NOVEL NUTRITION STRATEGIES TO MAINTAIN SKILL PERFORMANCE IN ACADEMY SOCCER PLAYERS

By

Paola Andrea Rodríguez Giustiniani

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“Only a few know how much one must know to know how little one knows”

Werner Heisenberg

DECLARATION

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself (unless otherwise acknowledged in specific chapters), under the supervision of Professor Stuart Galloway and Dr Iain Gallagher. Neither the thesis, nor the original work contained therein have been submitted to this or any other institution for a higher degree.

Paola Rodriguez Giustiniani

Paola Andrea Rodríguez Giustiniani

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ABSTRACT

The present thesis reports on 4 studies on soccer-specific skill performance in academy male soccer players and novel nutritional strategies that could be used in order to help maintain skill.

The first study reported in this thesis investigated how the consumption of 250 mL of a 12% carbohydrate-electrolyte beverage, both before the start of a 90-min soccer match simulation and at half time, influenced the preservation of soccer-specific abilities (specifically, dribbling, and passing proficiency), sprinting speed, and anaerobic endurance running capacity. The main finding from this study was that higher passing scores were consistently attained by players using both their dominant and non-dominant feet from the 60-min mark onward in the match simulation when ingesting the carbohydrate-electrolyte beverage. Notably, this sustained skill performance was achieved without a decline in passing speed. Interestingly, passing speed was better maintained particularly on the non-dominant foot when the players consumed the 60g of carbohydrates. Concomitantly, high-intensity running capacity was improved when the participants ingested the 12% carbohydrate-electrolyte solution compared with placebo; all of these positive findings occurred with minimal impact on gut comfort.

In the second study, the test-rest reliability of soccer-specific skills was assessed using a modified version of the Russell et al (2011) soccer match simulation protocol. The results suggested that the modified soccer match simulation protocol exhibited promising reliability for most of the evaluated skills. Good to excellent reliability was reported for passing speed and accuracy and sprint speed. However, dribbling and shooting variables showed lower reliability. Additionally, it was determined that the modified version of the protocol could be easily incorporated into professional club settings without the need for specialised equipment.

The third study identified that a single dose of a beverage containing 300 mg of coffeeberry ingested one hour before a soccer-specific skill assessment, conducted under rested conditions, did not result in any significant alteration in soccer skill performance compared with placebo. Moreover,

it was concluded that it would be prudent to explore similar studies involving activities that induce physical and/or mental fatigue to better understand how coffeeberry might mitigate the negative impact of fatigue on soccer-specific skill performance.

In the fourth and final study, the primary aim was to examine the impact of a single pre-exercise dose of a beverage containing 300 mg of coffeeberry on soccer skill performance throughout a 45-min period of simulated soccer-match play and on soccer skills evaluated after exercise-induced fatigue. The study confirmed that the consumption of coffeeberry extract could be advantageous in preserving some aspects of soccer-specific skill performance under exercise conditions. Specifically, it demonstrated notable maintenance of passing performance with retention of passing speed and accuracy during a 45-min simulated soccer match.

TABLE OF CONTENTS

LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AD	Activation Deactivation
ARE	Antioxidant Response Element
CB	Coffeeberry
CGA	Chlorogenic Acid
CHO-E	Carbohydrate-electrolyte
CI	Confidence Intervals
CNS	Central Nervous System
COX	Cyclooxygenase
CQA	Caffeoylquinic Acid
CV	Coefficient of Variation
FMD	Flow Mediated Dilation
HR	Heart Rate
ICC	Intra-class Correlation Coefficient
LIST	Loughborough Intermittent Soccer Test
LSPT	Loughborough Soccer Passing Test
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NO	Nitric Oxide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear factor-like 2
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPE	Ratings of Perceived Exertion
SD	Standard Deviation
SA	Skill Assessment
SMS	Soccer Match Simulation
SPSS	Statistical Package for Social Sciences
TMS	Transcranial Magnetic Stimulation
VAS	Visual Analogue Scale

CHAPTER 1
GENERAL INTRODUCTION

1.1 Overview of factors influencing skill performance in soccer

Knapp (1977) defined skill as the learned ability to bring about pre-determined results with maximum certainty often with the minimum outlay of time or energy or both. All sports, to varying extents, involve the application of cognitive, perceptual or motor skills. Since soccer is performed under a rapidly changing environment, it could be inferred that it is a sport that involves all three aspects of skill (Bate, 1996). It has been suggested that the crucial element of soccer performance lies in the adept execution of skilful movement patterns, carried out with efficiency and effectiveness. Players are required to employ cognitive, perceptual, and motor abilities in response to swiftly evolving scenarios. Concomitantly, the motor skills required to successfully control, pass, dribble, and shoot the ball at goal are fundamental skills of the soccer player as highlighted by Ali (2011). In soccer, players are tasked with executing an array of skills tailored to the sport, often while running or immediately following it, frequently at high sprinting velocities. The effectiveness of these skills is quick to be impacted by the extent of effort exerted in both offensive and defensive actions throughout the course of the match (Rollo and Williams, 2023).

As a match unfolds, the player's ability to maintain skill mastery and the frequency of particular technical manoeuvres tend to decline (Rampinini et al., 2009; Harper et al., 2014). Even the most exceptionally skilled players will suffer from the effects of fatigue, encompassing both physical and mental fatigue aspects. As a result of match duration and intensity, players tend to encounter the impacts of fatigue both on a physical level (running, sprinting, jumping) and in terms of mental aspects (concentration, decision-making); this frequently leads to a decline in their ability to execute skills effectively during critical moments in a match, as noted by Mohr et al. (2005) and Smith et al., (2016). Hence, it can be concluded that soccer players will experience physical and mental fatigue that could have a negative impact on the performance of specific skills (Rollo and Williams, 2023).

Nutrition can play a valuable role in optimising the physical and mental performance of professional players during match-play (Collins et al., 2021). As such, nutritional related factors such as hydration, carbohydrate provision, and the utilisation of ergogenic aids (e.g., caffeine) have a direct effect on fatigue (physical and mental) and its deleterious impact on soccer skill performance (Figure 1.1). Moreover, the physiological and psychological demands in modern soccer are evolving rapidly (Nassis et al., 2020), and this is likely to increase the degree of fatigue during games (Mohr et al., 2022). Therefore, the development and optimisation of strategies that aim to delay the impact of fatigue on soccer performance or to maintain the execution of its specific skills would be of interest to players and clubs.

