiological	0.995	-1236.9	850.7	<.001	-448.4	-141.4	<.001	-99.0	-27.0	<.001	-178.4	-122.0
						Light/Moderate	<.001	703.6	2424.0	0.935	-85.1	167.9	1	-27.8	31.6	<.001	-118.5	-72.0

Table 4.6 continued

Average Session Outputs FOR (Mean ± SD)					Sig. Differences (Alpha set to 0.05)													
					Session Type		TD			HSD			SD			Hi A/Ds		
							Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
Stimulus	**TD**	**HSR**	**SD**	**Hi A/Ds**				Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Match	10090 ± 434	1022 ± 201	301 ± 126	195 ± 25	Match	Mechanical	<.001	2939.7	4577.0	<.001	743.4	990.5	<.001	212.6	380.1	0.999	-20.7	26.5
						Physiological	<.001	3009.0	4609.1	<.001	391.2	632.7	<.001	86.7	250.4	<.001	27.3	73.4
						Mechanical & Physiological	<.001	2693.5	4457.1	<.001	376.6	642.7	<.001	134.1	314.5	0.915	-16.5	34.3
						Light/Moderate	<.001	4837.9	6197.4	<.001	801.9	1007.1	<.001	228.7	367.8	<.001	54.2	93.4
						Metabolic Conditioning	<.001	3073.5	4756.6	<.001	813.6	1067.6	<.001	129.1	301.3	<.001	161.7	210.2
Mechanical	6188 ± 735	222 ± 168	18 ± 19	195 ± 18	Mechanical	Match	<.001	-4577.0	-2939.7	<.001	-990.5	-743.4	<.001	-380.1	-212.6	0.999	-26.5	20.7
						Physiological	1	-692.7	794.0	<.001	-467.1	-242.7	<.001	-203.9	-51.8	<.001	26.0	68.9
						Mechanical & Physiological	0.988	-1013.9	647.6	<.001	-482.7	-231.9	0.148	-157.1	12.9	0.979	-17.9	29.9
						Light/Moderate	<.001	1147.2	2371.4	0.85	-54.8	130.0	1	-60.7	64.5	<.001	53.3	88.5
						Metabolic Conditioning	0.993	-631.2	944.6	0.479	-45.2	192.6	0.047	-161.8	-0.6	<.001	160.4	205.8
Physiological	5982 ± 1095	483 ± 136	124 ± 49	139 ± 22	Physiological	Match	<.001	-4609.1	-3009.0	<.001	-632.7	-391.2	<.001	-250.4	-86.7	<.001	-73.4	-27.3
						Mechanical	1	-794.0	692.7	<.001	242.7	467.1	<.001	51.8	203.9	<.001	-68.9	-26.0
						Mechanical & Physiological	0.962	-1046.2	578.6	1	-125.0	120.3	0.386	-27.3	138.9	<.001	-64.9	-18.0
						Light/Moderate	<.001	1121.7	2295.6	<.001	304.0	481.1	<.001	69.7	189.8	0.001	6.6	40.4
						Metabolic Conditioning	0.999	-662.5	874.5	<.001	312.6	544.6	0.528	-31.9	125.3	<.001	113.5	157.7

Table 4.6 continued

Average Session Outputs FOR (Mean ± SD)					Sig. Differences (Alpha set to 0.05)													
					Session Type		TD			HSD			SD			Hi A/Ds		
Stimulus	TD	HSR	SD	Hi A/Ds			Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval		Sig.	95% Confidence Interval	
								Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound		Lower Bound	Upper Bound
Mechanical& Physiological	6452 ± 865	538 ± 181	120 ± 46	192 ± 19	Mechanical & Physiological	Match	<.001	-4457.1	-2693.5	<.001	-642.7	-376.6	<.001	-314.5	-134.1	0.915	-34.3	16.5
						Mechanical	0.988	-647.6	1013.9	<.001	231.9	482.7	0.148	-12.9	157.1	0.979	-29.9	17.9
						Physiological	0.962	-578.6	1046.2	1	-120.3	125.0	0.386	-138.9	27.3	<.001	18.0	64.9
						Light/Moderate	<.001	1248.2	2636.7	<.001	290.1	499.7	0.036	2.9	145.0	<.001	44.9	84.9
						Metabolic Conditioning	0.862	-513.5	1193.1	<.001	302.2	559.8	1	-96.4	78.2	<.001	152.5	201.6
Training	4694 ± 1196	157 ± 132	17 ± 20	124 ± 33	Light/Moderate	Match	<.001	-6197.4	-4837.9	<.001	-1007.1	-801.9	<.001	-367.8	-228.7	<.001	-93.4	-54.2
						Mechanical	<.001	-2371.4	-1147.2	0.85	-130.0	54.8	1	-64.5	60.7	<.001	-88.5	-53.3
						Physiological	<.001	-2295.6	-1121.7	<.001	-481.1	-304.0	<.001	-189.8	-69.7	0.001	-40.4	-6.6
						Mechanical & Physiological	<.001	-2636.7	-1248.2	<.001	-499.7	-290.1	0.036	-145.0	-2.9	<.001	-84.9	-44.9
						Metabolic Conditioning	<.001	-2245.0	-960.3	0.893	-60.9	133.0	0.005	-148.8	-17.4	<.001	93.7	130.7
Metabolic Conditioning	6051 ± 787	252 ± 76	37 ± 388	22 ± 40	Metabolic Conditioning	Match	<.001	-4756.6	-3073.5	<.001	-1067.6	-813.6	<.001	-301.3	-129.1	<.001	-210.2	-161.7
						Mechanical	0.993	-944.6	631.2	0.479	-192.6	45.2	0.047	0.6	161.8	<.001	-205.8	-160.4
						Physiological	0.999	-874.5	662.5	<.001	-544.6	-312.6	0.528	-125.3	31.9	<.001	-157.7	-113.5
						Mechanical & Physiological	0.862	-1193.1	513.5	<.001	-559.8	-302.2	1	-78.2	96.4	<.001	-201.6	-152.5
						Light/Moderate	<.001	960.3	2245.0	0.893	-133.0	60.9	0.005	17.4	148.8	<.001	-130.7	-93.7

Table 4.7 Average positional internal load for on pitch exposures, expressed as time spent above 85% of individual HR max. Centre backs (CB) n=6, fullbacks (FB) n=7, midfielders (MID) n=7 and forwards (FOR) n=4. Values presented as means ± standard deviations in gey. Results of one-way ANOVA presented alongside in white.

Average Session Outputs CB (Mean ± SD)		**Sig. Differences (Alpha set to 0.05)**				
Stimulus	Mins Spent >85% Max HR	Session Type		**Mins Spent >85% Max HR**		
				Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mechanical	12 ± 6	Mechanical	Physiological	0.931	-8.301	4.581
			Mechanical & Physiological	0.975	-9.446	6.074
			Light/Moderate	0.128	-0.685	9.146
			Metabolic Conditioning	1.000	-6.230	6.358
Physiological	14 ± 10	Physiological	Mechanical	0.931	-4.581	8.301
			Mechanical & Physiological	1.000	-7.805	8.154
			Light/Moderate	0.014	0.835	11.346
			Metabolic Conditioning	0.928	-4.639	8.487
Mechanical & Physiological	14 ± 10	Mechanical & Physiological	Mechanical	0.975	-6.074	9.446
			Physiological	1.000	-8.154	7.805
			Light/Moderate	0.121	-0.891	12.725
			Metabolic Conditioning	0.973	-6.111	9.611
Training	8 ± 7	Training	Mechanical	0.128	-9.146	0.685
			Physiological	0.014	-11.346	-0.835
			Mechanical & Physiological	0.121	-12.725	0.891
			Metabolic Conditioning	0.162	-9.241	0.908
Metabolic Conditioning	8 ± 6	Metabolic Conditioning	Mechanical	1.000	-6.358	6.230
			Physiological	0.928	-8.487	4.639
			Mechanical & Physiological	0.973	-9.611	6.111
			Light/Moderate	0.162	-0.908	9.241

Average Session Outputs FB (Mean ± SD)		**Sig. Differences (Alpha set to 0.05)**				
Stimulus	Mins Spent >85% Max HR	Session Type		**Mins Spent >85% Max HR**		
				Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mechanical	16 ± 8	Mechanical	Physiological	0.999	-5.730	6.800
			Mechanical & Physiological	0.994	-6.406	8.689
			Light/Moderate	0.017	0.672	10.235
			Metabolic Conditioning	0.018	0.811	13.055
Physiological	15 ± 8	Physiological	Mechanical	0.999	-6.800	5.730
			Mechanical & Physiological	1.000	-7.155	8.367
			Light/Moderate	0.066	-0.193	10.030
			Metabolic Conditioning	0.049	0.014	12.781
Mechanical & Physiological	14 ± 6	Mechanical & Physiological	Mechanical	0.994	-8.689	6.406
			Physiological	1.000	-8.367	7.155
			Light/Moderate	0.380	-2.309	10.934
			Metabolic Conditioning	0.230	-1.855	13.438
Training	10 ± 7	Training	Mechanical	0.017	-10.235	-0.672
			Physiological	0.066	-10.030	0.193
			Mechanical & Physiological	0.380	-10.934	2.309
			Metabolic Conditioning	0.922	-3.456	6.415
Metabolic Conditioning	10 ± 8	Metabolic Conditioning	Mechanical	0.018	-13.055	-0.811
			Physiological	0.049	-12.781	-0.014
			Mechanical & Physiological	0.230	-13.438	1.855
			Light/Moderate	0.922	-6.415	3.456

Table 4.7 continued

Average Session Outputs MID (Mean ± SD)		Sig. Differences (Alpha set to 0.05)				
		Session Type		Mins Spent >85% Max HR		
Stimulus	Mins Spent >85% Max HR			Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mechanical	20 ± 9	Mechanical	Physiological	1.000	-7.493	7.256
			Mechanical & Physiological	0.344	-2.897	14.871
			Light/Moderate	0.002	1.963	13.220
			Metabolic Conditioning	0.002	2.573	16.985
Physiological	19 ± 11	Physiological	Mechanical	1.000	-7.256	7.493
			Mechanical & Physiological	0.353	-3.030	15.242
			Light/Moderate	0.005	1.693	13.728
			Metabolic Conditioning	0.003	2.384	17.412
Mechanical & Physiological	13 ± 9	Mechanical & Physiological	Mechanical	0.344	-14.871	2.897
			Physiological	0.353	-15.242	3.030
			Light/Moderate	0.980	-6.190	9.399
			Metabolic Conditioning	0.773	-5.209	12.792
Training	12 ± 8	Training	Mechanical	0.002	-13.220	-1.963
			Physiological	0.005	-13.728	-1.693
			Mechanical & Physiological	0.980	-9.399	6.190
			Metabolic Conditioning	0.837	-3.622	7.997
Metabolic Conditioning	12 ± 10	Metabolic Conditioning	Mechanical	0.002	-16.985	-2.573
			Physiological	0.003	-17.412	-2.384
			Mechanical & Physiological	0.773	-12.792	5.209
			Light/Moderate	0.837	-7.997	3.622

Average Session Outputs FOR (Mean ± SD)		Sig. Differences (Alpha set to 0.05)				
		Session Type		Mins Spent >85% Max HR		
Stimulus	Mins Spent >85% Max HR			Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mechanical	12 ± 6	Mechanical	Physiological	0.907	-8.101	4.213
			Mechanical & Physiological	0.849	-10.142	4.693
			Light/Moderate	0.144	-0.746	8.651
			Metabolic Conditioning	0.754	-3.407	8.625
Physiological	15 ± 9	Physiological	Mechanical	0.907	-4.213	8.101
			Mechanical & Physiological	0.999	-8.407	6.847
			Light/Moderate	0.012	0.873	10.920
			Metabolic Conditioning	0.270	-1.720	10.826
Mechanical & Physiological	15 ± 11	Mechanical & Physiological	Mechanical	0.849	-4.693	10.142
			Physiological	0.999	-6.847	8.407
			Light/Moderate	0.041	0.170	13.185
			Metabolic Conditioning	0.292	-2.181	12.847
Training	9 ± 6	Training	Mechanical	0.144	-8.651	0.746
			Physiological	0.012	-10.920	-0.873
			Mechanical & Physiological	0.041	-13.185	-0.170
			Metabolic Conditioning	0.941	-6.194	3.507
Metabolic Conditioning	9 ± 9	Metabolic Conditioning	Mechanical	0.754	-8.625	3.407
			Physiological	0.270	-10.826	1.720
			Mechanical & Physiological	0.292	-12.847	2.181
			Light/Moderate	0.941	-3.507	6.194

4.5 Discussion

This study aimed to identify and map intense sessions during the in-season phase in an elite U23s football team using novel ways to identify intense physiological and mechanical sessions. To the authors' knowledge, this is the first study that has attempted to identify and map the training stimulus in the context of the physiological and mechanical load-adaptation pathway across the in-season phase in football. The results from this study would demonstrate that intense sessions, as per the methods used in this study occur frequently and regularly across the season for each position. This could have potential implications on the mechanical and physiological load-adaptation pathways. However, these adaptive responses may occur at different time points throughout the in-season phase based on playing position. When compared to light/moderate sessions, it was demonstrated that intense sessions have significantly higher sprint distance and high-intensity accelerations & decelerations. This was also the case when internal responses were compared across sessions. Overall, if one considers the most intense stimuli as a proxy for adaptation, then the results of this study demonstrate that there could be the potential implications on the physiological and mechanical load-adaptation pathways throughout the season.

The findings of this study demonstrate that intense sessions, classed as either mechanical, physiological, or mechanical & physiological, demonstrated significantly higher external loads when compared to light/moderate training sessions. This suggests that these sessions may have the potential to stimulate certain adaptive responses since they differ significantly in demands to the more frequent light/moderate sessions. As mentioned previously, a single short, high-intensity stimulus implemented by Nyberg et al. (2016) and Jensen et al. (2009) was enough of a deviation away from normal training to lead to positive adaptations in football players. However it is difficult to draw comparisons between previous studies as what qualifies as 'normal training' would be different across different teams.

Nevertheless, for this squad, the high-intensity acceleration & deceleration demands of mechanical and mechanical & physiological sessions were not significantly different to matches. It is well-established that match days represent the most physically demanding days within a microcycle (Di Salvo et al., 2007). This would suggest that even though matches are held up as the biggest stimulus of the week (Morgans et al., 2018), in some cases the positional demands of mechanical, or mechanical & physiological sessions could have a greater number of high-intensity accelerations & decelerations.

The mechanical load-adaptation pathway could be stimulated as a consequence of drills such as small-games and rondos which may 'overload' certain physical components such as changes of direction in order to challenge players physically and stimulate responses in the mechanical load-adaptation pathway (Gaudino et al., 2014; Ross and Leveritt, 2001; Lopez-Felip., 2019; Ade et al., 2014). It has been demonstrated by Rebelo et al. (2016) that accelerations and decelerations (>2.0 $m \cdot s^{-2}$) were higher in 4v4+GK than in 8v8+GK. Thus, sessions where the main stimulus involves using SSGs with low player numbers and small pitch spaces, could have the potential to stimulate adaptive responses In the mechanical load-adaptation pathway Furthermore, it has been shown possession based SSGs lead to a greater number of decelerations. This is due to the multidirectional effect of the possession drills and the fact that having a goal to target creates a more linear focus to running (Gaudino et al., 2014). The SSG in this study contained goals and as such, may have benefitted from being possession based in order to increase the mechanical load, if such adaptations were desired.

Moreover, at this club Rondos were a staple in many training session, which may help explain the trends for Hi A/Ds observed in table 4.1. Overall, these drills may stimulate the mechanical load-adaptation pathway as a result of the biomechanical loads placed on the tissues of the body such as muscles, tendons and bones, due to the repetitive impact forces, joint forces, and muscle-tendon forces that result from impacts with the ground during acceleration (Verheul et al., 2019). Moreover, decelerations require large braking forces which require a high eccentric demands on muscles such as the quadriceps, hamstrings, and gastrocnemius to absorb these forces (Hodgson et al., 2014). Evidence of positive adaptations to SSGs such as increased muscle size, muscle fibre mass and muscle strength has been demonstrated when a 12-week football training intervention consisting of 5v5 SSGs was compared to moderate intensity running by Krustrup et al. (2010), highlighting the potential of this stimulus to lead to positive adaptations.

With regards to the physiological load-adaptation pathway, this pathway could be stimulated through the use of large-sided games. In terms of external demands a recent meta-analysis concluded that increases in the relative pitch area during SSGs increases the total distance, the distance covered at high speeds as well as increasing the mean heart rate (Praça et al., 2022). It has also been previously demonstrated that SSGs elicit greater HR, blood lactate and perceptual responses when pitch sizes increase (Hill-Haas et al., 2011; Rampinini et al., 2007). Previous studies report that drills using larger relative pitch areas or a reduced number of ball contacts per player before possession has to be released also elevated exercise intensity (Castellano et al., 2013; Dellal et al., 2011; Hill-Haas et al., 2011). However, other drills such as counter attacking drills and technical drills using speed endurance protocols would also potentially stimulate the physiological load-adaptation pathway. This has been demonstrated by Ade et al. (2014) showed that speed endurance drills elicited greater mean heart-rate responses, blood lactate concentrations, and subjective ratings of perceived exertion when compared with SSGs in elite soccer players. Furthermore, the use of position-specific speed endurance drills similar to the protocols used by Ade et al. (2020), were utilised throughout the season during training in this club.

It is important to note that players in different positions may experience different physiological responses to the same actions as a consequence of being adapted to their position. As mentioned previously players who play in positions that require a high density of changes of direction (e.g. central midfielders) training sessions may require a higher density of these actions to stimulate an adaptive response. The internal responses would suggest that CB experienced a higher internal response when they covered more sprinting in physiological sessions, whereas FB experienced a higher internal response when exposed to a high number of accelerations and decelerations in mechanical sessions. These actions would seem to differ from what could be classed as normal demands for these positions (i.e FB may generally have higher sprinting demands and CB may have more change of direction demands). Thus, these exposures may have been outside of normal demands for these players, which increase the internal physiological response. Thus, it would seem that the same external loads may not be appropriate to classify a physiological or mechanical session for different positions.

A stimulus must be intense enough and applied over a long enough period to lead to an adaptation (Hawley et al., 2014; Zierath and Wallberg-Henriksson, 2015). Thus, whilst it can be argued that intense sessions may be enough to stimulate responses in the physiological and mechanical load-adaptation pathway, how these responses manifest into an observable adaptation is unknown. It may be that the stimuli observed in this study is enough to stimulate a response at a low level of organisation, such as the cellular level, but needs to be repeated over time to manifest as an adaptation at the organism level (Balagué et al., 2020; Pol et al., 2020). However, without measuring internal responses it is difficult to suggest that this would occur in response to the stimuli observed in this study. Thus, it would seem imperative to adopt broader approaches to measuring adaptative responses to training by focusing on internal responses in order to truly understand how players respond to training in the in-season phase.

This would seem important given that this study found that across the in-season phase, different sessions qualified as intense from a mechanical, physiological, or mechanical & physiological standpoint depending on the position played. This is not surprising given that individual responses may vary to the same training stimulus (Morgans et al., 2014). However, when intense sessions were mapped across the season this study found that implications of training sessions may not simply lead to a training response or no response. In contrast, training sessions may stimulate unique adaptations in distinct load-adaptation pathways based on playing position, thus expanding the possible outcomes of any training session on individual player. This may expand how football practitioners think about the implications of a training stimulus on players in different positions. These implications could be dictated by a ‘theoretical individual threshold’ which is dictated by players adapting to the different demands of their position. For example, in this study the threshold for intense sessions differed by position (Table 4.1). Thus, the implications of a single could be a physiological response, a mechanical response, or no response (figure 4.5). This may have implications on the considerations taken when designing team training sessions.

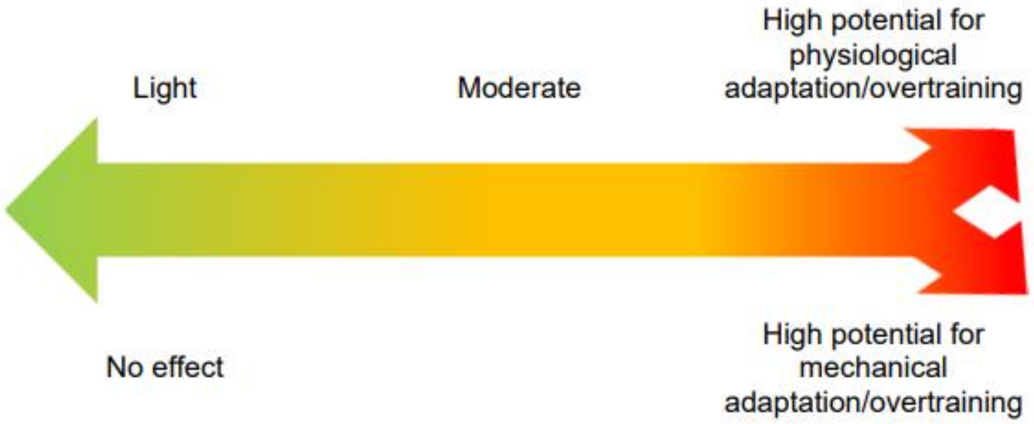


Figure 4.5 An updated outline that takes into consideration the potential for a stimulus to lead to adaptation in two distinct load-adaptation pathways.

Interestingly, for all positions, it would seem that the potential for training to lead to adaptations in the mechanical-load adaptation pathways was greater during the first half of the season. In contrast, the potential for training adaptations in the physiological-load adaptation pathways is greater during the second half of the season. The differences in where different types of sessions occur are most likely a consequence of training design, where intense mechanical sessions were planned more frequently during the early part of the season. However, the low number of mechanical sessions in the second half of the season is likely due to the congested fixture list, especially in weeks 24 to 29. Consequently, more light/moderate training sessions would have been planned to manage fatigue in this period (Anderson et al.,2016; Malone et al., 2015). Overall, this could have implications for the types of adaptations that potentially could have occurred throughout the season and highlights the need to monitor internal responses in both the mechanical and physiological load-adaptation pathways.

Overall, this study attempted to map where intense sessions occur across a single season for each playing position using an exploratory method for identifying football-based sessions with the highest external demands. This was an attempt to map the potential implications of the training on adaptations in the physiological and biomechanical load-adaptation pathways. The results of this study demonstrate that intense training sessions occur frequently and regularly across the season for each position yet differ in terms of where they occur throughout the in-season phase based on position.

4.6 Limitations

Despite this study being novel in nature, it is not without its limitations. From a conceptual standpoint, the categorisation of mechanical sessions using High-intensity accelerations & decelerations and physiological sessions using sprint distance has some notable limitations. Firstly, the external demands placed on the body cannot be captured by two external load metrics, there are many more metrics that could be used to capture the physiological and mechanical response (Vanrenterghem et al., 2017). It must be noted that any potential physiological adaptations that arise as a result of a session with a high sprint distance, does not indicate that sprint distance is the driver of a response in the physiological load-adaptation pathway. Rather this metric may be just one indicator of the nature of a training session, as there are other contextual factors to consider which could also be responsible for stimulating responses. For example, training sessions with a high volume of HSR could stimulate metabolic responses in the physiological load-adaptation pathway, independent of sprint distance. Furthermore, High-intensity accelerations & decelerations may also have implications for the physiological load-adaptation pathway this is evidenced by the HR responses found in this study.

Likewise, there are morphological adaptations that occur as a result of sprinting and the forces associated with sprinting could be considered highly mechanical (Ross and Leveritt, 2001). Furthermore, the thresholds used in this study for each GPS metric do not represent a threshold for an adaptive response to be triggered, therefore, a dose-response relationship between these metrics and any adaptation cannot be claimed with any strength. Furthermore, what qualifies as a ‘sprint’ may be different for different players based on individual maximum speeds. For example, a sprint at 7 m/s will not have the same physiological impact on a player with a max speed of 10 m/s versus a player with a max speed of 9 m/s. This point can also be extended to accelerations and decelerations as the impact of these actions will vary across individuals based on characteristics such as muscle size and strength, tendon, and bone properties, as well as movement proficiency when accelerating or decelerating. Therefore, it is impossible to ascertain a dose-response relationship between the magnitude of these actions and individual adaptation.

Furthermore, despite the common practice of using high velocity bands for acceleration and deceleration to monitor training loads (Delves et al., 2021), these metrics are not without their limitations. There is error associated with threshold-based counts for accelerations and decelerations, which is a consequence of using cut offs to define ‘intense’ actions and interunit variance. This means an acceleration near the cut off could be measured differently by two different devices, the bias of which has been shown to be 10-15% in a study using 15hz GPS devices (Bucheit et al., 2014).

However, the devices used in this study have a greater sampling rate (18hz) than the units used in the study by Bucheit.et al. (2014) (15hz), which may provide lower variability. However, to date, no data exists on the reliability of these specific units in terms of accelerations and decelerations. Nevertheless, this paper does not suggest that the thresholds used in this study represent the potential for dose-response relationships. In contrast, this study acts as a thought experiment, demonstrating the value of considering two separate load-adaptation pathways when analysing external and internal loads and the potential for different adaptations to occur across the season.

A further limitation relates to the classification of intense sessions using the 75th percentile. In theory, any threshold could have been used to classify intense training sessions. As mentioned in the methods section this threshold was chosen retrospectively to separate intense sessions for non-intense sessions. Of course, different thresholds would lead to a different number of intense sessions throughout the season. Moreover, it would be inappropriate to suggest a physiological session that fell under the 75th percentile by a sprint distance of 5m for example, would not qualify as enough to elicit an adaptive response. This is due to the inherent error in measurement associated with the devices used and the fact that no internal response was taken in this study to assess the true response to the stimuli. Nevertheless, this study does not claim that this threshold acts as the difference between a response and homeostasis. Moreover, since this threshold was enough to demonstrate that significant differences existed between intense and non-intense sessions, therefore suggesting the potential for an adaptive response, it would seem to have fulfilled its purpose as a though-experiment.

From a practical standpoint, the external loads for each position are dependent upon the individual players from those positions. At times throughout the season, certain players were not available through illness/injury/training with another squad. Therefore, the positional averages for each session may be made up of one or two players in some sessions, whilst others may have had the full number of players for each position in the squad. Thus, it is difficult to apply these findings to any individual player. Nevertheless, to map positional trends across the season, this was a necessary compromise.

Furthermore, the positions are not broken down further into types of players within those positions (i.e a number 8 vs a number 6 in MID which invariably have different physical demands, or a winger vs a defensive midfielder). A greater insight into internal loads could have been accomplished through the use of differential RPE (dRPE). This could have provided an easy to collect indirect measure of physiological and biomechanical load (Vanrenterghem et al., 2017). Finally, this study is representative of one club and cohort of players therefore generalisations to other clubs cannot be made with absolute confidence. Nevertheless the aim of this paper was not to determine the efficacy of the methods used in this study, hence why a power calculation was also deemed inappropriate based on the nature of this paper.

4.7 Conclusion

Overall, the methods used, and subsequent results highlight how the physiological and mechanical load-adaptation pathway could be stimulated as a result of the training program during the in-season phase. Despite its limitations, the methods used in this study provide a more holistic approach to mapping external loads in football. One of the key practical applications of this study is the information that could be provided to technical coaches to illustrate how planned training sessions may be affecting the adaptive responses of players in different positions. However, different metrics may need to be used to more accurately reflect the physiological and mechanical demands. However, based on the findings and ideas presented in this study, it is hoped the main takeaway is that broader approaches to measuring adaptive responses to training are needed to truly understand how players respond to football training stimulus during the in-season phase.

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CHAPTER FIVE

UNTARGETED METABOLOMICS IN FOOTBALL– A PILOT STUDY

5.1 Introduction

External load data from Chapter 4 demonstrates that there may be opportunities for adaptation during the in-season phase. Depending on their intensity, training sessions may lead to physiological and/or biomechanical adaptations. Investigations into the internal responses to intense training sessions are therefore warranted.

Evidence from Chapter 3 would suggest that broader approaches are needed to capture the full spectrum of internal responses that occur in a complex biological system to the demands of football. Measuring internal responses on the cellular level, using metabolomic approaches could be deemed advantageous when looking to observe the internal responses to football training. The advantages of this approach are related to efficiency and cost. This is due to the ease associated with taking a single sample, and the comprehensive amount of information regarding dynamic changes in metabolism that can be harnessed from a single sample (Wackerhage, 2014; Gomes et al., 2019). Thus, these approaches may be more favourable than measures that provide narrow insights into one parameter. such as analysing a blood sample for a single substrate.

Metabolites are substrates that provide insights into cellular metabolism. These products are essential for energy production, signal transduction and apoptosis (Duft et al., 2017). Thus, this approach can provide a broad picture of the function of a complex adaptive system (Hudson et al., 2021). Metabolomic studies have primarily been based on plasma or serum analysis drawn from the vein (Duft et al. 2017). However, these types of measurements can be a challenge to obtain in an elite football environment due to time restrictions and the invasiveness associated with these approaches. This is evidenced in chapter 3, where more pragmatic approaches are favoured in football research when mapping internal responses.

However, as alluded to in chapter 3, novel and broader insights into the adaptive response to football may be gained in future research by merging conceptual and pragmatic approaches to mapping responses to football. Distinct metabolic changes across several metabolic pathways have been demonstrated to occur in response to two exercise sessions with differing rest duration between repeated efforts, highlighting the potential of this method to identify stimulus-specific metabolic ‘fingerprints’ (Pechlivanis et al., 2010). Metabolic fingerprinting relates to the classification of changes that occur in a biological sample. As metabolomics research develops in football over time, this could lead to metabolic profiling, which focuses on specific metabolites that act as signalling molecules in specific pathways (Duft et al., 2017). This could lead to more focused research questions to understand how these pathways are stimulated by various football activities. Consequently, these approaches could provide detailed insights into the complex and dynamic nature of the internal responses to football activities and the signals that lead to adaptation.

Therefore, exploring ways of investigating metabolomic responses to football activities in a less-invasive and more time-efficient manner may provide more opportunities for greater insights into internal responses. Consequently, analysing capillary blood samples to investigate metabolic may provide advantages in elite football due to its ease of collection and less invasive nature than taking venous blood samples. This approach could allow for comprehensive mapping of internal responses across multiple time periods. Methodologically, salivary metabolomics has been reported as a valid, non-invasive, and comprehensive way of analysing the metabolic responses to football (Pitti et al.,2019; Ra et al., 2014). Furthermore, previous work in football has shown that associations exist between the external load and urinary metabolic profiles, with alterations of biochemical pathways associated with long-term adaptations to football training (Quintas et al., 2020).

However, despite the ease at which saliva, urine and capillary blood samples can be collected, caution must be exercised not to use results from these different biofluids interchangeably. This is due to the unique environments of the oral cavity and urinary tract (Williamson et al., 2012). Moreover, in a metabolomics study conducted on youth football players using blood, saliva, and urine, it was found that blood provided a more comprehensive picture of the metabolic changes in response to ‘moderately-intense’ training (Alzharani et al., 2020). Furthermore, Nishiumi et al. (2019), demonstrated that there are differences in the levels of certain metabolites when comparing blood taken from fingertip versus venous blood samples. However, the amount of blood taken from the fingertip in this study was limited to a few drops, unfortunately, the authors were not specific with the amount of blood taken. Despite this, the authors suggest metabolomics using fingertip blood samples may provide useful insights.

Consequently, this study aims to capture the metabolic response to a single intense training session in football using capillary blood samples taken from the fingertip. Furthermore, this is, to the author’s knowledge, the first study to investigate the metabolomic responses to football training using capillary blood samples. This study will provide novel insights into the utility of this method as well as add to the limited metabolomic literature in football. Overall, this study could help address issues raised in Chapter 3 regarding the challenges associated with tracking adaptation in football frequently over time.

5.2 Methods

5.2.1 Participants and Research Design

Following ethical approval and informed consent, 10 elite u23s football players (3 centre backs, 1 full back, 3 midfielders and 3 forwards were recruited for this study (mean ± SD, age; 22.0 ± 2.7 years, body mass; 74.4 ± 6.2 kg).

5.2.2 Research Design

10 elite u23s players from a premier league club academy took part in this study. The study took place over 1 day before and after a MD-3 session at the club’s training ground. Players arrived at the training ground at 9 am where fasted capillary blood samples were taken from the fingertip two hours before the commencement of the training session. The blood collection process took between 30 seconds to 3 minutes per player. The researcher was assisted by a group of sports scientists familiar with fingertip blood collection and as such, 5 players at a time had blood samples taken. Once taken samples were allowed to clot for 30 minutes and then placed on ice. Players then consumed breakfast, the breakfast was not standardised In terms of food content or amount for each player, however, the meal choices were limited to two options. Forty-five minutes after breakfast players completed a forty-minute pre-training gym session as part of their normal daily routine. This gym session consisted of mobility-based exercises, proprioception, and stability exercises, as well as some bodyweight strength and plyometric exercises. This session would be relatively low in terms of intensity when compared to gym sessions focused on strength development, which usually took place post-training. Twenty minutes after this gym session players began training. This session was designed to be an intense training session from a physiological and mechanical standpoint. The aim was to achieve the physiological stimulus through high-speed running and sprinting, whilst the mechanical stimulus

would come from small-sided games.

Capillary blood samples were collected within five minutes of the cessation of the training session using fingertip samples. As before, the blood collection process took between thirty seconds and three minutes per player. Samples were allowed to clot for thirty minutes and then put on ice. Both pre-training and post-training samples were then transported to a University laboratory where sample preparation took place before they were then analysed. A broad workflow for this study is outlined below.