It has been stated that fluid losses greater than 2% of body mass have detrimental effects on exercise performance; the potential explanations for this negative impact include augmented cardiovascular strain, heightened heat stress (hyperthermia), modified central nervous system function, or a blend of these factors (Cheuvront et al., 2003). Dehydration has the potential to negatively influence endurance performance particularly when coupled with heat stress (Sawka et al., 2007). Conversely, muscle strength and anaerobic task performance are less prone to impairment due to dehydration (Cheuvront and Kenefick, 2014; Ali and Williams, 2013). Dehydration tends to take place over the course of 90-min of soccer-specific exercise. This is evidenced by reductions in body mass (Ostojic and Mazic, 2002) as well as elevations in plasma osmolality and blood sodium concentration (Kingsley et al., 2014). Owen et al. (2013) conducted a study investigating the impact of dehydration on football skills such as passing and shooting, as well as intermittent high intensity running performance. Despite variations in fluid consumption (none, ad libitum, and prescribed volume) and levels of dehydration (2.5%, 1.1%, and 0.3% respectively), the performance of football skills and intermittent high-intensity exercise showed no differences. These findings, contradict earlier research that indicated that dehydration was related to a decline of 5% in dribbling performance (McGregor et al., 1999). It could be that these conflicting findings reflect the relatively poor reliability of dribbling skills in test-retest situations. In addition, it has been stated that soccer players typically replenish only around half of their fluid losses during training and matches, which can potentially lead to dehydration levels of more than 2%, particularly under warmer environmental conditions (Laitano et al., 2014). Therefore, it is advisable for players to maintain adequate hydration levels (limiting fluid losses to less than 2% of body mass) during both training sessions and matches (Shirreffs et al., 2006) as it appears that adhering to an effective hydration regime enables soccer players to avert performance declines linked to dehydration (Russell et al., 2011).

A decline in blood glucose concentration has been put forth as a potential mechanism for impaired physical and cognitive capabilities and prospective contributor to decreased soccer-specific skill performance (Winnick et al., 2005; Ali et al., 2007; Patterson and Gray, 2007). However, this notion has faced challenges, with some authors stating that hypoglycaemia is rare during 90-min of soccer-specific exercise (Baker et al., 2015). Notably, episodes of hypoglycaemia have been observed subsequent to the consumption of high glycaemic carbohydrate during a passive half-time period (Russell et al., 2015). Nonetheless, an elevation in blood glucose concentration has been correlated with improved visual discrimination, psycho-motor speed, and fine motor speed; all relevant to the successful execution of soccer-specific technical performance (Bandelow et al., 2011). The benefits of ergogenic aids that aim to enhance circulating glucose concentration, especially the acute provision of carbohydrate before or during soccer match-play, have been broadly reviewed (Rollo and Williams, 2023). It was concluded that enhancing muscle and liver glycogen reserves through carbohydrate intake prior to, and consuming carbohydrates during matches can postpone the onset of fatigue and promote the preservation of soccer-specific skill execution. The authors also concluded that strategic carbohydrate intake during competition could potentially offset adverse feelings and enhance focus, thereby helping players in maintaining skill performance throughout exercise execution (Rollo and Williams, 2023). It is worth mentioning that in the present thesis players were provided with standardized carbohydrate-rich meals on the day of the trials and these followed the current recommendations on pre-match carbohydrate ingestion (Collins et al., 2021) in order to mimic match day strategies and avoid insufficient fuelling.

Most researchers have employed soccer-specific protocols when evaluating the effectiveness of carbohydrate ingestion. A subset of studies have not observed beneficial effects of carbohydrate on soccer-specific technical performance (Zeederberg et al., 1996; Abbey and Rankin, 2009; Ali et al., 2007; Ali and Williams, 2009). However, certain authors have identified positive impacts of acute carbohydrate ingestion on passing and shooting performance (Northcott et al., Ali et al., 2007., Currell et al., 2009., Russell et al., 2012) as well as better maintained dribbling performance (Ostojic and Mazic, 2005., Currell et al., 2009; Harper et al., 2017) when compared to placebo. The discrepancies in outcomes might stem from various factors, including player skill levels or the reliability of the performance tests employed. Nevertheless, the provision of carbohydrate is more likely to maintain technical performance in soccer players when they are fatigued (i.e., during the latter stages of a match), as declines in technical performance tend to manifest under fatigued conditions (Russell and Kingsley, 2001; Baker et al., 2015).

The use of caffeine as an ergogenic aid is popular (Doherty et al., 2004). Extensive research has delved into the performance-enhancing effects of acute caffeine ingestion on endurance, intermittent, and resistance exercise (Graham, 2001; Burke, 2008), as well as basic cognitive functions (Lorenzo et al., 2021). To be more precise, caffeine has been demonstrated to improve the physical and technical components essential for successful soccer match-play (de Almeida et al., 2022). There is evidence indicating enhancements in repeated sprinting and jumping performance (Gant et al., 2010), passing accuracy (Foskett et al., 2009), and reactive agility (Duvanjak-Zaknich et al., 2011). These outcomes have probably contributed to the widespread use of caffeine among professional soccer players (de Almeida et al., 2022). However, a study examining the effect of caffeine supplementation on match activities and development of fatigue during a football match encountered that oral caffeine administration does not appear to have an ergogenic effect in young male football players during match-play (Pettersen et al., 2014). Conversely, acute caffeine ingestion before soccer small-sided games reduced defensive errors when compared to placebo. Concomitantly, a higher ball possession was also observed in the caffeine condition (de Almeida et al., 2002). Both studies utilised a caffeine supplement in similar doses (5-6 mg/kg) one hour before the main trials. Even though the study by Pettersen et al. (2014) showed that caffeine intake did not alter the activity pattern and fatigue profile during match-play for young male football players, despite the physiological effects that caffeine supplementation could have. The study by de Almeida (2002) showed positive effects on tactical performance in young players. The authors mentioned that this was the first study to assess the influence of caffeine on tactical performance and the results should be interpreted with caution.

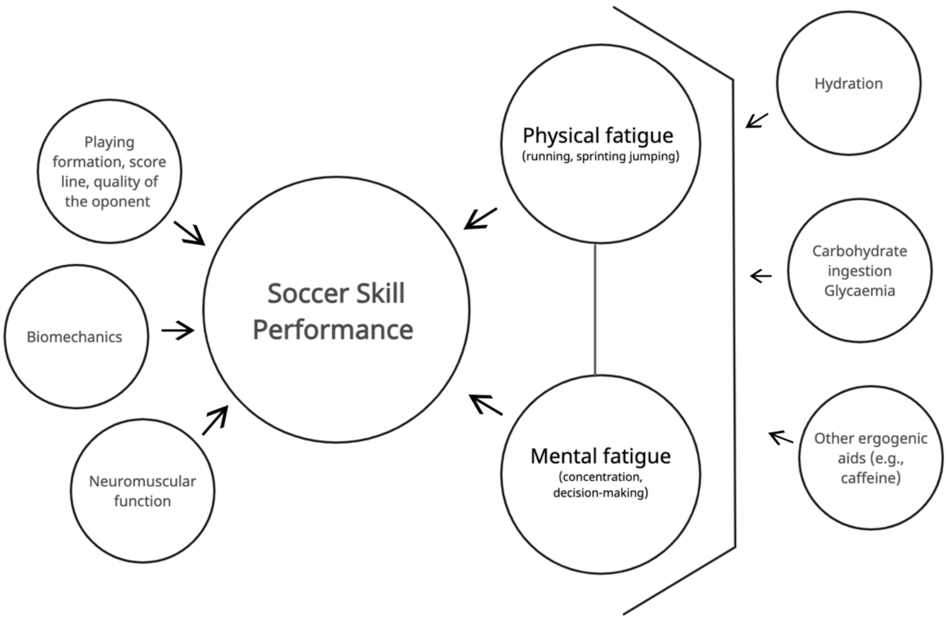

Figure 1.1. Factors affecting soccer skill performance

1.2 Aims of the thesis

This thesis describes a series of studies examining nutritional strategies to maintain soccer skill performance in professional academy players. Firstly, **Chapter 3** describes an experimental study which examined the impact that the ingestion of a 12% carbohydrate-electrolyte beverage, before kick-off and during the half-time period, had on the retention of soccer-specific skills (dribbling and passing performance), sprint speed, and anaerobic endurance running capacity during and after a 90-min soccer match simulation. Next, **Chapter 4** describes a study that examined the test-retest reliability of soccer-specific skills within a modified version of a soccer match simulation protocol. This modified version was utilised as the main testing protocol for the subsequent studies presented in this thesis. The study presented in **Chapter 5** aimed to determine whether a single dose of coffeeberry taken one hour before soccer specific exercise had an influence on soccer skills (dribbling, passing, and shooting) in a non-fatigued resting state. Thereafter, **Chapter 6** depicts a study that aimed to determine the effects that acute pre-exercise coffeeberry supplementation had on soccer skill performance during a period of simulated soccer match-play, and on high-intensity running capacity, and soccer skills assessed following exercise-induced fatigue. Finally, the practical implications of the performed research studies are discussed in **Chapter 7** and subsequent aims for future research are provided.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Soccer (association football) is the most popular team-based sport worldwide for audiences, attendance, participation, and commercial interest. Official matches consist of two halves of 45-min each, interspersed with a 15-min break (Bangsbo et al., 2006; Mohr et al., 2022). Soccer is characterised as an intense multicomponent intermittent sport challenging the entire performance spectrum by requiring athletes to execute a variety of explosive, technical, and tactical movements repetitively with high concomitant cardiovascular, metabolic, and musculoskeletal fitness demands (Turner and Stewart, 2014; Krustrup et al., 2018). Succeeding in this sport, which is accomplished by winning games, requires a high level of tactical, physical, cognitive, and technical abilities.

Performance in soccer match-play requires a complex physiological demand that relies on the interaction of anaerobic and aerobic energy systems (Dolci et al., 2018). The high energy demand is accompanied by measurements of high peak and average heart rate, elevated blood, and muscle lactate levels, increases in plasma free fatty acids, as well as acutely lowered muscle phosphocreatine concentrations that reflect anaerobic and aerobic substrate turnover (Krustrup et al., 2006). Although physical performance is crucial for soccer match-play success, skill performance is also a key factor, and therefore technical proficiency is a discriminating factor in match success (Lago-Peñas et al., 2011).

Understanding the multi-faceted nature of fatigue in the context of soccer match-play has received considerable interest. Fatigue plays a paramount role in the outcome of matches (Hulton et al., 2022). For example, the onset of fatigue is associated with an increase in goals conceded during the final 10-min of 90-min matches (Reilly, 1997), with extra time leading to additional demands that would further impact the effects of fatigue (Harper et al., 2014) . The decline in movement output and soccer-specific skill execution over the course of a match has predominantly been attributed to acute fatigue induced by high physiological, technical, and cognitive demands (Dolci et al., 2020). While there are many potential mechanisms responsible for fatigue during match-play, peripheral fatigue linked to nutritional implications (depletion of muscle glycogen, hypoglycaemia) could be of importance in the development of this phenomenon (Hulton et al., 2022; Mohr et al., 2022).

Based on extrapolation of current trends in modern soccer, the game will continue to become more physically demanding in the future which will exacerbate fatigue at the end of the game (Mohr et al., 2022). Thus, the ability to recover faster between high-intensity periods within the match, the ability to save energy, and the maintenance of skill performance might be relevant factors over the entire 90-min game (Dolci et al., 2020). The development of nutritional strategies such as the optimisation of carbohydrate ingestion before and during the match, as well as the utilisation of ergogenic aids, could ultimately maintain performance over the course of the game, and therefore warrant further investigation.

2.2 Physical and energetic demands of soccer match-play

The physiological demands of soccer and the capacity of a soccer player to meet the physiological demands of match-play provide a key determinant of soccer performance. According to Castillo et al. (2021), a myriad of factors influence the performance demands of a player, such as the player's physical capacity, technical qualities, playing position, tactical role, and the style of playing, as well as ball possession of the team, quality of the opponent, importance of the game, seasonal period, playing surface, and environmental factors.

In recent years, there has been a surge in knowledge regarding movement patterns and the activity profiles of professional soccer players. This trend stems from the need to maximise performance (Milanović et al., 2017). Accordingly, the demands of a soccer player during match-play can be determined from match analysis and physiological measurements during a game. Due to advances in sport technology, the demands of 90-min of match-play are now well characterised. Overall, elite male outfield soccer players typically run between 9 and 14 km during a 90-min game, the majority of this distance is covered by walking and low-intensity running (Bangsbo et al., 2006; Krustrup et al., 2005; Di Mascio and Bradley, 2013; Sarmento et al., 2014). However, 22-24% of total match distance is run at speeds higher than 15 km/h (high intensity threshold), 8-9% at higher than 20 km/h (very high intensity threshold), and 2-3% at higher than 25 km/h (sprinting threshold) (Rampinini et al., 2007; Gualtieri et al., 2023). Moreover, the data on high-intensity running does not include a number of energy-intensive activities such as short accelerations, turns, actions with the balls, tackles, and jumps. For example, most maximal accelerations do not result in speeds associated with high intensity running but are still metabolically demanding. Hence, it has been stated that players complete > 700 turns, 30-40 tackles and jumps, and 120-250 brief intense muscle contractions over the course of a 90-min game (Bloomfield et al., 2007).

Sprints and high intensity running during match-play are critical in terms of soccer performance. In addition, available data suggest a 28% difference in high intensity exercise running distance between elite and amateur players (Riboli et al., 2020). This statement is consistent with Molinos-Domene (2013) that demonstrated an additional 58% more sprints performed by elite soccer players. Comparing the physiological demands among players in the Danish league, Castillo et al.'s (2021) examination of the implication of small-sided game models on physiological and physical needs among players furthers Molino-Domene's (2013) argument by stating that teams in the top half of the league had a higher high speed running distance (30-40%) in comparison to the teams in the lower half. These statistics imply that the increased high speed sprinting, the better the performance of the teams and players. However, it is intuitive that this statement only applies in matches played against opponents of similar ability.