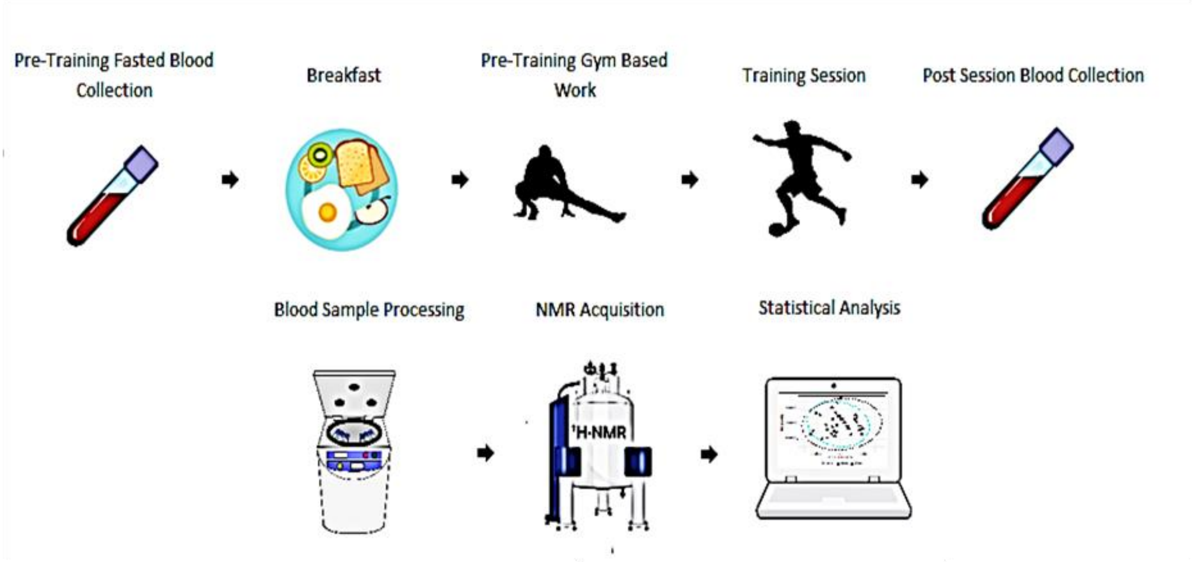


Figure 5.1 Workflow for this study from initial pre-training sample collection to statistical analysis.

5.2.3 Training Demands

Training loads were quantified using GPS. The GPS units (Apex, StatSports, Ireland) were placed between the scapulae of the players in bespoke vests. The process for this session is the same as outlined in Chapter 4.

5.2.4 Dietary Intake

The pre-training meal was analysed using the nutrition analysis software Nutritics (Nutritics Ltd., Dublin, Ireland) by a registered sports and exercise nutritionist (SENr). This control measure allowed us to account for any metabolites that may have appeared in the analysis due to dietary intake. Macronutrient and energy intakes are reported here as mean (±SD).

Table 5.1 Macronutrient and energy intakes from breakfast consumed after fasting blood sample.

Energy(Kcal)	Energy(kJ)	Carbohydrate (g)	Protein (g)	Fat (g)
546 ± 155	2286 ± 642	47 ± 16	28 ± 8	27 ± 15

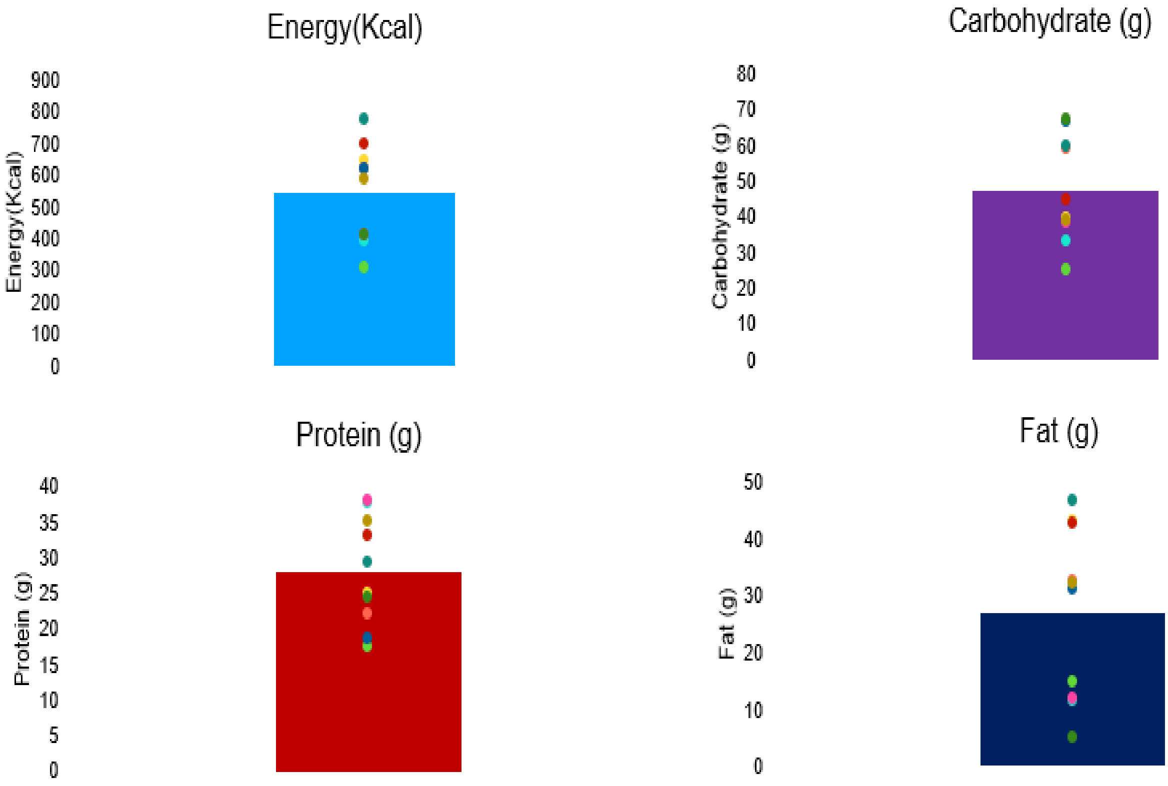


Figure 5.2. Mean + Individual SD for each macronutrient is presented in table 5.1. Each dot represents an individual player whilst column represents the mean.

5.2.5 Capillary serum sample collection

Capillary blood samples were collected from the fingertip in 1 ml untreated microvette tubes, inverted 5-6 times and allowed to clot at room temperature for 30 minutes before centrifugation (1300 x g, 10 min, 4ºC). Serum was aliquoted and stored at -80ºC until analysis. All blood samples (pre-and post-training) were processed in the same manner (with time between blood draw and freeze consistent throughout).

5.2.6 Serum preparation for NMR

Serum samples were prepared according to standard protocols (Beckonert et al., 2007) with NMR samples consisting of 50% serum, 10% ^2H_2O with 100mM sodium phosphate buffer pH 7.4 and 0.1% azide.

5.2.7 NMR set-up and acquisition

Spectra were acquired on Bruker 700MHz avance IIIHD spectrometer equipped with TCI cryoprobe and chilled autosampler (SampleJet). Standard vendor pulse-sequences were applied to collect 1D ^1H NMR spectra (cpmg1dpr). A Carr-Purcell-Meiboom-Gill (CPMG) edited pulse sequence was employed to attenuate signals from macromolecules present (proteins etc.). Serum spectra were collected at 37^{o}C with 32 transients whereas tissue extract spectra were collected at 25^{o}C with 128 transients for optimal sensitivity, with all other parameters kept constant.

5.2.8 Spectra Processing and Quality Control

All spectra were automatically pre-processed at spectrometer by Fourier-transformation, phase correction and baseline correction using standard vendor routines (apk0.noe) and referenced indirectly via anomeric glucose signal. Spectra were subjected to quality control criteria as recommended by Metabolomics Standards Initiative [MSI] (Salek et al., 2013; Sumner et al., 2007). Quality control criteria consisted of appraisal of baseline, linewidth, residual water signal width, phase, and signal-to-noise. Spectra were bucketed according to peaks boundaries defined with each bucket the sum of the integral for that region divided by the region width.

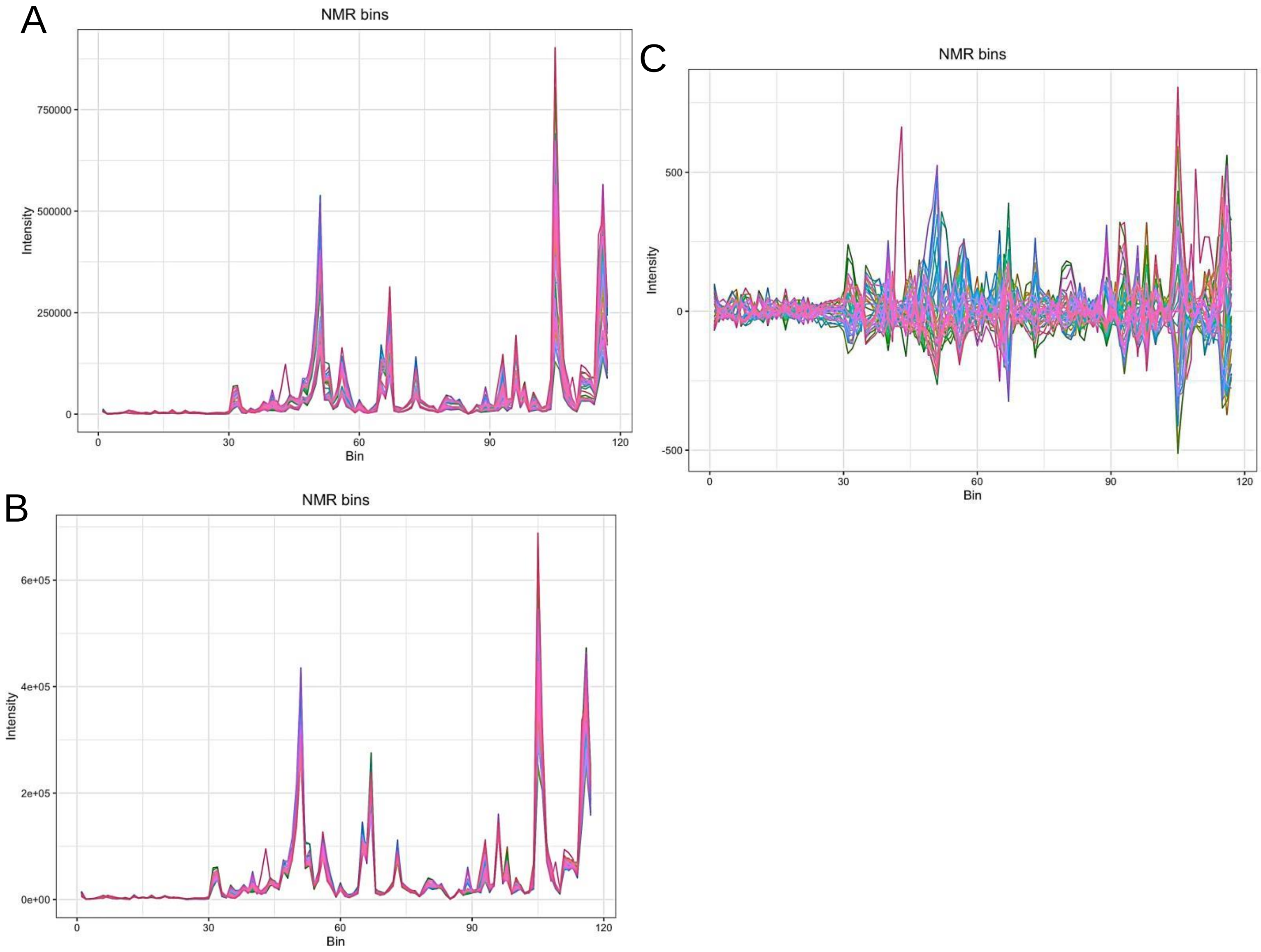


Figure 5.3 Panel A shows the raw spectra following spectral processing and quality control described in methods. Panel B shows the spectra following probabilistic quotient normalization and panel C shows the spectra following normalization and pareto scaling.

Metabolites were annotated via the use of metabolite recognition software Chenomx (Chenomx v 8.2, Chenomx Ltd, CA) and the respective buckets were annotated before statistical analysis. Metabolite identities were confirmed (where possible) via comparison to the in-house metabolite library

5.3 Statistical Analysis

Spectra were normalised via Probabilistic Quotient Normalisation (PQN) method (Kohl et al., 2012). This is a pre-processing step required when dealing with complex biofluids. This method accounts for different dilutions of samples by scaling the spectra to the same virtual overall concentration, Improper normalization methods can significantly impair data collected. Otherwise, unwanted biases and variances could occur in samples without this scaling (Uh et al., 2020). PQN calculates the most probable dilution factor by comparing the distribution of the quotients of the amplitudes of a test spectrum against a reference spectrum (Dieterle et al., 2006).

Samples of participants before and after training were compared via paired Welch tests which p-values were corrected for false discovery rate by Benjamini-Hochberg method and an adjusted p-value of <0.05 was considered significant and the multivariate approach included principal component analysis (PCA). All statistical analyses were performed with the statistical software R (RStudio v.1.1.383, RStudio, Boston, MA). Significant metabolites after the univariate tests were used for metabolite set enrichment analysis. MetaboAnalyst 3.0 (Xia et al., 2015) was used to enquire about the enrichment of the default pathways sets.

5.4 Results

5.4.1 Training Demands

Overall session outputs are presented as mean (±SD) in table 5.2 below. The session consisted of a warm-up, a technical passing drill, a linear conditioning drill which consisted of box-to-box runs and finally 6 x 5-minute small-sided games (5v5). The small-sided games took place in a 35x24 yard grid, with no offsides and goalkeepers included. The outputs for each drill are presented as mean (±SD) in table 5.3. When compared with the positional thresholds for intense physiological or mechanical sessions outlined in chapter 4, this session was physiologically intense for all positions, whilst also being mechanically intense for FB and CB.

Table 5.2 Team external and internal demands presented as mean ± sd. Abbreviations :TD – Total Distance, HSR – High Speed Running, SD – Sprint Distance, Hi A/Ds – High Intensity Accelerations & Decelerations,

Time	TD	SD	HSR	Hi A/Ds	AV HR %	Mins Spent >85% Max HR
63	5354 ± 291	157 ± 80	558 ± 57	140 ± 28	73% ± 4%	23 ± 7

Table 5.3 Positional external and internal demands presented as mean ± sd. * indicates that session was intense as per thresholds in chapter 4. Thus * in SD column indicates threshold for positional sprint distance exceeded indicating a physiological session. * in Hi A/Ds column indicates threshold for positional Hi A/Ds exceeded, indicating a mechanical session. There was only 1 full back in this study, hence there is no standard deviation for this position. Abbreviations :TD – Total Distance, HSR – High Speed Running, SD – Sprint Distance, Hi A/Ds – High Intensity Accelerations & Decelerations,

	Time	TD	SD	HSR	Hi A/Ds	AV HR %	Mins Spent >85% Max HR
FB	63	5695	165*	368	151*	74%	16.6
CB	63	5143 ± 309	106 ± 96*	339 ± 9	137 ± 28	79% ± 8%	29.1 ± 5.2
MID	63	5420 ± 329	157 ± 74*	345 ± 12	90 ± 55	72% ± 3%	21.4 ± 6.2
FOR	63	5407 ± 314	181 ± 72*	347 ± 9	150 ± 17	76% ± 1%	25.9 ± 7.1

Table 5.4 Team external and internal drill demands throughout the session. Drills are in the order completed throughout the session. Values presented as mean ± sd. Abbreviations :TD – Total Distance, HSR – High Speed Running, SD – Sprint Distance, Hi A/Ds – High Intensity Accelerations & Deceleration,

Drill	Time	TD	SD	HSR	Hi A/Ds	Av HR %	Mins Spent >85% Max HR
Warm-Up	12	600 ± 58	35 ± 11	80 ± 7	11 ± 4	62% ± 8%	1.3 ± 0.4
Technical Pass & Move	11	903 ± 40	0 ± 0	0 ± 0	18 ± 8	68% ± 4%	1.9 ± 1.3
Box to Box Runs	5	683 ± 31	119 ± 73	435 ± 40	8 ± 1	76% ± 3%	2.3 ± 0.9
5v5 x 5 Mins - 6 Reps (35x24) No Offsides	35	3168 ± 268	3 ± 6	43 ± 24	103 ± 22	79% ± 5%	20.2 ± 5.9

5.4.2 Metabolomics

Unsupervised multivariate analysis of the groups by Principal Component Analysis (PCA) was performed to determine if any underlying structure was present in the data (Figure 5.4) and we observed no clear structure when comparing pre-to-post-training. However, within-group differences pre-and post-training were then explored by univariate tests for all serum buckets. Overall, 117 metabolites were identified, from which 18 non-overlapped metabolites were found to have significantly changed pre-to post-training (Table 5.5). Metabolites involved in energy metabolism predominate the significantly altered metabolite dataset; Isobutyric acid, citric acid, lactic acid, and glucose were all significantly altered as well as the glucogenic amino acids alanine, glycine, and arginine.

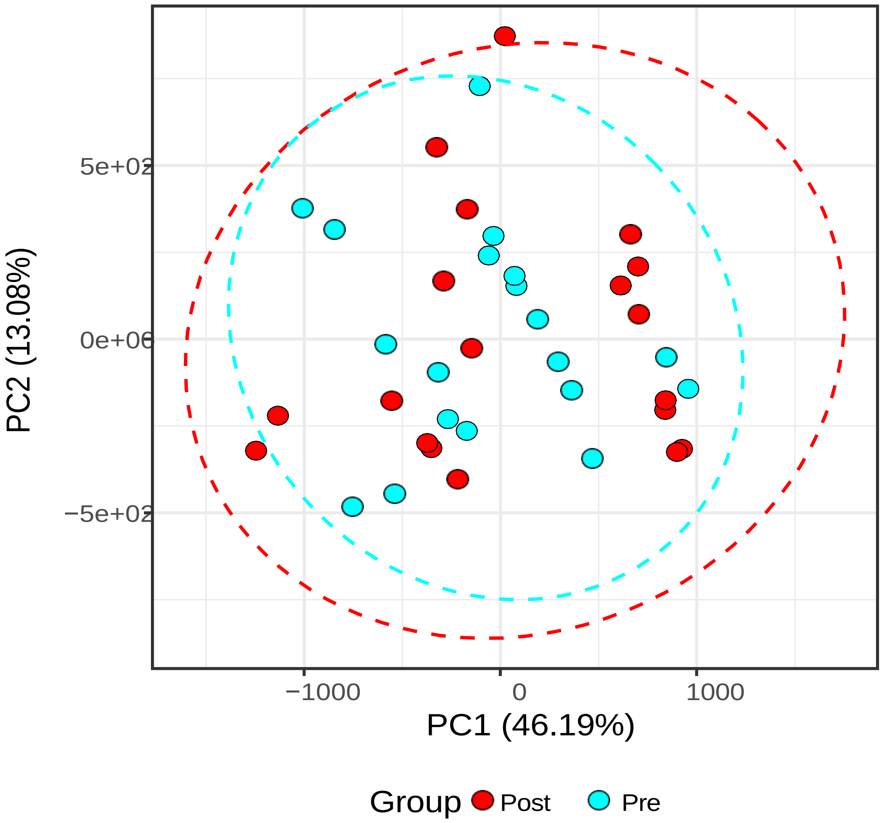


Figure 5.4 Pre- and post-training comparison via principal component analysis of serum, ellipses show the 95% confidence region.

Table 5.5 A list of metabolites that significantly changed pre- to post-training in capillary serum samples. Only metabolites with a Benjamini-Hochberg p-value of <0.05 are reported. Arrows represent direction of change, red arrows indicate decreases, whilst green arrows represent increases.

Metabolite/Bucket	95% Conf. Int. Mean (Pre to Post)	Unadjusted pvals	Benjamini-Hochberg pvals	Bonf vals
Isobutyric acid ⬆	(28.26451, 55.07861)	0	0.00045	0.00045
Citric.acid ⬆	(46.01387, 85.83987)	0	0.00104	0.00223
unknown_161 ⬇	(-48.10532, -27.88455)	0	0.00104	0.00312
unknown_ 139 ⬆	(43.12751, 86.82351)	0	0.00112	0.00446
Lactic acid ⬆	(68.75899, 167.09708)	1.00E.04	0.00125	0.00625
Alanine ⬆	(81.04434, 166.2303)	1.00E.04	0.00141	0.00848
unknown_ 91 ⬆	(22.60949, 65.08412)	2.00E.04	0.0024	0.01919
Creatinine ⬆	(35.49332, 89.56649)	2.00E.04	0.0024	0.01919
Acetic.acid ⬇	(-159.04959, -50.45465)	3.00E.04	0.00347	0.03124
unknown_ 81 ⬆	(46.33848, 159.22842)	3.00E.04	0.00393	0.03928
Tyrosine ⬆	(15.39018, 43.5576)	5.00E.04	0.0051	0.06115
Mobilełipids_152 ⬇	(-51.15925, -15.24603)	5.00E.04	0.0051	0.06115
Arginine ⬇	(-50.19144, -22.90022)	6.00E.04	0.0058	0.07543
unknown_ 22 ⬇	(-29.67513, -8.01947)	0.0033	0.02793	0.39098
Glucose ⬆	(66.11089, 319.50814)	0.0053	0.04157	0.62351
Threonine ⬆	(15.14595, 74.71155)	0*0071	0.04644	0.83596
Glycine ⬇	(-137.90317, -23.55783)	0,0071	0.04644	0.83596
unknown_ 162 ⬇	(-21.95842, -3.08737)	0,0071	0.04644	0.83596

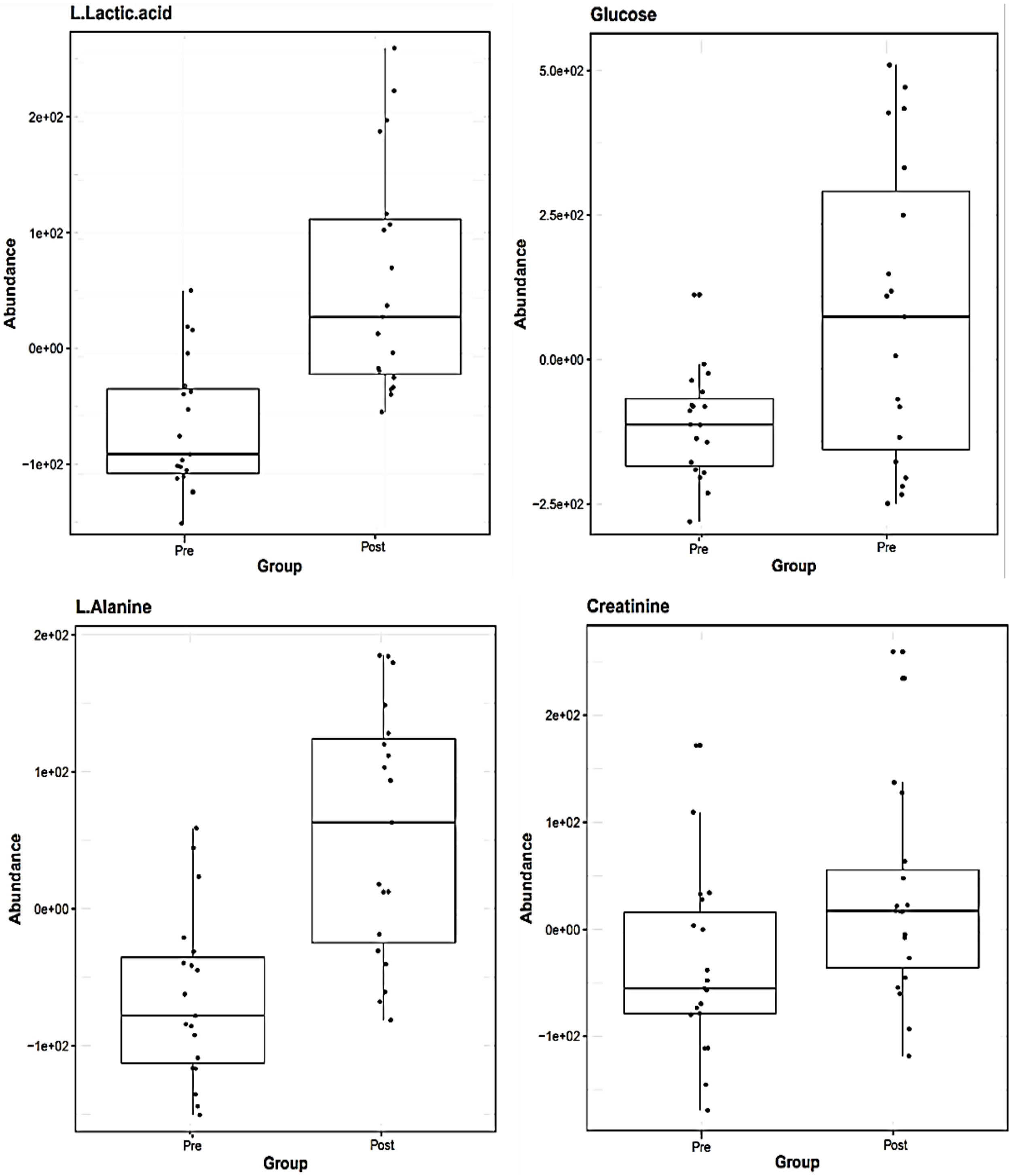


Figure 5.5 Boxplots for selected metabolites lactic acid, glucose, alanine and creatinine with both the A and B samples for each player at pre and post

MSEA analysis revealed that the shortlisted metabolites were enriched predominantly in energy metabolism pathways such as tRNA biosynthesis, glyoxylate and dicarboxylate metabolism, pyruvate metabolism, glycolysis/gluconeogenesis amongst others (Figure 5.6).

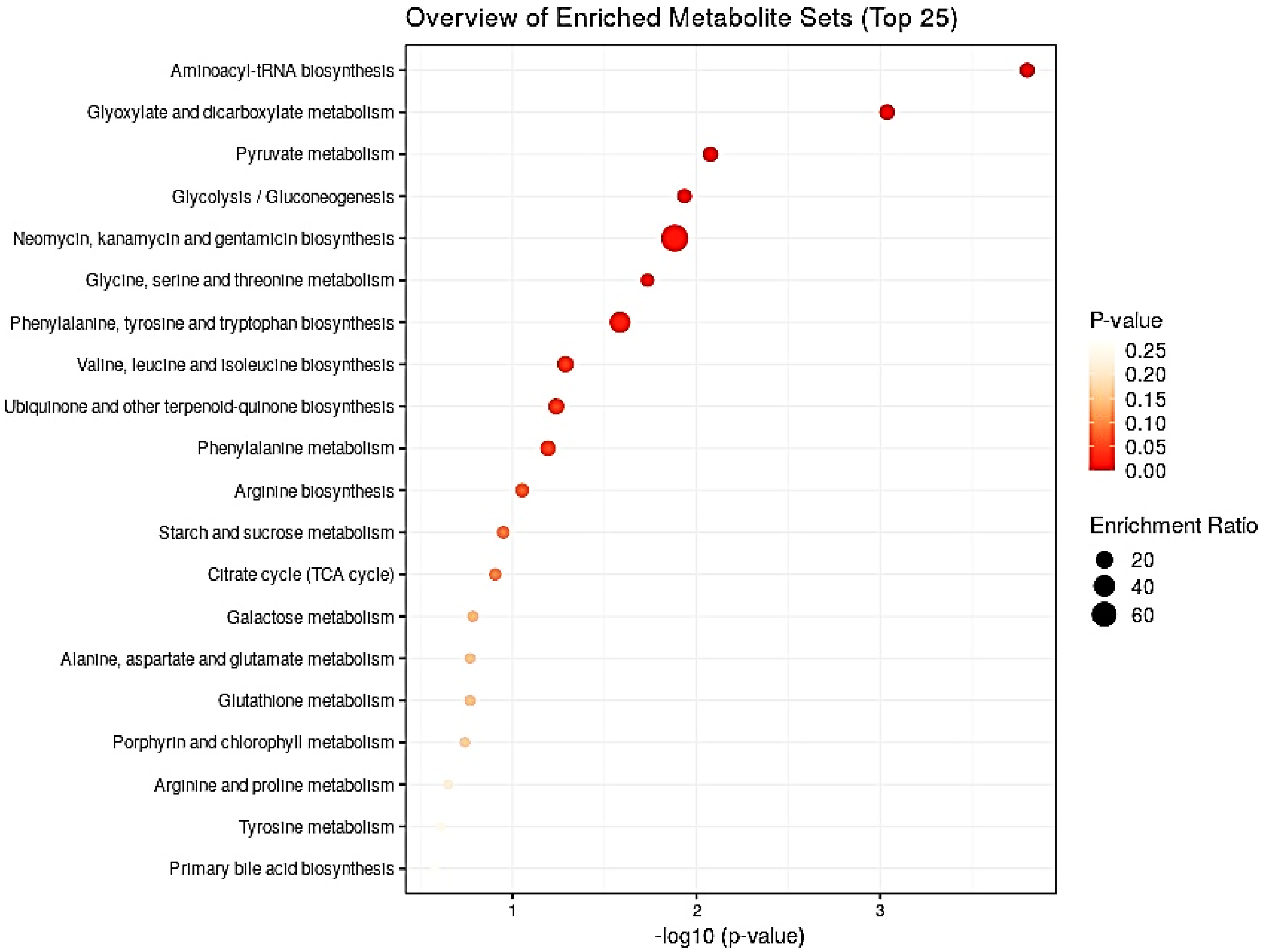


Figure 5.6 Metabolites with a Benjamini-Hochberg p-value of <0.05 as determined by Welch's test were shortlisted and used for metabolite set enrichment analysis (MSEA) via MetaboAnalyst, displayed above. MSEA analysis was performed with MetaboAnalyst v5.0

5.5 Discussion

This pilot study aimed to capture the metabolic response to a single intense training session in football using capillary blood samples taken from the fingertip. The external and internal loads would suggest that this session was intense, especially when compared to the findings in chapter 4. The results from the metabolomics analysis revealed that serum metabolomics, collected using fingertip blood samples and analysed using NMR spectroscopy, was sensitive enough to detect changes in 18 metabolites following this intense session. Previous research has demonstrated that metabolomic analysis using other biofluids such as saliva and urine, have been reported as a valid and comprehensive way of analysing the metabolic responses to football (Pitti et al.,2019; Ra et al., 2014; Quintas et al., 2020). From a methodological standpoint, this method may be an attractive research area in football due to the ease and speed at which these samples can be collected in the field, as well as the insights into the metabolic response to football that can be obtained.

The activity profile of this training session demonstrates that players were subjected to a mixture of high intensity running and sprinting demands. Moreover, players were also exposed to a high number of intense changes of direction, as indicated by the high-intensity accelerations and decelerations. When compared with other MD-3 sessions in the literature this session demonstrates a lower average TD than a study in elite La Liga footballers by Martin-Garcia et al. (2018) (5354m vs 5602). However, high-speed distance and sprint distance were higher (558m vs 217m and 157m vs 35m). High-intensity accelerations and decelerations were combined in this study, whereas Martin-Garcia et al. (2018) separated accelerations and decelerations. Nevertheless, their study had average session high-intensity accelerations of 118 and decelerations of 108, which is higher when compared to the average high-intensity accelerations and decelerations of 140 found in this study. This may be explained by the prevalence of Rondos, Which are a common drill in adopted by the FCB in the paper by Martin-Garcia et al. (2018). This drill is conducted with a set of exterior players forming a circle and passing the ball to keep it away from two interior players who try recover it which would cause a high rate of accelerations and decelerations (Lopez-Felip., 2019). However, it could also be a consequence of time as the duration in this study is shorter than the MD-3 found in the paper by Martin-Garcia et al. (2018) (63 vs 83 minutes, respectively). This leaves less time to accumulate the same number of intense actions.

Nevertheless, when this study is analysed against the methods and thresholds used in chapter 4, this session as a whole was physiologically and mechanically intense for FB, whilst physiologically intense for CB, MID and FOR. When this session is analysed drill by drill, it reveals that players were exposed to drills with different activity profiles throughout the session. The warm-up and technical passing drill were relatively low in terms of intensity and would most likely be highly aerobic in nature. Whereas the box-to-box runs and 5v5 small-sided games contained more high-intensity actions. The time-motion characteristics would suggest that the box-to-box drill, a HIIT drill which consisted of a work to rest ratio of 1:2 (10s:20s), may have placed a high demand on the physiological load-adaptation pathway. Moreover, the frequent and intense changes of direction that occurred because of the small sided-games would have placed high demands on both the physiological and mechanical load-adaptation pathway (Vanrenterghem et al., 2017; Praça et al., 2022; Hill-Haas et al., 2011; Rampinini et al., 2007; Dellal et al., 2011).

From an energy provision standpoint carbohydrate oxidation, particularly from muscle glycogen, dominates at higher exercise intensities, whereas fat oxidation is more important at lower intensities (Hargreaves and Spriet, 2020; Knuiman et al., 2015). The contribution of carbohydrates and fats used to fuel exercise will depend on the intensity and duration of a training session, the amount of carbohydrate stores available, as well as the training status of a player (Jeukendrup, 2003). The major intramuscular and extramuscular substrates are muscle glycogen, blood glucose (derived from liver glycogenolysis and gluconeogenesis, and from the gut when carbohydrate is ingested) and fatty acids derived from both muscle intramuscular triglyceride and adipose tissue triglyceride stores (Fig. 5.7). The results of the metabolomic analysis found that the energy demands of this training session stimulated energy metabolism pathways such as aerobic glycolysis, the tricarboxylic acid cycle, glyoxylate and dicarboxylate metabolism and gluconeogenesis. These metabolic perturbances are indicative of an intense training stimulus which required ATP to be generated through a variety of metabolic pathways.

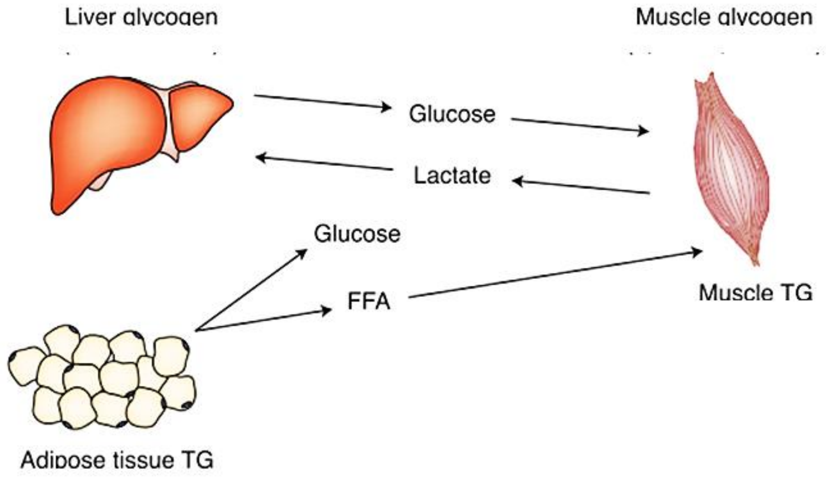


Figure 5.7. intramuscular and extramuscular fuel sources for exercise metabolism. Major sources of carbohydrate in the muscle and liver and of fat in the muscle and adipose tissue during exercise. TG, triglyceride; FFA, free fatty acids. Adopted from (Hargreaves and Spriet, 2020).