Soccer is an intermittent sport that places significant demand on both the aerobic and anaerobic systems in order to perform at the highest level (Iaia et al., 2009). These energy systems are highly challenged with average peak heart rates of ~85% and ~98% of maximal values, respectively. Such data suggests that the average oxygen uptake is around ~70% $\dot{V}O2$ max with approximately ~90% of match-play performed at low to moderate intensity (Bangsbo, 2014). The consensus is that aerobic metabolism clearly provides the greatest energy supply during a game as the estimated oxygen consumption accounts for approximately 90% of total energy required from match activity (Bangsbo et al., 2006; Bangsbo, 2014). The aerobic system provides most of the energy, with a focus being on recovery between bouts of high intensity activity (Krustrup et al., 2013). High intensity activities in soccer, such as accelerations and jumping, require use of energy from the anaerobic system (Bangsbo, 2014). These high intensity actions often lead to crucial moments of match-play, such as straight-line sprinting within goal scoring situations (Faude et al., 2012). Although the anaerobic system provides a minor contribution to energy supply over the duration of a 90-min match, it still needs to be highly taxed during specific periods of a game (Dolci et al., 2020). This is evident within the movement demands and profile of soccer, involving periods of high intensity activities, mainly relying on the glycolytic pathway, and brief explosive movements, mainly relying on phosphocreatine energy pathway (Gastin, 2001; Turner and Stewart, 2014). Therefore, the role of anaerobic system in soccer should not be undervalued and should remain targeted for training prescription by coaches (Dolci et al., 2020).

As previously stated, fatigue occurs at different stages throughout a match, with temporary fatigue present after intense periods of the game, to more prolonged fatigue identified towards the end of match play (Mohr et al., 2003), Although there are many potential mechanisms responsible for fatigue, two of the main explanations which have a nutritional implication may be attributed to a depletion of muscle glycogen and to hypoglycaemia (Mujika and Burke, 2010). The link between the degree of fatigue at the end of match-play and depleted muscle glycogen stores is based on early observations of association between initial muscle glycogen content and physical game performance (Saltin, 1973). Other researchers have demonstrated a substantial game-induced muscle glycogen depletion, especially at the single-fibre level, with two thirds to three-quarters of individual type 1 and 2 fibres depleted of glycogen immediately post-match play (Krustrup et al., 2006). Moreover, statistical associations between the degree of muscle glycogen degradation and the decrease in post-game exercise tolerance have accompanied these findings (Mohr et al., 2022). Moreover, multiple studies that have manipulated dietary carbohydrate intake prior to games, or game simulations, have demonstrated an indirect relationship between muscle glycogen content and performance (de Sousa et al., 2021).

Furthermore, a strong correlation has been observed between muscle oxidative capacity and the ability to maintain sprint performance toward the end of a game (Mohr et al., 2016). These data indicate a performance-enhancing effect of increased capacity for fat oxidation is likely due to muscle glycogen sparing (Mohr et al., 2022). Indeed, reductions in blood glucose concentrations due to inadequate carbohydrate availability invariably leads to hypoglycaemia, and once liver glycogen is depleted there is an inability to produce sufficient glucose for circulation from the lower rates of gluconeogenesis (Hulton et al., 2022). As the brain primarily uses glucose from the blood as an energy source, hypoglycaemia has been considered a factor contributing to central fatigue (Christensen and Hansen, 1939). Consequently, hypoglycaemia may be identified as a nutritional factor that may underpin the onset of fatigue or suboptimal performance in soccer (Mujika and Burke, 2010).

2.2.1 Considerations about growth and development

It is important to acknowledge that young male athletes encounter distinct challenges related to the variability in the velocity of physical growth (Naughton et al, 2000; Malina et al., 2000). While some may share similar maturation levels, their chronological ages can vary, a discrepancy particularly noticeable between the ages of 13 and 16 (Malina et al., 2004). The transitional phases encompass childhood, pre-puberty (the phase preceding puberty), adolescence (marked by physiological changes in the musculoskeletal, cardiorespiratory, and reproductive systems), and adulthood (Naughton et al., 2000). Moreover, it is widely recognised that adolescents should not be regarded or treated as miniature adults (Castagna et al., 2003; Teplan et al., 2012). Consequently, differences in physical performance can be anticipated among young players of varying maturation levels as well as between young and adult players (Atan et al., 2014).

Comprehending the age-related physical requirements is vital for understanding individual needs reflective of in-game demands (Mendez-Villanueva et al., 2013). Moreover, there persists a lack of consensus in analysing youth soccer matches, with many studies relying on findings from adult research. However, it is crucial to recognise that young soccer players cannot simply be viewed as scaled-down versions of adults; they undergo distinct physical and physiological processes that influence their soccer performance (Rosenbloom et al., 2006). These distinctions stem from factors such as lower aerobic and anaerobic capacity, reduced glycogen reserves, less advanced thermoregulatory mechanisms, and varying levels of maturation (Meylan et al., 2010; Rowland, 2007). Consequently, it is anticipated that the performances of young players will differ from those of their adult counterparts.

The intermittent nature of soccer demands a robust aerobic capacity to sustain activity throughout extended periods of game-play (Randers et al., 2010). Existing literature suggests that young soccer players typically cover a range of 5 to 8 kilometres per match, which is 3 to 4 kilometres less than their adult counterparts (Mohr et al., 2005; Capranica et al., 2001; Carling et al., 2009; Rebelo et al., 2014). The distinguishing factors between young and adult players appear to lie in differences in aerobic capacity values and limited glycogen reserves. In adults, higher aerobic capacity signifies superior cardiorespiratory fitness levels, enabling sustained performance during soccer matches (Bangsbo et al., 2006). This correlation between aerobic power and metrics like distance covered, competitive ranking, and quality of play is well-documented in adult match-play (Krustrup et al., 2003). Moreover, enhancements in aerobic performance correlate positively with time spent in high-intensity activity, number of sprints, ball touches, and improved recovery during intense activity (Impellizieri et al., 2005).

Young individuals typically exhibit lower aerobic capacity due to factors like smaller heart size, resulting in lower maximal cardiac output compared to adults (Stolen et al., 2005). Currently, there is insufficient evidence to establish a relationship between aerobic capacity and total distance covered among young players, and little is known about their 'trainability' (Naughton et al., 2000). Studies have suggested that sexual maturity significantly influences intermittent shuttle run performance, with improvements in aerobic capacity progressing during the maturational process. However, post-maturation, aerobic capacity improvements may rely more heavily on training interventions (Malina et al., 2004).

A decline in distance covered, ranging from 5-18%, is commonly observed in the second half compared to the first half across different playing positions and age groups in youth soccer (Di Salvo et al., 2009). Fatigue in adult soccer players has been linked to glycogen depletion and reduced distances covered towards the end of matches (Mohr et al., 2005). Young athletes possess glycogen stores only about 50-60% of those in adults (Alvarado, 2005), resulting in faster depletion rates (Boisseau and Delamarche, 2000) and potentially smaller distances covered during match play. However, research on this issue specifically for young players during matches is lacking due to ethical concerns regarding invasive procedures like blood sampling and muscle biopsies (Malina et al., 2004). Consequently, differences in energy metabolism between children and adults during exercise remain incompletely understood.

Additionally, anaerobic power in young players is approximately 50% less than in adults (Nikolaidis, 2011), leading to limited ATP supply during high-intensity exercise and reduced glycolysis due to lower phosphofructokinase (PFK) activity and immature muscle fibre distribution (Malina et al., 2004). Consequently, it can anticipated that young players exhibit less sprinting, slower speed, or reduced distance covered in high-intensity activities due to their immature anaerobic capacity (Alvarado, 2005). However, anaerobic capacity typically improves progressively with age, primarily due to increases in body size, muscle mass, and enzymatic activity (Malina et al., 2004). Nonetheless, it has been consistently reported that young players demonstrate the ability to maintain high-intensity running performance in both halves of matches (Rebelo et al., 2014; Castagna et al., 2010; Di Salvo et al., 2009).