The evidence of this session being intense in nature can be found through the increases in metabolites related to carbohydrate metabolism observed in this study. These include citric acid, lactate, alanine, and glucose, which would be expected to increase after high intensity exercise (Iaia and Krustrup, 2007). It has been demonstrated that the supply of glucose from the liver is enough to compensate for the blood glucose demands as a result of high intensity activities during match play, as demonstrated by increases in blood glucose at the end of match play (Krustrup et al., 2006; Bangsbo et al., 2007). Thus, the demands for glucose are most likely a consequence of the high intensity drills used this study.

Moreover, the increases in creatinine may reflect the damaging nature of certain intense actions, such as accelerations and decelerations on the muscular system (Wyss and Kaddurah-Daouk, 2000).

Furthermore, an increase in lactate would be expected given its importance in oxidative metabolism and gluconeogenesis (Rabinowitz and Enerbäck, 2020). Gluconeogenesis may have occurred to support the continuation of glycolysis to support the energy demands of this session, again most likely during the higher intensity drills. For gluconeogenesis, a degradation of glucogenic amino acids occurs, converting them to pyruvate, which is then transaminated to alanine, leading to an increase of alanine in the blood. Alanine is then transformed to pyruvate again in the liver, and then into glucose through the glucose-alanine cycle (Ishikura et al., 2013; Schranner et al., 2020; Hargreaves and Spriet, 2020). The changes in alanine observed in this study, have been shown to increase as a consequence of high-intensity exercises, as opposed to moderate intensity exercise (Hudson et al., 2021; Schranner et al., 2020).

Furthermore, the intense demands of the session could also explain the increase in glyoxylate and dicarboxylate metabolism observed in this study, which has been suggested to occur during exercise due to substrate changes with increasing exercise intensity and cellular stress (Osswald et al., 2021). This may explain the changes in metabolites of the glycine, serine, and threonine metabolism pathway. The enrichment of tRNA biosynthesis may be a consequence of the changes in amino acid activity observed in this study. The aminoacyl-tRNA synthetases function set the genetic code during the initial stage protein synthesis. However, these enzymes have a wide impact on other metabolic pathways and cell signalling processes such as cell wall formation, protein labelling for degradation, aminoacylation of phospholipids in the cell membrane (Raina and Ibba., 2014; Pang et al., 2014). The increases in tyrosine observed in this study are most likely related to the conversion of phenylalanine into tyrosine during exercise, most likely a consequence of protein breakdown (Hudson et al., 2021; Ishikura et al., 2013).

Overall, the results outlined would suggest that the methods used in this study were able to reflect the metabolic demands of the training session. Further insights may be gained by analysing the metabolomic response on an individual and drill by drill basis to create metabolomic fingerprints to specific actions. From a practical standpoint, the biggest challenge with collecting data for research in a professional football setting, would seem to be having access to players. Therefore, this method is would seem to be attractive due to its relative ease in terms of collection and less invasive nature when compared to venous blood samples for example.

Invasiveness and the speed and ease at which a measurement can be taken are important considerations when working with athletes in a team-sport setting (Bok and Foster, 2021). It must be noted that the amount of blood taken from the fingertip in this study (~1ml yielding 200-300 μl of serum) is a lot more than a ‘few drops’ reported by Nishiumi et al. (2019) and thus, is a slightly more time-consuming method. However, fingertip blood sampling does not require special training, making it more accessible in football. The insights provided by this study highlight the potential for metabolomics, analysed from capillary blood samples, to provide insights into the response to football activities.

Nevertheless, despite the ease of collection, complexities with this method arise as a result of the analytical techniques and equipment needed to obtain results, which are costly and time-consuming. Analysis of samples requires nuclear magnetic resonance spectroscopy (NMR) or mass spectrometry (MS), which are most commonly used in metabolomics research (Duft et al., 2017). Furthermore, data analysis and interpretation requires specialised statistical and graphical applications to extract the relevant information. However, the cost and time-consuming nature of the analysis could potentially be mediated through the collaboration between a football club and an academic institution with the equipment and skilled personnel to perform these analytics.

5.6 Limitations

Whilst this pilot study provides insight into the potential for metabolomics using capillary blood samples from the fingertip, a noteworthy limitation of this study is the lack of comparison between capillary blood samples and more established methods using venous blood. Additionally, multiple samples for test-retest reliability were not collected during this pilot study. This would have helped provide more robust insights into the validity of this method. Furthermore, analysing metabolites using mass spectrometry would potentially identify more metabolites due to its increased sensitivity when compared to NMR spectroscopy (Reo, 2002).

Another limitation arises due to the lack of a sample collection between the pre-training session and the pitch session, which would have provided greater insights into the metabolomic response of this activity. Furthermore, a measure of fatigue at the organism level would have helped provide further context to the metabolomic results of this study and their impact on fatigue and performance at the organism level. Moreover, this study reports average changes, not individual changes or changes by position. This is an important limitation, because individual resting blood metabolite concentrations vary greatly in-between individuals and are strongly dependent on DNA sequence variation (Schranner et al., 2020). Grouping responses by position would have provided greater insights and practical application.

Finally, it is clear that factors such as diet and hydration status will affect the metabolomic responses to training. Therefore, it is important the nutrition in the lead up to training is carefully quantified and controlled where possible. Whilst in this study meal choices were controlled, the amount of intake varied on an individual basis, which may have affected the results of this study, such as the variation in the changes in glucose observed, and is an important to consider.

5.7 Conclusion

This is, to the author's knowledge, the first metabolomics study conducted in football using fingertip blood samples. Results demonstrated that the training session was intense enough to stimulate several metabolic pathways to support the energy demands of the session, namely tRNA biosynthesis, glyoxylate and dicarboxylate metabolism, glycolysis, and gluconeogenesis. Overall, this study demonstrates that metabolomics using fingertip blood samples was sensitive enough to detect changes in metabolites following a training session in football players. Elite football players are hard to access, thus, it would seem these types of collaborations have the potential to be mutually beneficial to both practitioner and researcher in the future. Therefore, due to the relative ease of sample collection in the field, it would seem that the methods used in this study could help provide the perfect blend of pragmatics and broad insights into the response to football. This may help researchers specialising in the field of metabolomics and football clubs find common ground, allowing further research to be pursued in this area. However, despite the practical advantages of this method, caution must be applied when interpreting the results of this pilot study as more research is needed to establish the reliability and validity of this method used.

CHAPTER SIX

A PROTOCOL FOR COMPREHENSIVELY MAPPING PHYSIOLOGICAL & BIOMECHANICAL ADAPTATION IN FOOTBALL.

Following the pilot study outlined in Chapter 5, it was important develop the test-retest reliability of this method, in order to then be able to profile the metabolomic response to training sessions with different external demands. Furthermore, it was also imperative to determine if acute responses helped explain any potential changes in adaptations and changes in performance in football.
Consequently, an intervention study was planned for chapter 6. However, due to the worldwide pandemic, this intervention became unfeasible. As a result, this chapter has developed into a study protocol. Publishing study protocols has been an important development in recent years as a way to keep researchers in the same field up to date with what studies are being done. Furthermore, these types of studies can encourage collaboration and increase transparency (Ohtake and Childs, 2014). Therefore, this chapter serves to replace the intervention study that was originally planned and instead aims to develop a thorough protocol for an observational study that maps adaptation across numerous levels, tissues, and time-courses in response to football training.

6.1 Introduction

Chapter 2 demonstrated that adaptations are required across multiple systems to provide support for the intermittent and unpredictable demands of football. These systems have been referred to as physiological and biomechanical throughout this thesis. Within these systems are structures that are governed by their own unique adaptive processes. These processes occur across the cellular, tissue, organ, organ system and ultimately lead to changes in performance and load tolerance at the organism level (Balagué et al., 2020; Pol et al., 2020). As a consequence, it would seem important to map the response and interactions across systems and levels if broader insights into adaptive responses are to be gained. Chapter 3 demonstrated that these types of broad conceptual approaches to adaptation are lacking in football research.

Chapter 3 also highlighted the need to quantify the stimulus footballers are exposed to in detail. Footballers may be exposed to stimuli such as strength training alongside regular football training. These different stimuli have unique implications on the tissues and systems within the physiological and biomechanical load- adaptation pathways (Vanrenterghem et al., 2017). Therefore, it would seem important to quantify the stimulus in detail to evaluate their impact on responses. Furthermore, different stimuli may have unique implications for adaptation as outlined in chapter 2. Thus, there seems to be a requirement for using different outcome measures, on different levels in the body in order to provide more comprehensive insights into adaptive process. This has implications for study design.

These implications will also affect the duration of the study, as adaptations across different levels may not manifest over the same time courses. This would also extend to the adaptations of different physiological tissues. This demonstrates the importance of considering the nature of the stimulus and its potential implications for adaptation. Furthermore, it is important to consider how best to quantify a stimulus in detail before deciding what outcome measures or durations to use to assess the response.

A lack of broad approaches to adaptation is most likely due to the constraints of elite football. These constraints are most likely related to the time that practitioners and researchers have to access players. Another constraint relates to the level of invasiveness that is ethically justifiable and that players/managers are comfortable with. Thus, conceptual approaches to mapping adaptation in football also require pragmatic approaches. One such approach may be to find measures that are time-efficient and non-invasive yet provide comprehensive insights. However, to date, no attempts have been made to design a protocol for a study using this integrated approach. Therefore, this study aims to provide a protocol that satisfies the broad conceptual view of adaptation present in this thesis, with some considerations made regarding the practicalities of taking measurements. This study will be an observational study in elite football that maps responses across multiple levels of organisation in both the physiological and biomechanical load-adaptation pathways.

6.2 Methods

The following section will provide a brief overview of the study design and duration, the participants, how the stimulus will be quantified and how internal and external loads will be measured. Following this, the transient and adaptive outcome measures to be used in this study will be outlined before a more extensive overview of these measures and their purpose within this study is presented. The test-retest reliability of each measure will need to be initially established before this protocol begins, this is especially warranted where tester variability may compromise results.

6.2.1 Study Design

This is an observational study with repeated measurements over 12 weeks. Measures will be collected at baseline, 6 weeks, and 12 weeks. Measurements collected at baseline, 6 weeks and 12 weeks will involve measurements of bone, tendon and muscle properties, cardiovascular fitness, muscular strength, jumping performance, acceleration, and sprinting performance. Venous and capillary blood samples will also be taken at these time points for metabolomic, and proteomic analysis, as well as analysis of bone turnover. Furthermore, capillary blood samples for metabolomic and proteomic analysis, external loads and internal loads will be collected at each training session throughout the study duration. To account for any changes in height or weight that could influence study results the player's height and weight will also be measured at baseline, 6 weeks, and 12 weeks.

6.2.1.1 Study Duration

The study duration will last 12 weeks. This study duration has been selected to allow enough time for potential tendon and bone adaptations to manifest. The examination of adaptations of bone and tendon are part of a more holistic approach to mapping adaptations in the mechanical-load adaptation pathway, as outlined in Chapter 2. Based on evidence outlined in chapter 2 it is expected that any potential muscular and cardiovascular adaptations will be apparent after 6 weeks. Whilst tendon and bone adaptations may not manifest until the 12-week measurement point. The approach with this study design is to map transient responses consistently over time (daily training data collection), against adaptive responses taken over longer durations (baseline, 6 weeks, and 12 weeks). This will allow us to determine if baseline characteristics determine the response to a given stimulus. Furthermore, it will also help determine if acute responses repeated over time are linked with adaptive changes in outcome after 6 and/or 12 weeks.

6.2.1.2 Study Participants

Male U23s players from an elite football academy will be recruited. Only outfield players will be recruited due to the differences in demands of goalkeepers. Players must be over 18 years of age. Players with long term injuries >12 weeks will be excluded.

6.2.1.3 Stimulus

Players will be exposed to a wide variety of training stimuli such as football training, matches, strength training and metabolic conditioning sessions over the 12-week study period. These sessions will be documented in detail in this study. As alluded to in chapter 3, any concurrent stimuli must be comprehensively captured. The frequency and duration of football training, matches, strength training and metabolic conditioning sessions will be captured. From a football perspective, players will train approximately four days per week, with one day of the week dedicated to competitive match-play. The drill content of each football session will be recorded. The content of each training session will be determined by the team's technical soccer coaches and fitness coach in line with the physical, tactical, and technical objectives for each given session. The modality of exercise and duration will also be recorded for off- pitch conditioning sessions.

6.2.1.4 External and Internal loads

External loads for pitch-based sessions will be quantified using GPS, with data collected from all on-pitch training sessions and matches. The GPS units (Apex, StatSports, Ireland) will be placed between the scapulae of the players using bespoke vests. These GPS units' sample at 18 Hz and the accelerometers at 100 Hz. Each GPS unit will be switched on prior to the warm-up for each session to ensure that a satellite link is established before the beginning of the activity. Following each session, the data will be downloaded using the manufacturer's software package (Apex Sonra). External loads for gym-based sessions will be quantified using the volume of load lifted. The volume will be quantified for the lower body by multiplying the reps, sets and weight lifted by each player. Off-feet conditioning sessions will be quantified by recording the mode and duration of the session.

6.2.1.5 Internal loads

To assess cardiovascular response to training heart rate (HR) based measures will be collected during training sessions using HR belts (Polar H1 system). Internal loads for off-pitch conditioning sessions will also be captured using HR monitors.

Session ratings of perceived breathlessness (sRPE-B) and leg muscle exertion (sRPE-L) will also be collected post session as an indicator of internal training load. Players will be asked to how their breathing was affected or how much their legs were affected by the training session. sRPE-B will be used as an indicator of physiological load, whilst sRPE-L will be used as an indicator of biomechanical load (Weston et al., 2015; Vanrenterghem et al., 2017).

6.2.1.6 Responses

The purpose of the outcome measures used to map these responses in this study will now be outlined before a more detailed examination of each measure follows. During testing at baseline, 6 weeks and 12 weeks, players will have fasted venous and capillary blood samples taken for metabolomic, proteomic analysis as well as bone turnover markers. Capillary samples will be assessed against venous samples to observe the differences in metabolites and proteins found. These markers will provide a comprehensive insight into the cellular response to football training.

At the tissue/organ level lower body bone size and mineral density will be assess using a DXA scan. Tendon mechanical and morphological properties of both the Achilles and Patellar tendon will be examined using ultrasonography. Ultrasonography will also be used to assess hamstring and quadriceps muscle properties. These measures will act as a reference point for future responses to determine if baseline properties determine the magnitude of the training response to a given load. Furthermore, these measures will be used as markers of tissue adaptation at 6 and 12 weeks. The test-retest reliability of these measures will need to be established initially to ascertain tester reliability, which can vary depending on operator. For example, potential sources of error using ultrasonography can arise due to probe placement pressure and orientation (May et al., 2021). To reduce any potential tester variation, the same tester will be used throughout the study.

To understand how any potential changes at the cellular and tissue/organ level translate to performance at the organism, players will perform strength, jump, speed and change of direction testing. These tests will help establish if musculoskeletal adaptations from the mechanical loads experience manifest as changes in performance. Finally, a submaximal YOYO IR2 test will be implemented to assess the cardiovascular response to the physiological demands of training. Tests at the organism level have been chosen due to their practicality as well as their specificity to football performance.

The outlined tests performed at baseline, 6 weeks and 12 weeks will be combined with more consistent measurements of the metabolomic and proteomic response to each training session. This allows us to map the response to training consistently over time to understand if trends in the acute metabolomic and proteomic response manifest as adaptations over time.

Capillary blood samples will be taken from players in a fasted state each morning before training as well as immediately post-training over the 12-week period. To account for any metabolites or proteins detected in blood samples, players pre-training meal will be analysed using the nutrition analysis software Nutritics (Nutritics Ltd., Dublin, Ireland) by a registered sports and exercise nutritionist (SENr). Samples will not be collected on matchdays to avoid any unwanted interference on matchday performance. However, fasted capillary samples will be collected within 24 hours post-match before players undertake recovery sessions. Overall, figure 6.1 provides an outline of the methods used to quantify the stimulus, transient responses, and adaptive responses across multiple levels. A more extensive overview of these responses will be outlined in the next section.

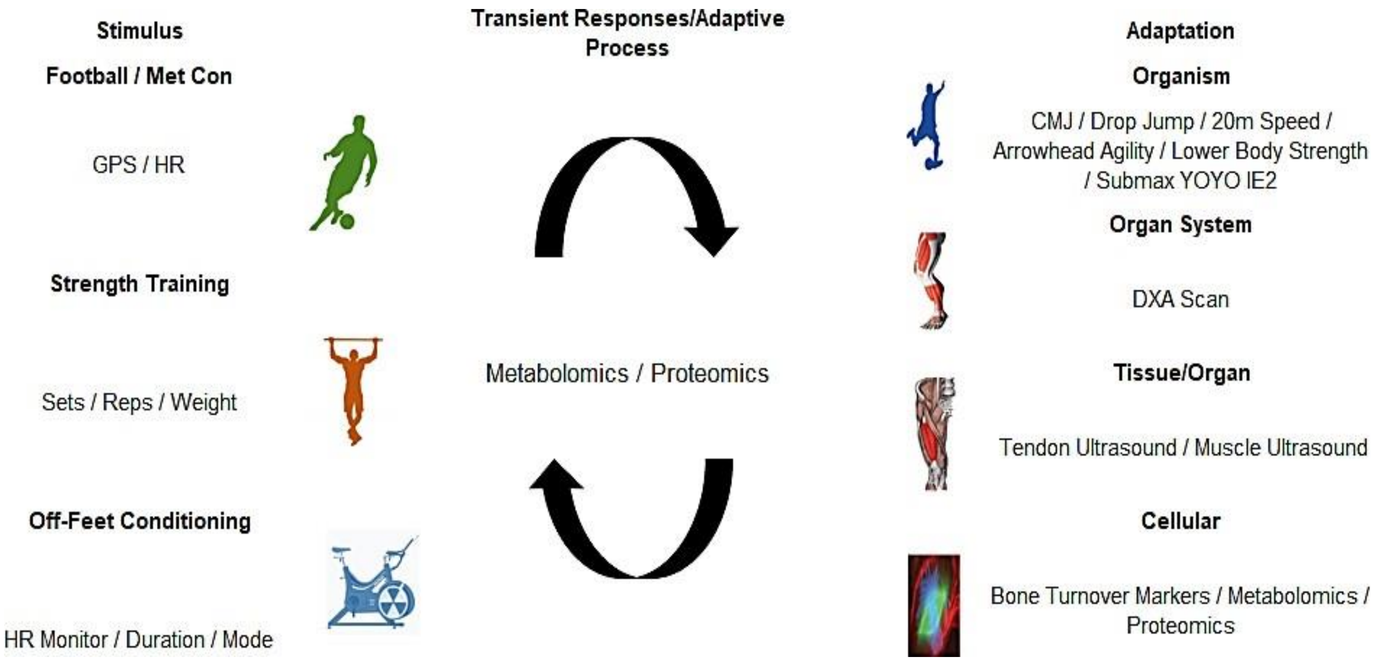


Figure 6.1. Outline of the methods used to quantify stimulus, adaptive response, and adaptation across multiple levels of organisation.

6.3 Outcome measures

6.3.1 Cellular level

Fasted venous blood samples will be collected from the subjects at the start, midpoint, and end of the study. This collection will involve two samples which will be drawn using standard venipuncture techniques. One sample will be used to analyse metabolomic and proteomic profiles, whilst the second sample will be used to analyse bone turnover markers. The metabolomic and proteomic results from these samples will be compared with results from fasted capillary fingertip samples taken at the same time. This will help establish if any differences in detected metabolites or proteins exist between the two samples. Nishiumi et al. (2019), demonstrated that some differences in certain metabolites when comparing blood taken from fingertip versus venous blood samples. Thus, it would be pertinent to establish the differences that exist in these samples when conducted in football players. Both samples will be prepared from proteomic and metabolomic analysis according to standard protocols (Shin et al., 2008; Beckonert et al. 2007) and analysed using NMR spectroscopy.

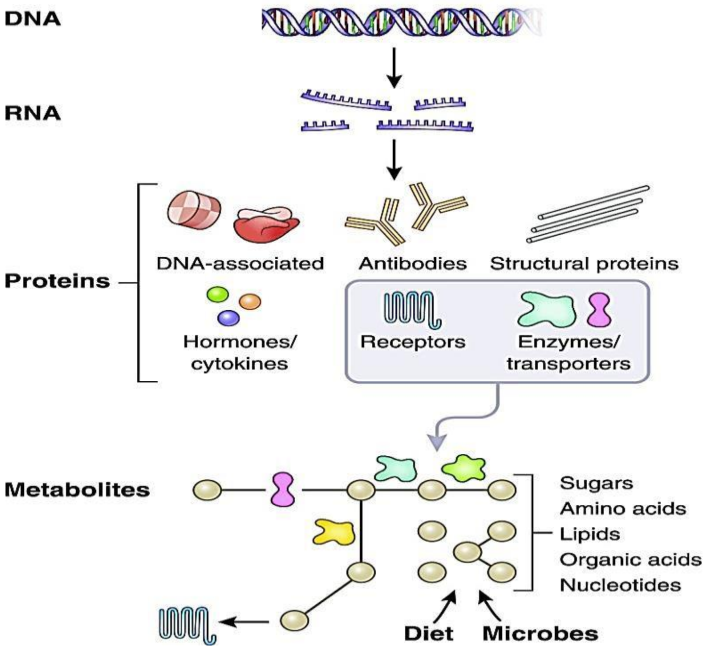


Figure 6.2 Relationship between the genome, proteome, and metabolome. DNA transcription and RNA translation yield numerous different proteins, each with its own structure and function. Subsets of proteins, particularly enzymes and transporters, plays a prominent role in modulating metabolites. which are derived from endogenous metabolism and exogenous sources such as diet and the microbiome. (Adopted from Dubin and Rhee, 2020).

Both the proteome and metabolome provide dynamic insights into the consequences of the genome (Figure 6.2). Proteomics can provide insight into changes in multiple proteins and isotypes, which can provide insight into the function of cells and tissues as well as the mechanisms behind any disruptions to their function (Kriet and Eils, 2006; Sanchez et al., 2011). Metabolites represent the process of cellular metabolism and as such metabolomics provides insight into the disturbances to cells as a result of exercise (Duft et al., 2017). In relation to exercise, it is the changes in the proteome and metabolome that is of most interest as opposed to static snapshots. The changes in proteomics and metabolomics of most interest in this study will be related to energy metabolism, oxidative stress, amino acid metabolism and inflammatory responses in response to football training.

An important contributor to bone mineral density and bone strength is the rate of bone remodelling, which can be assessed by measuring bone turnover markers. These markers provide acute insights into this metabolic process which can provide useful insights into the direction of the adaptive process (Högström, 2007). Markers of bone turnover, specifically serum Carboxy-Terminal Collagen Crosslinks and Procollagen Type 1 Amino-Terminal Propeptide will be measured by electrochemiluminescence immunoassay (ECLIA). Serum Procollagen Type 1 Amino-Terminal Propeptide and Carboxy-Terminal Collagen Crosslinks have been well established as reliable and informative markers of bone formation and resorption (Seibel, 2005; Florencio-Silva et al.,2015; Shetty et al., 2016; Högström, 2007). Football studies have demonstrated bone turnover markers are sensitive to changes in training in an 8-week off-season period (Weiler et al., 2012) and the first 10 days of pre-season training after a 4-week off-season period (Karlsson et al., 2003).

Overall measurements taken at the cellular level will provide insight into the influence of football training of different energy pathways and bone metabolism which will provide useful insights when compared with the external and internal demands of training. It may be possible to build proteomic and metabolomic profiles which could distinguish between physiologically intense sessions and biomechanically intense sessions.

6.3.2 Tissue/Organ Level

Hamstring and quadriceps muscle size, fascicle length and fascicle pennation angle will be determined using ultrasonography. The force generating ability of muscle is determined by its CSA (Attwaters and Hughes, 2022; Wisdom et al., 2015). Thus, muscle size would seem to be a key determinant for explosive actions. Indeed, higher levels of muscle CSA have been found in speed and power athletes (Fukutani et al., 2020; Miller et al., 2020). The architecture of muscle, as well as its size, is also an important determinant of its force producing capabilities. Longer muscle fascicles and a greater fascicle pennation angle have been identified as important for explosive actions (Wickiewicz et al., 1983; Abe et al., 2001). Longer fascicles seem to result from an increase in serial sarcomere number, which may influence force production (Walker et al., 2020).

Furthermore, this increase in length may be important for injury risk reduction, as the muscle can lengthen over a greater range during eccentric contractions (Wisdom et al., 2015). Changes in muscle fiber CSA translate into changes in anatomical CSA via the pennation angle, where the angle changes to allow more room for ‘fiber packing’ to occur (Wisdom et al., 2015; Farup et al. 2012). Overall, the baseline properties and potential changes in these muscle properties assessed by Ultrasonography will provide context for any changes in performance at the organism level. Moreover, the baseline properties may provide interesting insights mapped against metabolomic and proteomic responses to different types of training sessions. Finally, these any change in muscle properties could have further implications on tendons due to the potential for increased force transmission.

Thus, Achilles and patellar tendon length and cross-sectional area measurements will be obtained using ultrasonography. Evaluating tendon CSA provides some insight into the force tolerance and strength capabilities of the tendon (Magnusson et al. 2008). Cross-sectional research has demonstrated that elite athletic populations demonstrate larger Patellar tendon CSA than non-elites (Murtagh et al., 2018); Furthermore, this trend has been observed in the Achilles tendon when comparing runners with non-runners (Magnusson and Kjaer, 2003). Overall, this suggests favourable adaptations as a consequence of the differences in loading. Increases in tendon CSA have also been shown to lead to increases in tendon stiffness in youth athletes (Mersmann et al., 2015), demonstrating the usefulness of CSA as a potential indicator of tendon stiffness. The benefits of any positive adaptations in these tissues relate to improved force transmission and load tolerance. In a football context, this could lead to a player being able to accelerate, decelerate, change direction and sprint more quickly, jump higher, and run further during a game.

6.3.3 Organ System Level

Lower body and lumbar spine bone mineral density will be measured using a DXA scanner. Bone size affects bone density measurements this is because it measures areal bone mineral density to establish bone quality (aBMD), this differs from true volumetric density (vBMD). As a result, two different sized bones could have the same volumetric density, but the bigger one will show a higher aBMD. However, this measurement can still be useful when combined with blood turnover markers to assess the role of bone size on adaptation. As alluded to in chapter 2 to compensate for a lack of cross-sectional area, slender bones develop higher density cortices and are stiffer than larger bones (Hart et al., 2015; Wallace et al., 2012; Beck et al., 2000). This stiffness at the expense of ductility and robustness, meaning slender bones are less resistant to overload than their larger counterparts (Hart et al., 2015; Tommasini et al., 2005; Beck et al., 2000). Thus, whilst not providing a comprehensive indicator of bone strength, the size of bone as measured via aBMD can provide a useful reference point to potentially explain changes in bone turnover markers.

Overall, baseline characteristics and changes at the tissue/organ level will provide insights into the relationships between muscle, tendon and bone and the implications of football and strength training on their adaptive responses. Furthermore, the insights gained from proteomic and metabolomic trends at the cellular level may provide insights into the magnitude of responses needed to have implications at the tissue/organ level.

6.4.4 Organism Level

The strength, jump, speed, change of direction and submaximal test have been chosen as pragmatic approaches that aren’t overly time-consuming whilst also avoiding having players perform maximally to exhaustion. These decisions come as a result of potential scheduling conflicts that could occur throughout the season, which could impact the players willingness to perform maximally. Furthermore, technical coaches may not be willing to risk the potential implications of this test to impact training or match performance.

Changes in strength will be examined using a combination of strength tests routinely implemented as part of regular performance testing with the cohort of players that will be recruited for this study. As a consequence players are familiar with these tests and their procedures. Furthermore, each of these tests can be performed in a short amount of time for each player. These strength tests involve an assessment of hip abduction and adduction strength in a supine position with their knee joints at an angle of 60°. Force outputs from this test will be recorded in Newtons using the GroinBar Hip Strength Testing System (Vald Performance, Albion, Australia). Tests using the same protocols have been shown to have a high level of reliability (CV: 6.3% (4.9–9.0%) and an ICC of .94, with CV exceeding the SWC (5%) on both limbs (Ryan et al., 2019). Tests of eccentric hamstring strength will be conducted by performing a bilateral eccentric nordic, with peak force outputs recorded in Newtons using a Norbord (Vald Performance, Albion, Australia). Tests using the same protocols have been shown to have acceptable levels of reliability (CV<10% (9.0-9.1%) (ICC = 0.823-0.834, 95% CI = 0.666 – 0.926 (Ross et al., 2020).

Players will perform three maximal contractions for each test to obtain peak force outputs. A back-squat 3-RM test will be used to assess overall lower body strength using free-weights and a squatting rack. Players will perform the squat to a depth where the knee angle is 90 degrees with the heaviest weight possible for 3 repetitions. Verbal encouragement will be provided throughout all efforts.

To examine the implications of any change in tendon and muscle properties on jumping ability at the organism level, bilateral vertical countermovement jump, and vertical drop jump performance will be assessed. This will be achieved using a portable force platform sampling at 1000 Hz. The countermovement jump is commonly used to assess lower limb muscular power. This is evidenced in Chapter 3. However, as opposed to focusing solely on jump height, a force platform allows the kinetics of this action to be unpacked and thus, provides deeper insights into an individual’s lower limb muscular performance (Harper et al., 2020). The outcome measures used to assess lower limb performance on top of jump height will be countermovement jump height, rate of force development, time to take-off and countermovement depth. Time to take-off provides a useful understanding of the duration of time between the initiation of the countermovement and take-off, whilst countermovement depth may also help to explain why any changes in time have occurred. This will help unpack how the jump has been performed, helping us unpack how the jump height was achieved (Bishop et al., 2021). Rate of force development will be used to assess the speed at which the contractile elements of the lower limb muscles can produce force (Aagaard et al., 2002). Moreover, muscle-tendon unit (MTU) behaviour seems to be similar when countermovement jumps are compared with acceleration actions, this helps paint a greater picture of the force producing capabilities of athletes (Aeles et al., 2018).

With regards to the drop jump measurement, unlike the countermovement jump, this test focuses on the assessment of the stretch-shortening cycle force capacity of the muscle-tendon units on the leg extensors (Pedley et al., 2017). This tests exposes players to large braking and concentric forces (Flanagan and Comyns, 2008). Consequently, this test provides a deeper insight into the explosive capabilities of an individual due to the emphasis on short ground-contact times and the rapid application of force that can be observed during high-speed running and changes of direction (Morin et al., 2015). This test will be performed using a 40cm box from which players will step off and make contact with a force platform sampling at 1000 Hz. Players will be instructed to jump for maximal height whilst spending the shortest amount of time possible on the floor. The outcome measures used will be jump height and ground contact time, the latter of which will help unpack the strategy of the jump and distinguish between slow and fast stretch shortening cycle timeframes (Bishop et al., 2021).

A key component of football performance is the ability to sprint over relatively short distances (Reilly, 2005). As mentioned previously the function of the MTU would seem important for acceleration, high-speed actions and change of direction tasks (Aeles et al., 2018; Morin et al., 2015). To test acceleration and sprinting ability and the implications of any adaptations on these key components of performance, 20m sprint times will be measured using electronic timing gates. Timing gates will be placed at 5m, 10m and 20m. A standing start with the lead-off foot placed 1 m behind the first timing gate will be used. The test will

For change of direction ability, a reactive agility test will be implemented. The test will use the Y-shaped format widely used in literature (Green, Blake, & Caulfield, 2011;Veale, Pearce, & Carlson, 2010), however this test will involve a reactionary element using live tester acting as an opponent delivering a stimulus to react to as per the protocol outlined in Trecroci et al. (2019) (Figure 6.3). After an initial sprint, participants will be required to react to the left or to the right gates according to the tester's movements within a Y-shape course. Time to complete each trial will be recorded using a timing gate. This version of the test has been shown to distinguish between elite and sub-elite youth soccer players when compared with a light stimulus and is more in line with perceptual cues found in match play. This may override any issues with tester variability due to its more ecological approach (Trajkovic et al., 2020).

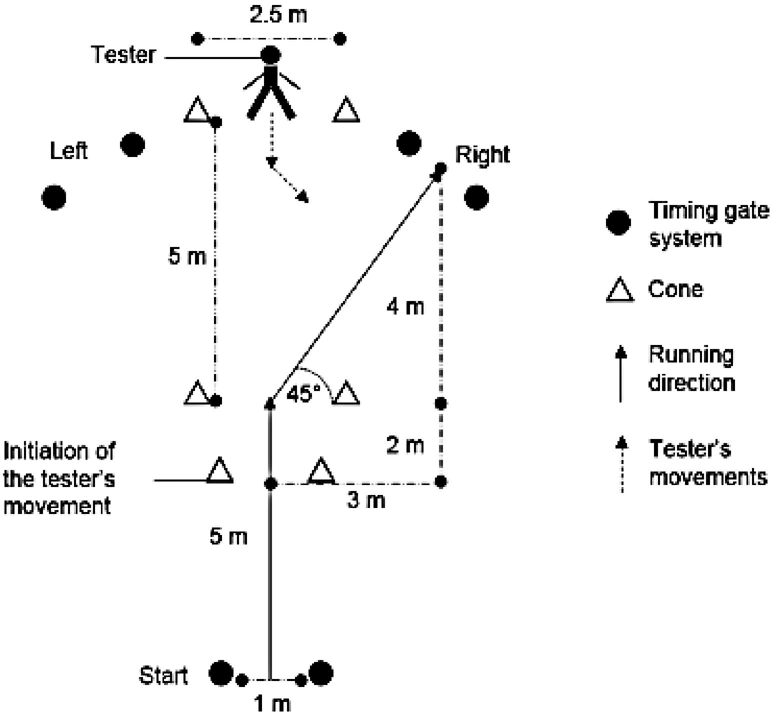
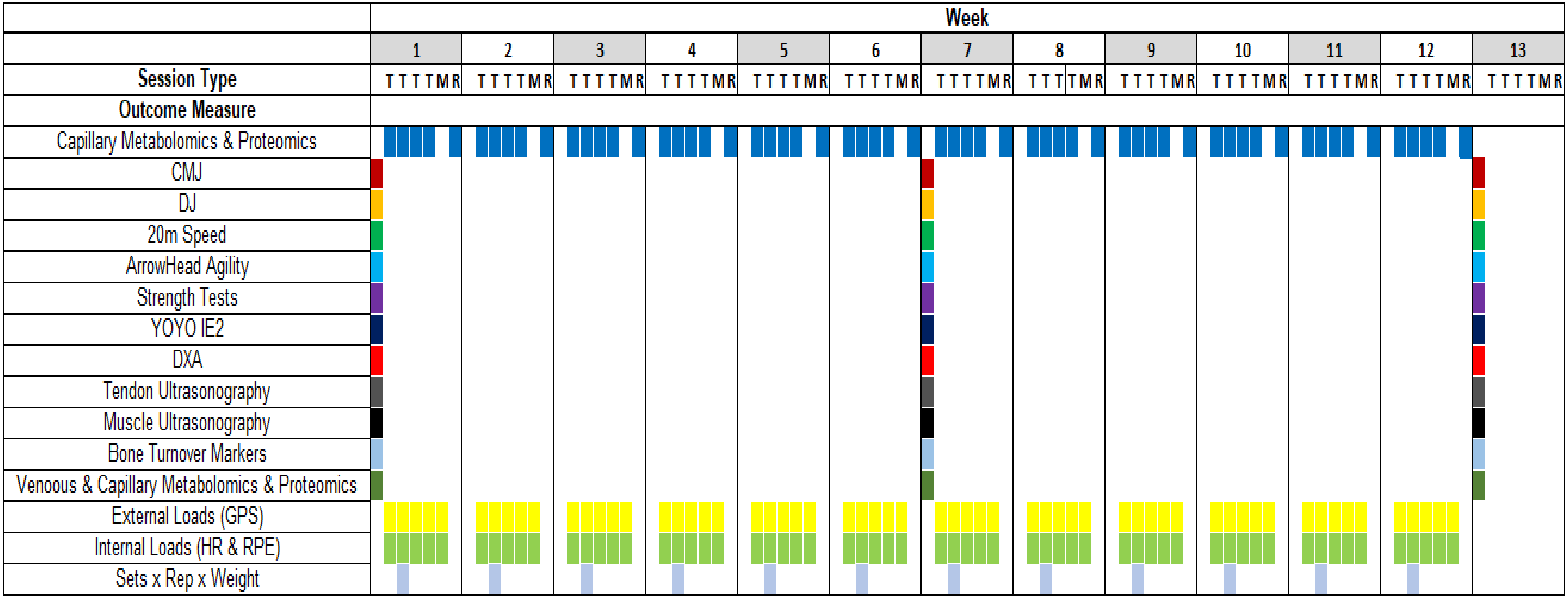