Another consideration is the anatomical and physiological traits of adolescents, which have been noted to hinder thermoregulatory responses. These factors encompass reduced sweating capacity, a high ratio of body surface area to mass, and lower cardiac output (Rowland, 2007). Throughout extended periods of soccer match participation, young players may encounter challenges relative to adults in terms of thermoregulation. Therefore, meticulous attention is warranted in planning and executing sports activities for adolescents. Consequently, it is advised that young players hydrate themselves whenever opportunities arise during soccer matches (Alvarado, 2005).

As previously mentioned, disparities in soccer performance can stem from differences in maturity (Meylan el al., 2010). Research indicates that elite players tend to exhibit greater maturity compared to non-elite youth players when examining physical and physiological characteristics (Meylan et al., 2010). Elite players consistently demonstrate taller stature, leaner physique, enhanced power, and greater aerobic capacity in comparison to their non-elite counterparts (Meylan et al., 2010). These advantages are attributed to maturity-related developments in body size, strength, speed, and endurance (Rowland, 2007). These findings align with Stroyer et al. (2004), who noted significant disparities in anthropometric measurements and $\dot{V}O2max$ values between elite players at the conclusion of puberty and players at the onset of puberty. Consequently, it is reasonable to anticipate that elite young players exhibit greater physical and physiological advancements at the elite level and are likely to display superior match-running performances (Atan et al., 2014).

In summary, it can be inferred that young players cover shorter distances, engage in fewer and less frequent high-intensity activities during match play compared to adults, owing to physiological variances, maturity status, and the shorter duration of playing time during games. Existing research has inadequately addressed the distinctions between chronological versus relative age, elite versus non-elite youth players, and children versus adults in match analysis. Further exploration is necessary to gain a deeper understanding of match-play demands in young soccer players (Atan el al., 2014). Moreover, there is a lack of data concerning players aged 16 to 20 years old. This age range encompasses individuals who may be experiencing significant growth and development, while others may have already reached full maturity.

2.2.2 Carbohydrates and soccer performance

As previously stated, soccer match-play involves players engaging in a myriad of activities such as walking, jogging, sprinting, changing direction, jumping, striking the ball, among others (Collins et al., 2021). Outfield soccer players maintain an average heart rate of 85% of maximum and the average relative intensity is at 70% of maximal oxygen uptake over the duration of a game (Bangsbo et al., 2006). This exercise stimulus is equivalent to an energy expenditure of which ~1300-1600 kcal (Ferrauti et al., 2006), and thus carbohydrates contribute ~60-70% of the total energy supply (Anderson et al., 2017). Since carbohydrates represent the primary fuel for muscle during high-intensity exercise it is a key macronutrient when preparing for match-play (Collins et al., 2021). It has been shown that carbohydrate intake strategies are associated with improved performance during match-play (Bangsbo et al., 1992), small-sided games (Balsom et al., 1999), and simulated soccer activities (Souglis et al., 2013; Russell et al., 2012; Harper et al., 2017). Therefore, the main goal to focus on for pre-match day or match-day is to ensure that both muscle and liver glycogen stores are optimally increased in preparation for match-day.

Although the glycogen cost of elite soccer match-play is still unknown, early investigations demonstrated that players starting a soccer match with low glycogen availability went on to fully deplete their stores by the end of the match (Saltin, 1973). It was observed that glycogen stores were nearly depleted at half-time, leading to early fatigue characterised by a significant increase in time spent in low intensity movement such as walking (50% vs. 27% of total distance), and an inability to complete sprinting activities (15% vs 24% of total distance) when compared to those who started the match with normal glycogen availability. Moreover, data from a friendly match from a lower division Danish male players (Krustrup et al., 2006) demonstrated that although total muscle glycogen was not depleted, a reduction from 449±23 mmol/kg/dry weight to 225±23 mmol/kg/dry weight was observed and accounted for approximately 47% of muscle fibres depleted or almost depleted of glycogen. Interestingly, following fibre type analysis, it was shown that depletion was apparent within type IIa and type IIx fibres that are related with high intensity actions. It was then suggested that this could explain the significant reduction in sprinting performance observed during the match (Hulton et al., 2022). It has been suggested that the prevalence in glycogen depletion may be higher in elite players because they perform more high intensity activities than those performed in sub-elite standard soccer (Mohr et al., 2005). These studies and others measuring glycogen use in soccer match-play (Jacobs et al., 1982) or simulated game activity (Nicholas et al., 1999; Foskett et al., 2008) emphasise the need for optimal glycogen storage prior to match-day.

Eccentric activities (such as those involved in soccer) result in a reduced capacity to replenish muscle glycogen stores (Bangsbo et al., 2006; Zehnder et al., 2004). Muscle glycogen stores have been observed to have been recovered by around 80% following a soccer match in elite players after 3 days of high carbohydrate intake (Bagsbo et al., 2006), and this confirms the 50% reduction in muscle glycogen 48-h after a competitive match (Jacobs et al., 1982). Hence, in order to elevate muscle and liver glycogen stores, it has been suggested that during the day prior to a game carbohydrate intake should be at least 6-8 g/kg body mass (Hulton et al., 2022).

On match-day, carbohydrate intake is one of the most important considerations. The overall guidance is for players to consume 6-8 g/kg body mass (Souglis et al., 2013). Specifically, a high carbohydrate pre-match meal should be ingested 3-4 hours before kick-off, and it should contain 1-3 g/kg body mass in order to ensure that players begin the match with adequate glycogen stores (Anderson et al., 2017; Collins et al., 2021). The pre-match meal is of particular importance for the promotion of liver glycogen stores, given that such stores have been observed to decrease significantly following an overnight fast, with total depletion expected after 36-h. This means that approximately 33% of liver glycogen could be lost during an overnight fast of 12-hour, and up to 50% if no carbohydrate is consumed until kick-off (Nilsson, 1973). The benefits of high carbohydrate intake on match-day could go beyond the enhanced capacity for high intensity exercise and the delay of fatigue.

It has been suggested that these might extend to players' technical performance, since a study found an increased dribbling speed when professional youth soccer players consumed a larger breakfast (500 vs 250 kcal, with 60% of carbohydrate) 135-min before a match (Briggs et al., 2017).

A primary nutritional consideration during soccer match-play is carbohydrate ingestion. Carbohydrate ingestion during soccer-specific exercise augments plasma glucose availability and maintains rates of carbohydrate oxidation (Russell et al., 2012). It has been suggested that ingestion of carbohydrates during exercise improves exercise capacity possibly due to prevention of hypoglycaemia, maintenance of high carbohydrate oxidation rates, glycogen sparing, and effects on the central nervous system that ultimately delay the onset of fatigue (Holway and Spriet, 2011; Phillips et al., 2011).