Figure 6.3 Outline of agility test (Adopted from Trecroci et al., 2019)

A 4-minute submaximal intermittent YOYO endurance test will be used to assess the cardiovascular and response to training. Unpublished data from Rice (2021), has demonstrated that this test was reliable and was sensitive enough to identify changes in fitness between starters and non-starters throughout a season in elite academy football, highlighting the potential to detect changes in fitness in football players. Furthermore, this test is time-efficient and does not require players to perform maximally. Moreover, sub-maximal and maximal versions of the YoYo tests have been shown to have robust reproducibility, as well as being able to demonstrate the intermittent exercise capacity of elite soccer players (Bradley et al., 2011).

The heart rate recovery 60 seconds post the cessation of this test will be used as a marker of cardiovascular adaptation. Heart-rate recovery is regulated by autonomic nervous system and is characterized by parasympathetic reactivation and sympathetic withdrawal (Daanen et al., 2012; Borresen and Lambert, 2008). When the exercise stops, cardiac output is reduced through parasympathetic nervous system reactivation and inhibition of sympathetic impulses. The speed at which heart rate recovery occurs has been shown to distinguish between trained and untrained individuals, supporting its use as a measure of fitness (Daanen et al., 2012). Heart rate monitors (Polar H1 system). will be used during the test to assess heart rate responses. Within the context of this study, this provides a baseline measure of aerobic fitness, which will provide an appropriate reference point when contextualising the individual physiological responses to the demands of training throughout the study period.

Overall outcome measures at the organism level are primarily dictated by pragmatics. However, given the comprehensive insights that will be obtained from measures at the cellular and tissue/organ level, there may be more freedom for pragmatic measures at the organism level. If one considers a scenario where more robust, time-consuming, and detailed measures are taken at the organism level, whilst single markers are taken at the cellular level, it would seem limited insights into the mechanism behind any responses at the organism level could be obtained. Therefore, it would seem the approach mapped out in this study has the potential to provide mechanistic insights into various practical measures of performance at the organism level. This approach may be more favourable for practitioners in the field working with athletes and thus, may be more likely to be adopted and modified in future research. To encapsulate this study design in its entirety figure 6.3 outlines the outcome measures and times at which they will be obtained throughout the study.



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Outcome Measure																																																																														
Capillary Metabolomics & Proteomics	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■						
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DJ	■																																				■																																				■					
20m Speed	■																																				■																																				■					
ArrowHead Agility	■																																				■																																				■					
Strength Tests	■																																				■																																				■					
YOYO IE2	■																																				■																																				■					
DXA	■																																				■																																				■					
Tendon Ultrasonography	■																																				■																																				■					
Muscle Ultrasonography	■																																				■																																				■					
Bone Turnover Markers	■																																				■																																				■					
Venoous & Capillary Metabolomics & Proteomics	■																																				■																																				■					
External Loads (GPS)	■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■							
Internal Loads (HR & RPE)	■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■		■	■	■	■	■							
Sets x Rep x Weight		■						■						■						■						■						■						■						■						■						■						■						■										

Figure 6.3 Schematic of proposed study timeline and measurements to be taken over the 12-week study period. Session type: T=Training, M=Match, R=Recovery Session. Outline represents an ideal scenario where matches and training sessions occur regularly at the same point in the week over 12 weeks. This would not be a realistic portrayal of the study outline as matches may fall on different days. However, for illustrative purposes this graphic highlights the frequency of data collection required for the stimulus and response. Strength training sessions mainly occur on a MD-3, but may be subject to variation depending on the fixture schedule.

Statistical analyses

All data analyses will be conducted by using the statistical software IBM SPSS Statistics. The aim of the analysis will be to look for significant changes in responses over the course of the study as well as establish if relationships exists between external and internal loads and responses. Initially it will be important to establish the normally of data, thus a Shapiro-Wilk test will be conducted to assess normality of data for each variable, followed by a Levene's test for homogeneity of variance. A one-way ANOVA will be used to assess the magnitude of change between baseline measurements and any subsequent changes in measurements over time. Post-hoc tests will then be used to identify where the any differences between baseline measures and subsequent measures lie.

Associations between external and internal load variables and changes in adaptive responses will be assessed using Pearson’s correlation coefficients and linear regression analysis. This will help understand if the external and internal loads explain any changes in response at 6 and 12 weeks. The same methods (persons correlation and regression analysis) will be applied to establish which external or internal load variables have the highest influence on changes in responses will be conducted. The same method will be applied to understand how metabolomic and proteomic responses influence adaptive responses measured at 6 and 12 weeks. Moreover, this method will be applied to understand how external and internal training load influences metabolomic and proteomic responses.

Discussion

The aim of this study was to provide a protocol for an observational study in elite football that maps responses across multiple levels of organisation in both the physiological and biomechanical load-adaptation pathways. To the authors’ knowledge, this is the first study protocol developed that illustrates the depth of consideration needed when mapping adaptive responses across multiple levels. This study will characterise the cellular activity and signalling response to football and map these responses with adaptive changes at the tissue/organ, organ system and organism level. Overall this will provide unique insights into how football stimulates the processes behind adaptation in individuals and whether these processes lead to changes at higher levels of organisation. Furthermore, it will help establish if baseline tissue properties and performance at the organism level help explain the magnitude of the cellular response to training.

This protocol demonstrates the detail required when mapping the stimulus, as well as the tissues that need to be observed and the level responses need to be measured. This approach can help characterise the stimulus-response relationship in more detail and provide insights into the potential mechanisms behind changes in performance at the organism level. Overall, the stimulus needs to be well defined and captured in a way that allows for comparison between sessions, such as being able to compare external GPS loads between football sessions, or the volume lifted in different strength training sessions. This detail will help characterise the stimulus-response relationship between training and responses at the cellular level. Furthermore, baseline characteristics across multiple systems and tissues are needed. As well as being starting points for the study, these baseline measurements may provide extra insights into the responses to training. For example, baseline muscle, tendon and bone properties may have some part in explaining metabolomic, proteomic or bone turnover responses to training.

Finally, outcome measures need to be measured across multiple levels of organisation to help provide insights into the mechanisms behind changes on each level as well as the potential relationship between changes across levels. Ultimately if practitioners and researchers desire to be able to understand how individuals will respond to training, these types of comprehensive approaches are required.

The outcome measures in this study are chosen for the information they provide as well as their benefits in terms of efficiency and accessibility for the researcher. However, there is scope for different outcome measures to be used in this protocol, as other measures may provide more insights into those chosen in this study. As an example, tibial bone adaptations, namely increases in trabecular density, cortical density, cross-sectional area, and strength-strain index have been shown to increase as a result of 12 weeks of football training in elite u18 football players (Varley et al., 2017). However, these changes were detected using peripheral quantitative computed tomography (pQCT) which was not available to the researcher at the time of design. This would be a favourable tool for measuring bone adaptation if accessible. Furthermore, it can be argued that measuring tendon stiffness by observing the change in length during a ramp contraction using an isokinetic dynamometer and ultrasonography simultaneously provide greater insights into tendon stiffness (Maganaris, Narici and Maffulli, 2008). However, the time associated with obtaining such measurements provides a barrier in elite football.
This is especially the case in a study of this nature. Practitioners and researchers with greater freedom of access to players or more sophisticated equipment may be able to ‘plug in’ different measurements whilst still using the same approach in terms of design as in this study.

In summary, this study provides insights into the broad approaches required when mapping adaptation in football. This level of detail is required if insights into the causal mechanisms behind changes in physical performance are to be gained. It is important to note that the test-retest reliability of all measures would first need to be established prior to utilisation within this study, especially where tester variability exists. Nevertheless, it is hoped this protocol will help stimulate ideas for better research design in order to further develop our understanding of adaptation in football.

CHAPTER SEVEN

SYNTHESIS OF FINDINGS

The purpose of this following chapter is to consider the findings of this thesis in relation to the original aim and objectives. The practical implications of this research relative to adaptation in football will be discussed based on the synthesis of the major findings. Finally, the limitations of this thesis and recommendations for future research based on the findings will also be examined.

7. 1 Realisation of aims and objectives

The primary aims of this thesis were to describe and critically evaluate approaches to investigating adaptation in football-related research and explore novel approaches to measure adaptation in football. This was met through the completion of four separate studies (Chapter 3, 4, 5 and 6) investigating the following objectives:

Objective 1: To understand how adaptation is measured in football.

To find novel approaches to measure adaptation, which were explored in Chapter 5 and Chapter 6, it was first considered imperative to thoroughly investigate current approaches to measuring adaptation in football. To achieve this initial objective a scoping review was conducted. This review focused on extracting information from 461 studies related to adaptation in football. The information extracted allowed us to map the stimulus, response type, study duration, outcome measure and the level of organisation an outcome measure was taken in all 461 studies. The approaches taken in this review provided a novel and broad insight into adaptation in football.

Overall this review demonstrated that approaches taken to measure adaptation to the stimuli faced by football players are diverse. This could be the result of different pragmatic and conceptual views of adaptation. The findings of this study justify the exploratory approaches to measuring adaptation taken in chapter 5. Furthermore, the findings from this study will allow practitioners and researchers in football to consider more broadly the approaches they take when measuring adaptation in football.

Objective 2: Demonstrate how the physiological and biomechanical load-adaptation pathways could be stimulated during the in-season phase.

The description of the demands of football in chapter 2 outlined the potential implications of these demands on two distinct physiological and biomechanical load-adaptation pathways. This helped frame the argument that the dose-response relationship in football needs to be considered more broadly. In chapter 3, the data presented demonstrated the breadth of considerations that must be considered when measuring the stimulus-response relationship. Consequently, the overarching aim of chapter 4 was to attempt to expand the usefulness of GPS training load data in order to consider the potential implications of training on both the physiological and mechanical load-adaptation pathways. This was an attempt to capture the stimulus in more detail as suggested in chapter 3.

As a means of demonstrating the potential for in-season training to stimulate adaptive responses, an analysis of the demands of in-season football-based training was undertaken. The information provided in chapter 2 demonstrated that footballers are subjected to demands that stimulate the physiological and biomechanical load-adaptation pathways. Research is scarce on adaptation during the in-season phase in football. It was concluded that if there is potential for physiological and biomechanical adaptations to occur during the in-season phase, these would occur due to specific stimuli and intensities.

Furthermore, the variability in any potential responses is likely to be a function of playing position. It was therefore postulated that the most intense in-season training sessions could have the potential to stimulate these pathways. This study found that sessions classified as intense from a physiological and biomechanical standpoint had significantly greater external loads than non-intense sessions for each position. Furthermore, SD and Hi A/Ds were not significantly higher in matches when compared to physiological and mechanical sessions, respectively. Overall, this suggests that intense physiological and mechanical sessions have the potential to stimulate adaptive responses.

Objective 3: To explore the utility of metabolomics as a pragmatic method for mapping responses.

Chapter 4 highlighted the potential for varied responses to occur based on the demands of different training sessions across the season. This study presented the idea that the most intense stimuli could stimulate adaptive responses in different load-adaptation pathways. Furthermore, this study demonstrated a potential way to think about the stimulus in a broad way. However, without directly observing the internal responses to training it is impossible to truly understand the bodies response to the multi-faceted demands of football. Thus, the purpose of chapter 5 was to follow on from chapter 4 and explore a way of mapping the internal response in a broad way. However, based on chapter 3 it was also deemed important that any method used to map the internal response provided a blend of a pragmatic and a comprehensive approach. Thus, a pilot study was conducted to explore the potential use of metabolomics using capillary blood samples to assess the response to a single training session.

Considering the outcomes of both chapters 2, 3 and 4, a pilot metabolomic study was conducted to assess the utility of using capillary blood samples to provide broad insights into the metabolomic response to football training. Chapter 2 highlighted the need for broader thinking when considering adaptation in football. Whilst Chapter 3 demonstrated that merging conceptual and pragmatic approaches to adaptation could provide interesting insights. The football session chosen for this study was intense based on positional data captured in chapter 4.

The results from the metabolomics analysis revealed that serum metabolomics, collected using fingertip blood samples and analysed using NMR spectroscopy, was sensitive enough to detect changes in metabolites following a single football training session. From a methodological standpoint, this method may be attractive for practitioners and researchers in football due to the relative ease at which these samples can be collected in the field and the broad range of insights this method provides. Together these results illustrate the potential use of this less invasive and quick method for collecting and analysing broad metabolic changes to football training. However, it is acknowledged that further reliability testing with regards to this method needs to be performed in future studies.

Objective 4: To develop a study protocol that broadly captures the adaptive response to football training

Chapter 6 was developed as an attempt to merge the ideas presented in previous chapters in this thesis and provide a study design that may reflect the level of detail needed to understand the dose-response relationship between football activities and adaptation more broadly.

Chapter 2 highlighted how adaptation occurs across different levels of organisation in the body, over different time courses. To provide broader insights into the adaptive process, outcome measures taken at multiple levels over multiple time courses are necessary to capture the full spectrum of the adaptive process. Unfortunately, due to the worldwide pandemic caused by COVID-19, a study of this nature became unfeasible. However, the protocols outlined in this study provide an approach to mapping adaptation that satisfies the blend of pragmatics and broad conceptual thinking evident throughout the previous chapters in this thesis.

7. 2 General Discussion

Overall this research programme critically evaluated approaches that have shaped our understanding of adaptation in football and outlined the importance of considering the implications of football on adaptation in a broad context. This project spawned from a desire to understand what it would take to be able to predict how players would adapt to football training. Ultimately to achieve such a feat means that the adaptive processes of multiple tissues and systems in the body need to be considered.

A first step in the right direction in terms of thinking broadly about adaptation was presented by Vanrenterghem et al. (2017) who used the idea of separate physiological and biomechanical load-adaptation pathways to demonstrate how to consider the implications of football in a broad context. This thesis is an attempt to challenge conventional thinking about adaptation in football, much like the paper by Vanrenterghem et al. (2017) was. This research project comprehensively mapped the approaches taken regarding measuring adaptation in football. This was an important first step before making recommendations about how to progress the area of adaptation in football forward. Furthermore, this thesis has also attempted to provide comprehensive yet pragmatic solutions to mapping adaptation in a more holistic way in elite football. Overall, this thesis provides a novel and broad perspective on how adaptation has been thought about in football, as well as alternative approaches to study design.

The key messages from the literature review provided in chapter 2 are that football is a complex sport that requires the coordination of multiple physiological systems. However, this review provided some evidence of a lack of a broad approach when it comes to measuring adaptation in football. This was framed in the context of the physiological vs mechanical load-adaptation pathways. The evidence presented suggested that there is a primary focus on understanding the adaptive responses related to the physiological load-adaptation pathway. However, to fully understand the breadth of this area, it was important to comprehensively map how adaptation has been approached in football.

Chapter 3 revealed that adaptation is typically measured at one level without thinking about the interconnection between responses at other levels in the body. The results indicate a trend toward pragmatic approaches to measuring adaptation in football, which is understandable due to the difficulties of taking measurements in the field. However, to explain the mechanisms behind adaptation at the organism level, the dynamic interactions that occur across all levels in the body need to be considered. Overall the evidence presented in this chapter would suggest that there is a primary focus on observing changes in performance measures as opposed to trying to comprehensively understand the broad science behind these changes.