Most of the evidence on the effect of carbohydrate intake on soccer-specific exercise performance have been positive, with greater distance covered following soccer-specific protocols while ingesting carbohydrates versus placebo (Foskett et al., 2008; Phillips et al., 2010; Alghannam, 2011; Phillips et al., 2012). However, mixed findings have been presented regarding the impact of carbohydrate intake on sprint performance during soccer-specific activity, with some studies demonstrating small improvements in sprint time (Ali et al., 2007; Kingsley et al., 2014) and some reporting no benefit (Phillips et al., 2010; Phillips et al., 2012), nevertheless, the differences in outcomes may be due to age group differences as indicated earlier in this chapter. Performance benefits in protocols simulating soccer match-play have been reported when carbohydrate is consumed during exercise at rates of ~30-60 g/hour (Collins et al, 2021). However, current practices of elite players appear to be at the lower end of the recommended intakes. Players in the English Premier League reported mean carbohydrate intakes of 32 g/hour during a match. This has been attributed to match rules which limit intake to warm-up and half-time periods, and to the potential gastrointestinal issues during games (Anderson et al., 2017).

2.3 Technical demands of soccer match-play

Technical performance (skill performance) is a critical factor associated with sport performance. The importance of successful technical proficiency is a discriminating factor for match performance as executing a successful pass, dribbling with control and speed, and shooting accurately, will likely contribute to the outcome of a match (Lago-Peñas et al., 2010). For instance, teams finishing highest in the English Premier League completed the most successful passes (Bradley et al., 2013), and number of passes and pass completion rates have been associated with team success in other leagues (Lago-Peñas and Lago-Ballesteros, 2011; Castellano et al., 2012., Lago-Ballesteros et al., 2012; Collet, 2013). However, extrinsic factors such as league level, match location, quality of the opposition, score line, and ball possession, could all affect technical performance (Taylor et al., 2008; Dellal et al., 2011; Lago-Peñas and Lago-Ballesteros, 2011; Bradley et al., 2013).

Accumulating evidence suggest that technical performance tends to decline in the latter stages of 90-min matches. For example, significant reductions in the number of possessions and passing were recorded when comparing the first half of English Championship matches to the second half; and the last 15-min compared to the first 15-min (Russell et al., 2013). Moreover, Carling and Dupont (2011) have encountered declines in the number of completed passes and ball possessions in the last 5-min compared to the first 5-min of a match by elite soccer players of a team in the highest league in France. Likewise, significant declines in involvements with the ball and the number of successful short passes in the second half compared with the first half have been observed in Italian Serie A matches (Rampinini et al., 2009).

There are three major components of soccer-skill execution that have been evaluated during actual and simulated match-play, namely dribbling, passing, and shooting. Dribbling ability is considered a discriminating factor between low- and high-level (Harper, 2016) players (Stone and Oliver, 2009; Deprez et al., 2014) but surprisingly only few studies have measured dribbling performance during matches (Harper, 2016). There is evidence indicating that there were no changes in dribbling performance between the first and the second half of 416 Italian Serie A matches (Rampinini et al., 2009), likewise, studies have shown no decrements in time to complete dribbling tasks or dribble accuracy when evaluating performance during 90-min of soccer-specific exercise (Currell et al., 2009; Russell et al., 2011a).

Although passing performance (success, number of passes, passing sequence leading to a successful shot) is influenced by extrinsic factors including but not limited to, score line (Paixão et al., 2015), quality of opponent (Taylor et al., 2008), and playing formation among others (Bradley et al., 2011), it has been suggested that the ability to maintain possession through accurate passing discriminates between successful and unsuccessful teams (Adams et al., 2013). This notion is confirmed by the finding that passing accuracy scores can help differentiate between teams that score goals versus teams that concede goals (Rampinini et al., 2009; Taylor et al., 2008). In a study assessing passing performance that utilised the Loughborough Soccer Passing Test (LSPT) following high-intensity exercise that replicated the most intense 5-min period of match-play, passing proficiency was found to be negatively affected (Rampinini et al., 2008). However, other studies that also used the LSPT did not find changes in passing performance during 90-min of soccer-specific exercise (Ali et al., 2007; Ali and Williams, 2009). There is also evidence of perturbations in passing performance during match-play are evidenced by a reduced number of passes during the second half of matches (Rampinini et al., 2009) and during simulations of soccer-match play as shown by a decline in passing performance assessed during a reliable passing test (Russell et al., 2010).

It has been proposed that the ability to shoot and thus score goals is the most important and valued skill in soccer (Ali, 2011). There is conflicting data regarding effects of fatigue on shooting performance, with some studies showing reductions in shot speed and accuracy at 90-min compared to pre-exercise during simulated match-play (Ali et al., 2007; Russell et al., 2011a). Other studies show no changes in total number of shots, shot accuracy, and shot speed over 90-min of simulated match-play (Bendiksen et al., 2012; Delextrat et al., 2013) or in actual match-play (Rampinini et al., 2009).

However, all the studies mentioned above have utilised different shooting assessment protocols, this could be a reason for the discrepancy in the results.

2.4 Protocols simulating soccer match-play

Protocols simulating soccer match-play have been developed with the aim of replicating physical and technical patterns of actual match-play. One of the most popular protocols is the Loughborough Intermittent Soccer Test (LIST) which was developed by Nicholas et al. (1995) and further adapted to replicate match-play. The LIST consists of 15-min blocks of exercise, interspersed with 4-min rest periods; each block includes repeated cycles of walking, jogging, and sprinting. However, this protocol does not include a 15-min half time period and does not contain technical actions (Nicholas et al., 2000). Although several studies have incorporated soccer-specific skills testing within the LIST execution (Ali, 2011), these modified versions of the LIST lack some ecological validity, and according to Russell et al. (2011b) only measuring the time to complete a task is an incomplete representation of the skill executed.

Russell et al. (2011b) adapted the LIST to create a more ecologically valid version, with the inclusion of a half-time period and technical soccer-specific actions with applicable outcome measures. The Soccer Match Simulation (SMS) consists of two 45-min halves of soccer-specific activity separated by a 15-min passive recovery period (half-time). The protocol incorporates frequent changes of direction, lateral and backwards movements in addition to dribbling, passing, and shooting tasks, with measures of speed, accuracy, and success as measures of performance. The soccer-specific skills tested within the SMS have been shown to be reliable and valid as they were evaluated by Russell et al. (2010) in isolation before the development of the protocol. The SMS protocol of Russell et al (2011b) has been used to assess performance, physiological outcomes (Russell et al., 2011a), and the impact of nutritional interventions (Russell et al., 2012; Kingsley et al., 2014; Harper et al., 2017).

2.5 Nutrition and motor skill performance

Understanding the role of diet in modulating cognitive function is fundamental to team sport athletes because performance is at least partially dependent on motor control, coordination, decision-making, timing, and other cognitive tasks (Baker et al., 2014). Moreover, there is a large requirement for efficient technical performance in soccer which requires optimal motor control and coordination between the brain, nervous system, and the exercising musculature (Badin et al., 2016). Although many dietary constituents have been reported to enhance acute motor skill and/or cognitive performance, carbohydrate and caffeine have the greatest number of published reports supporting their ability to improve acute motor skill and cognitive performance in athletes (Baker et al., 2014).