If the opportunity arose in the future to give a practitioner the opportunity to predictably ‘program’ the desired response and adaptation to a stimulus in an easy manner, it would seem impossible to turn down. Thus, even if a practitioner was not interested in the cascade of responses that occur below the organism level, it would seem hard to not be interested in the idea of a predictable response to a stimulus, which ultimately will require knowledge of the interconnection between responses on and across all levels in the body. The type of insights that could be gained from this type of approach could result in being able to optimise adaptations to training as well as optimise recovery in the build up to gamedays, which could in turn optimise performance.

Nevertheless, the data presented in this study would suggest there is interest in understanding the responses that occur on levels below the organism. Some of the more common outcome measures found at the cellular level will be familiar to many football practitioners. Indeed, football practitioners may use insights gained at the cellular level to dictate practice already. Insights gained from cellular responses relating to muscle damage and substrate use to different types of conditioning/football drills is common practice. However, this thesis demonstrates that the complex nature of adaptation requires adaptation to be mapped across multiple levels, systems and tissues in order to truly understand how training impacts adaptation.

Chapter 3 also demonstrated the importance of mapping the stimulus in detail as well as the responses to different stimuli in football. From a practical perspective the insights developed from a deeper understanding of how different stimuli affect adaptive responses could influence how and where a stimulus is applied within the training week. In practice, training is constrained by the schedule of the players both on and off the pitch. Thus, in many cases, a pragmatic approach is taken due to time-constraints and different stimuli are applied in close proximity to each other. It is interesting to note the growth of players at the elite level who hire their own personal coaches to supplement their physical training programme. Invariably players would meet with these coaches outside of contact hours with their club.

Thus, it is plausible that these ‘extra’ sessions could take place far enough away from football training to negate any potential metabolic blunting. In the future it may be the case that the strength training stimulus is solely applied by personal coaches of the player outside of the club. This opens up more challenges as a collaborative approach would need to be taken between the club and external coach. However, the positives of such collaborations could be the fact that the optimal conditions for adaptations could be in place with this approach. However, further research is needed to ascertain how impactful any potential blunting is on long term adaptations and if it is practically feasible to separate the influence of multiple stimuli.

. Chapter 4 attempted to capture the importance of this type of thinking regarding the stimulus by mapping the physiological and biomechanical stimulus during the in-season phase. Results from this novel approach revealed that intense sessions from a mechanical and physiological standpoint occur regularly across the season. Physiological and biomechanical adaptations are a function of repeated exposures to stimuli that stress these systems; thus the repeated intense exposures may present opportunities for adaptation. However, the type of session, and therefore the nature of the response seems to be dependent upon position. This is not surprising but demonstrates how broadly one must consider the stimulus being applied in a team setting. However, these findings imply that different adaptive processes may occur at different time points for different individuals across the season. Thus, it may not be the case that there are ‘responders’ and ‘non-responders’ to training. Rather it is plausible that based on the stimulus there could be ‘physiological responders’, ‘mechanical responders’ or ‘non-responders’.

It is interesting to consider if the lack of research that exists demonstrating that adaptations could occur during the in-season in football, is a function of narrow views of adaptation. There may be subtle adaptations or adaptive processes occurring throughout the season that are not detected by measurements of performance at the organism level. It is conceivable that the training stimuli are intense enough to stimulate adaptive responses, but these responses do not result in adaptations that permeate to the organism level. This could be due to a lack of consistent application of an intense stimulus or the lack of adequate rest to allow the adaptive process to occur without causing injury due to accumulated damage or fatigue.

Furthermore, any performance testing at the level of the organism may come at a good time for tissues at the positive end of the adaptive process, but not for others in the middle of their adaptive process. This highlights the importance of considering the unique adaptive responses and time-courses of tissues within the physiological and biomechanical load-adaptation pathways. Thus broad approaches that map these more subtle responses on levels below the organism may detect the signals behind biomechanical or physiological adaptations.

Chapter 5 was an attempt to explore the use of capillary metabolomics as a way of merging the comprehensive approach with pragmatics to further understand responses in elite football. Results found this method was sensitive enough to detect changes in 18 different metabolites to a session that was physiologically and mechanically intense for some positions based on the criteria explored in chapter 4. This highlights the potential of this approach to provide comprehensive insights, whilst being practical from a collection standpoint. However, further work is needed to establish the reliability of this method in football. Similar approaches using other OMICs, such as Genomics and Proteomics, could be collected to provide a more comprehensive picture of the body's response to football.

Practically speaking each player's sample took no more than 5 minutes. With some players taking less than 1 minute. When one considers the practicalities of some tests routinely performed as part of daily monitoring such as screening, countermovement jumps etc. then the speed at which this sample can be taken would not seem to be an obstacle in the field. Moreover, this approach does not require staff to have specialised training for more invasive approaches. The analysis of the samples and data is the most time-consuming and costly part of this process. As such these approaches are more suited to research as opposed to informing any decision-making process daily in football at this moment in time. A logical first step to utilising metabolomics in football may be to analyse the changes in responses to a standardized fitness test over a period of time.

This information could be used to inform training and nutritional strategies to improve the adaptations to training as well as provide nutritional strategies for matches. In time as processes become most time and cost efficient, the use of metabolomics could extend to the analysis of players resting metabolic profile to indicate fatigue and immune system status. Processing and analysing these samples would require the collaboration between a football club and an academic institution with the equipment and skilled personnel to perform these analytics. This was the case for this study.

Building and developing these types of collaborations would seem to be a natural progression for practitioners seeking to merge conceptual views of adaptation with pragmatics to develop their understanding of the ‘science behind performance’.

The final study in this thesis attempted to demonstrate how a conceptual and pragmatic approach to adaptation might look in terms of a practical study. This study protocol demonstrated the level of detail required when capturing the stimulus and response to football activities. The choices made regarding outcome measures in this study demonstrate a necessary trade-off that must occur when doing research in the applied world. However, the comprehensive approach to capturing the stimulus and mapping responses across multiple levels and time courses using pragmatic approaches is the main take-away from this study.

The key themes discussed in this general discussion centre on the premise that a broader methodological approach is needed if our understanding of adaptation in football is to be developed. The chapters in this thesis demonstrate the importance of broadly considering the approach to the problem of measuring adaptation. This includes considering the stimulus, response type, level measured, duration of the study and outcome measures used. Figure 7.1 illustrates some key questions that need to be asked before studies are conducted.

Figure 7.1.Thinking broadly about adaptation in football research. Some key questions to ask that will drive the design of more robust research in future studies on adaptation in football.

Attempts have been made throughout this thesis to capture the stimulus and response using comprehensive yet pragmatic approaches. Ultimately when measuring adaptation to a stimulus, the ideal scenario is to establish a dose-response relationship. These questions demonstrate the necessary considerations regarding the stimulus and response that come as a result of a broad approach to designing research. Considering these questions may allow for better insights into the dose-response relationships between football and adaptation. Each chapter in this thesis has provided novel ways to answer some of these questions. Nevertheless, the key takeaway from this thesis is encapsulated into a simple framework demonstrating how to design better studies in football. This approach is presented in figure 7.2.

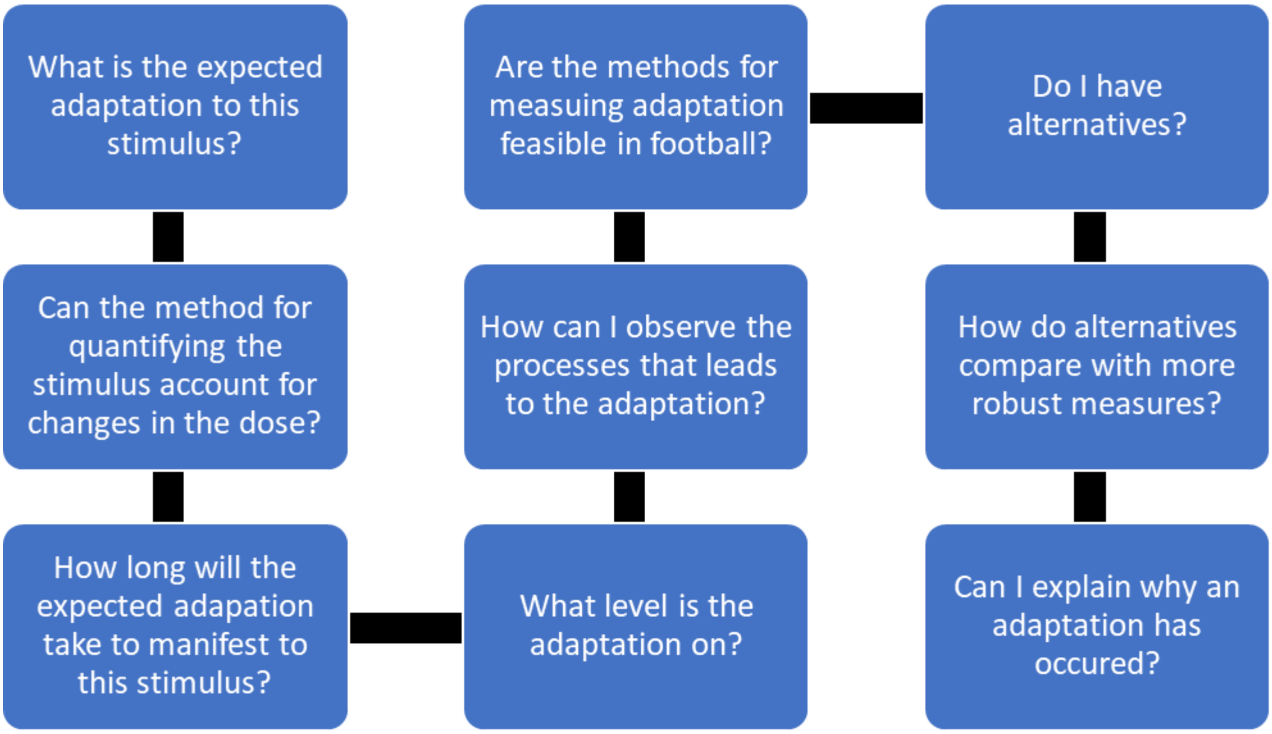

Figure 7.2 A framework outlining the key questions when designing future research. The black line joins up each question in the order in which it should be considered.

1. The first key question is to ask what should this dose of stimulus do? If the stimulus is repeated sprint training, then it would be logical that an increase in repeated sprint ability would occur.
2. However, if trying to establish a dose-response relationship then it would seem logical to be able to quantify the stimulus in enough detail to detect small changes. Otherwise, it is difficult to understand the reasons behind any observed change. Thus, it would seem illogical to continue with a study design if the stimulus cannot detect change easily.
3. If the stimulus can be quantified in detail, then the potential adaptations can be

considered further. These questions relate to the duration over which the stimulus should be applied to see the adaptation. As figure 7.1 illustrates, this means considering the effect of concurrent stimuli, which may influence these durations.

4. Next the level at which the adaptation will be measured should be considered. This will tie into the first question as an expected adaptation could be a change in jump height, which dictates that the level of the measurement is at the organism level.
5. However, this question sets up the next question which asks if the processes behind the adaptation can be observed. This question should guide the researcher to consider if measuring responses on levels below question 4 can provide insights into the processes driving adaptation. Thus, the researcher might conclude that changes in protein synthesis and muscle CSA are important measures to consider if mechanistic insights and potential dose-response relationships are to be established.
6. As is evident throughout this thesis, these measures ideally need to be captured with ease in football
7. Thus if invasive measures that provide insight into muscle protein synthesis are not available, such as a biopsy, then alternatives that could be implemented in football may need to be explored.
8. These alternatives would need to be compared to ‘gold standard’ measurements.
9. If these alternatives can provide greater insights into the ‘why’ behind adaptive responses, then question 9 can be answered.

It is hoped this framework provides a broader methodological process for mapping adaptation in football. Overall, this framework demonstrates how thinking about the stimulus in a broad way will allow for more robust studies that can map the potential reasons behind any response in detail. This may also allow for more reproducible research. Furthermore, considering the response broadly will provide greater insights into why adaptations occur.

7. 3 Project Limitations

This research project has provided a novel appraisal of adaptation in football and provides potential solutions to develop this research area. However, the methods used to do so are not without their limitations, these limitations will be outlined, from which recommendations for future research will be made.

The framework used to classify outcome measures by level and measurement category in Chapter 3 could be open to criticism as in some instances measures could be allocated to a different level or categorised differently based on subjective opinion. If this type of categorisation is to be adopted in future research, a consensus will need to be established for consistency regarding the level and category an outcome measure falls into.

In chapter 4 the GPS metrics used to define mechanical and physiological sessions are based on interpretations that may seem rational, yet it is difficult to determine if these metrics are indeed the most appropriate to use. Separating high-intensity accelerations and decelerations may be more appropriate as decelerations may provide a greater mechanical stimulus than accelerations (Harper et al., 2019). Future research may be able to develop the methods used in this study by using principal component analysis to bring together several external load metrics which could provide greater insights into what is mechanically or physiologically intense.

This method could be extended to include more detailed internal responses to different session types. Mapping responses and external loads to sessions specifically designed to be highly mechanical (i.e high number of changes of direction and ground reaction forces) and physiological (high metabolic demands), could provide more robust thresholds for the types of sessions that are deemed intense from a physiological and mechanical standpoint. Another limitation in this study was the fact that internal loads were based on the entire training session, including rest periods. Whilst rest periods do not alter the intense external loads, players' heart rates could significantly drop during these periods. Thus, the average heart rates and average maximum heart rates presented in this study may not reflect the true intensity of the training sessions.

In chapter 5 results using capillary blood samples were not compared with results from venous samples to establish the validity and sensitivity of this method. Future research needs to establish the validity and sensitivity of this method by comparing measurements using capillary blood versus venous blood. Furthermore, the pre-training meal was not completely standardised which may have influenced the results. It would have been useful to collect information regarding meals in the previous days to establish levelling of 'fuel' available for this session. Furthermore, a pre-and post-training muscle biopsy could have provided useful insights into the glycogen stores within the muscle which would have provided useful information to complement the metabolomic results. Finally, an analysis of the proteomic responses may have helped identify tissue-specific proteins that could have been stimulated by the mechanical demands of the training session. This would have provided a nice insight into the implications of this session on the physiological and biomechanical load-adaptation pathways.

7. 4 Recommendations for Future Research

The main theme of this thesis is centred on thinking broadly about adaptation. In the spirit of this approach, several recommendations for future research will be outlined below.

In Chapter 4 the attempt to map potential responses across the season demonstrates how varied the adaptive processes may be for individual players based on different stimuli. Using internal responses, future studies may be able to map the most intense responses related to the physiological and mechanical load-adaptation pathways as opposed to mapping the stimulus alone. This would provide extremely useful insights into internal responses relative to different session types throughout the microcycle, as well as the season as a whole.

The potential for metabolomics using capillary blood samples has been put forward in this thesis. Overall chapter 5 demonstrated that this method was sensitive enough to detect changes in metabolites that reflected the intense nature of the session. However, future research is needed to establish the reliability and validity of OMICs using capillary blood samples. A further limitation in the design of chapter 5 is the lack of comparison between individual demands and individual metabolic response. This would have provided interesting insights into the relationship between demands and metabolic response.

Future research using OMICs approaches is needed to map the metabolomic, proteomic and genome response to multiple football training sessions and drills with different physiological and mechanical demands. This could help identify session-specific biomarkers. This would be an acute study that would capture the profiles of different sessions. For example, the changes in metabolomic, proteomic and gene expression responses to a ‘mechanical’ session comprised of a high volume of accelerations and decelerations, could be compared to a ‘physiological’ session comprised a high volume of high-speed running and sprinting. This could identify ‘fingerprints’ for different types of sessions and allow for more targeted analysis of specific metabolites, tissue-specific proteins, and gene expression in response to football activities.

Such results could then be mapped against changes in tissue-specific properties at the tissue and organ level and changes in fitness or strength and the organism level. This would be a more longitudinal type of study using a similar protocol to that proposed in chapter 6, where responses would be mapped across multiple levels. The possibilities of these types of approaches could lead to studies that demonstrate the ability predict responses to specific stimuli based on individuals’ metabolomic, proteomic and genomic fingerprints (Zierath and Wallberg-Henriksson, 2015). This was demonstrated by Kuehnbaum et al., (2014) who predicted changes in glucose tolerance after high-intensity interval training in obese women based on metabolomic profiles. However, the stimulus in this study was tightly controlled as it was treadmill-based. This reiterates the importance of quantifying the stimulus in detail if predictions methods are to prove fruitful.

Consequently, future research could consider implementing other forms of external-load quantification tools to help map the stimulus in more detail. From a mechanical standpoint, it Is difficult to extrapolate any meaningful information regarding the stresses experienced by the lower extremities, from a

GPS device placed between the scapulae (Verheul et al., 2019). Inertial measurement units attached to the lower limbs demonstrate the potential to capture the movements and ground-reaction forces of the lower body more accurately. These tools may provide more insights into the mechanical demands of football and the potential implications on the tissues within the mechanical load-adaptation pathway. However, future research first needs to validate these tools against muscle, tendon, and bone responses to mechanical load before they can provide useful estimates of mechanical loading.

7. 5 Conclusion

This thesis aimed to examine and evaluate how adaptation has been considered in football. The outcomes from this thesis demonstrate that the implications of football on adaptations need to be considered from a broad perspective. The complex demands of football and the complexity of the body's responses mean this is not a straightforward problem to solve. This complexity is further compounded by the constraints that practitioners and researchers have to deal with when trying to measure adaptation in football players. However, progress towards greater insights may be made by adopting a different approach to the problem at hand. This thesis offers an alternative perspective and some approaches that may help guide better research and progress in the area of adaptation in football.

CHAPTER EIGHT

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