2.5.1 Carbohydrates

The overall evidence indicates that the ingestion of carbohydrate before and/or during practice or competition may improve skill performance in athletes (Jeukendrup, 2014; Baker et al., 2014). It has been suggested that carbohydrate consumption enhances performance tests that simulate skills and cognitive demand of various team sports (Collardeau et al., 2001; Welsh et al., 2002; Winnick et al., 2005). Carbohydrate feedings impact the levels of brain serotonin, a neurotransmitter associated with central nervous system fatigue during exercise. Hence, it is intuitive that carbohydrate intake during exercise would decrease serotonin synthesis in the brain and limit central fatigue (Davis and Bailey, 1997). Nevertheless, the role of serotonin in central fatigue during exercise is yet to be fully understood and warrants more research (Barker et al., 2014). Alternatively, the ergogenic effect of carbohydrate on skill performance may be due to carbohydrate-sensitive receptors in the mouth which affect the central nervous system and improve central command (Jeukendrup, 2013). It has been demonstrated that regions of the brain linked with reward and motor control during exercise are activated by the oral exposure to a 6.4% glucose or maltodextrin solution (Chambers et al., 2009). Furthermore, a recent review (Pomportes and Brisswalter, 2020) concluded that carbohydrate mouth rinse has a beneficial effect in motor ability and that this might foreshadow a substantial improvement in terms of performance in a sport like soccer.

Research on the acute ingestion of carbohydrate and its effects on changes in technical performance in soccer has revealed mixed results, this could be due to a number of factors including level of player, nutritional status, and the sensitivity and validity of the tests used to measure performance (Harper et al.,2017). In addition, carbohydrate provision is more likely to help maintain technical performance when soccer players are in a fatigued state. Most studies have utilised soccer-specific protocols when investigating the efficacy of carbohydrate ingestion on skill performance. Various studies in semi-professional and professional soccer players have found improvements with carbohydrate intake versus placebo in soccer-specific skills such as shot speed, shooting, passing (Northcott et al., 1999; Ali et al., 2007; Currell et al., 2009; Russell et al., 2012), and dribbling performance (Ostojic and Mazic, 2002; Currell et al., 2009; Harper et al., 2017). However, some authors have found no beneficial effects of carbohydrate on certain indices of soccer technical performance (Zeederberg et al., 1996; Abbey and Rankin, 2009; Ali and Williams, 2009), this is most likely due to differences in protocols used for the assessment of soccer specific skills or some studies reporting data from real matches rather than from simulated match protocols.

Table 2.1. Summary of studies assessing carbohydrates and soccer skill performance.

Author, year	Title	Sample	Age	Performance assessment protocol	Outcomes
Abbey and Rankin, 2009.	Effect of ingesting a honey-sweetened beverage on soccer performance and exercise-induced cytokine response.	10 male soccer players.	23 ± 3 y.	The performance test consisted of five identical blocks approximately 15 min in duration with a 10-min “halftime.” The protocol was based on that described by Kingsley et al. (2005), with a few modifications.	Acute ingestion of honey and a carbo- hydrate sports drink before and during a soccer-simulation test did not improve performance, although honey attenuated a rise in IL-1ra.
Alghannam, 2011.	Carbohydrate–protein ingestion improves subsequent running capacity towards the end of a football-specific intermittent exercise.	6 male soccer players.	26 ± 2 y.	5 × 15-min identical intermittent-activity cycles with a half-time 15-min period between cycle 3 and 4, immediately followed by run to fatigue.	Carbohydrate-protein resulted in longer run to fatigue than did carbohydrate on its own and placebo. Ratings of perceived exertion were lower in the carbohydrate-protein

					subjects at the onset of exercise and towards the end of intermittent exercise when compared with the placebo and carbohydrate subjects. When protein was added to a CHO supplement, subsequent running capacity following limited recovery from intermittent exercise was enhanced. This improvement suggests that protein co-ingestion may exert an ergogenic benefit upon endurance capacity during intermittent activity.
Ali et al., 2007.	The influence of carbohydrate-electrolyte ingestion on soccer skill performance.	16 male soccer players.	21 ± 3 y.	Loughborough Intermittent Shuttle Test (LIST), Loughborough Soccer Passing Test (LSPT), and Loughborough Soccer Shooting Test (LSST).	Ingestion of a carbohydrate–electrolyte solution during exercise enabled subjects with compromised glycogen stores to better maintain skill and sprint performance than when ingesting fluid alone.
Ali and Williams, 2009.	Carbohydrate ingestion and soccer skill performance during prolonged intermittent exercise.	17 male soccer players.	21 ± 3 y.	Loughborough Intermittent Shuttle Test (LIST), Loughborough Soccer Passing Test (LSPT), and Loughborough Soccer Shooting Test (LSST).	Skill performance during the simulated soccer activity appeared to deteriorate in the last 15–30 min of exercise. However, providing carbohydrate during exercise showed a tendency to better maintain soccer skill performance than a taste-matched placebo.
Currell et al., 2009.	Carbohydrate ingestion improves performance of a new reliable test of soccer performance.	11 male soccer players.	21 ± 1 y.	The protocol comprised a series of ten 6-min exercise blocks. Each exercise block was separated by the performance of two of the four soccer-specific tests, immediately after which the next block was undertaken. In total the duration of the protocol was 90 min, spilt into two 45-min halves. During the exercise periods participants were required to perform a field test that mimicked the movements used during soccer match play.	There was a significant improvement in performance for dribbling, agility, and shooting when carbohydrate was ingested compared with placebo.
Harper et al., 2017.	The influence of a 12% carbohydrate-electrolyte beverage on self-paced soccer-specific exercise performance.	15 male soccer players.	22 ± 2 y.	90-min of soccer-specific exercise (modified Soccer Match Simulation; SMS (Russell, 2011)) requiring audio-	A 12% carbohydrate-electrolyte beverage increased self-paced exercise performance, and improved dribbling speed in the final 30-min

				prescribed (ten blocks) and self-paced (four blocks) activity equally split across two halves. A 15-min passive recovery period separated two 45-min halves.	of exercise compared to water and placebo.
Kingsley et al., 2014.	Effects of carbohydrate-hydration strategies on glucose metabolism, sprint performance and hydration during a soccer match simulation in recreational players.	14 recreational male soccer players.	24 ± 1 y.	Soccer Match Simulation (SMS) which includes 90 min of intermittent free-running activity and ball skills (dribbling, passing and shooting), completed in two 45-min halves separated by 15-min recovery (half-time).	Mean sprint speed was 3 ± 1% faster in the players than ingested carbohydrate-electrolyte gels when compared with placebo.
Nicholas et al., 1995.	Influence of ingesting a carbohydrate-electrolyte solution on endurance capacity during intermittent, high-intensity shuttle running.	9 trained male games players.	25 ± 1 y.	Prolonged intermittent, high-intensity shuttle running test (PIHSRT).	Drinking a carbohydrate-electrolyte solution improves endurance running capacity during prolonged intermittent exercise.
Northcott et al., 1999.	Effect of a carbohydrate solution on motor skill proficiency during simulated soccer performance. Sample: 10 college male soccer players.	10 college male soccer players.	22 ± 1 y.	Two 45 minutes halves of intermittent exercise and soccer skills (passing and shooting).	Soccer skill proficiency was significantly improved in the last 15 minutes of the simulation under the carbohydrate condition. Total distance covered also improved but there was no effect on maximal sprinting speed.
Russell et al., 2012.	Influence of carbohydrate supplementation on skill performance during a soccer match simulation. Sample: 15 professional academy soccer players.	15 professional academy soccer players.	18 ± 1 y.	Soccer Match Simulation (SMS) which includes 90 min of intermittent free-running activity and ball skills (dribbling, passing and shooting), completed in two 45-min halves separated by 15-min recovery (half-time).	Carbohydrate supplementation influenced shooting, where carbohydrate attenuated the decline in shot speed. Supplementation did not affect passing or dribbling.
Ostojic and Mazic, 2002.	Effects of a carbohydrate-electrolyte drink on specific soccer tests and performance.	22 professional soccer players.	CHO: 23 ± 2 y; Placebo: 24 ± 2 y.	A match consisted of 90 minutes of action: two 45-min periods with a short break (15 min) in between, with technical and tactical demands same as on the real competitive soccer match (running, kicking a ball, jumping, heading, throwing, goal-keeping) followed by four soccer-specific skill tests (dribbling, precision, coordination, and power).	Subjects in the carbohydrate-electrolyte trial finished the specific dribble test faster in comparison with subjects in the placebo trial. Ratings of the precision test were higher in the carbohydrate-electrolyte trial as compared to the placebo trial but there were no differences in coordination test and power test results between trials. Supplementation with carbohydrate-electrolyte solution improved soccer-specific skill performance and recovery after an on-field soccer match

					compared with ingestion of placebo.
Phillips et al., 2012.	Ingesting a 6% carbohydrate-electrolyte solution improves endurance capacity, but not sprint performance, during intermittent, high-intensity shuttle running in adolescent team games players aged 12-14 years.	7 team games platers (5 males, 2 females).	13 ± 1 y.	Four 15-min periods of part A of the Loughborough Intermittent Shuttle Test (LIST) followed by an intermittent run to exhaustion (part B).	Intermittent endurance capacity increased by 34% with ingestion of the 6% carbohydrate-electrolyte solution compared with the 10% solution. Carbohydrate concentration did not significantly influence mean 15-m sprint time.
Phillips et al., 2010.	Ingesting a 6% carbohydrate-electrolyte solution improves endurance capacity, but not sprint performance, during intermittent, high-intensity shuttle running in adolescent team games players aged 12–14 years.	15 team games platers (10 males, 5 females).	13 ± 1 y.	Four 15-min periods of part A of the Loughborough Intermittent Shuttle Test (LIST) followed by an intermittent run to exhaustion (part B).	Time to fatigue was increased by 24.4% during part B when carbohydrate was ingested with distance covered in part B also significantly greater in the carbohydrate trial. No significant between-trials differences were observed for mean 15-m sprint time or peak sprint time during part A.
Zeederberg et al., 1996.	The effect of carbohydrate ingestion on the motor skill proficiency of soccer players.	Male players of two professional soccer teams.	Under 19 years old.	Two teams played against each other on two separate occasions.	Carbohydrate ingestion did not improve tackling, heading, dribbling, or shooting ability. There are no measurable effects of carbohydrate ingestion for the motor skill proficiency of soccer players during games played in a cool environment.

2.5.2 Caffeine

Caffeine (1, 3, 7-trimethylxanthine) is a substance found naturally in coffee, chocolate, guarana, and other plants such as cassina, kola nut, and yerba mate (Baker et al., 2014). The effects of caffeine in reducing fatigue and enhancing alertness have been recognised for many centuries. These properties have been targeted by shift workers, long-haul lorry drivers, members of the military forces, athletes, and other populations who need to fight fatigue or prolong their capacity to undertake their occupational activities (Burke, 2008). Indeed, caffeine is one of the most popular supplements among athletes for its potent stimulant effects and due to its easy availability in the market in different commercial forms (energy drinks, caffeinated beverages, pills, pre-workout, and thermogenic supplements, etc.) (Mielgo-Ayuso et al., 2019).

The past 40 years has seen the publication of a substantial number of studies of caffeine supplementation and exercise or physical activity, it is probably the supplement most extensively researched for effects on sport performance (Burke, 2008). Indeed, caffeine is a widely used substance in sports with most research and subsequent meta-analyses suggesting that it is ergogenic for endurance exercise (Southward et al., 2018; Souza et al., 2017), anaerobic-based exercise (Grgic, 2018a), and strength/power activities (Grgic et al., 2018b). Likewise, there is evidence that caffeine might be ergogenic in team sport athletes (Chia et al., 2017; Salinero et al., 2018). A systematic review on the effects of caffeine on ball games suggested that caffeine may be ergogenic by sparing muscle glycogen, increasing force production, decreasing perceptions of pain, and stimulating the central nervous system, which delays fatigue and enhances performance, and that the acute ingestion of caffeine doses between 3 and 6 mg/kg of body mass improved sprint performance and vertical jump in both net-barrier and invasion games without any major side effects (Chia et al., 2017). Also, team-sports performance could be positively affected by acute ingestion of caffeine as it has an ergogenic effect on several performance variables like jumping height, sprint speed, high-speed running distance covered, endurance, and agility during sport-specific testing and matches (Salinero et al., 2018). Therefore, one might presume that caffeine is also ergogenic in soccer. In a recent consensus on nutrition in elite soccer, caffeine is listed as one of the supplements that are potentially useful within this sport (Collins et al., 2021). Even though there is a highly individual response to caffeine, it has been shown that 3-6 mg/kg consumed ~60-min prior to exercise, or lower doses of < 3 mg/kg or ~200 mg provided both at half-time, could reduce perception of fatigue, enhance endurance, repeated sprint performance, skill and fine motor control, and improve cognitive function in soccer players (Collins et al., 2021).

Research indicates that caffeine may confer benefits even among individuals typically categorised as non-responders based on their physiological reactions post-consumption. For instance, in a study by Apostolidis et al. (2019), soccer players were classified as 'responders' or 'non-responders' depending on their post-caffeine intake biological responses, including blood pressure, plasma glycerol, free fatty acids, and adrenaline levels, which typically spike following caffeine ingestion. Participants received either caffeine (6 mg/kg) or a placebo before engaging in simulated matches, during which various performance metrics such as time to exhaustion and jump ability were evaluated. The findings revealed that caffeine supplementation enhanced performance metrics compared to the placebo, irrespective of whether individuals were classified as 'responders' or 'non-responders', although it's noteworthy that 'responders' reported lower perceived exertion.

Genetics, particularly variations in the CYP1A and ADORA2A genes, are suggested to influence individual responses to caffeine consumption. For instance, individuals categorised as rapid or poor metabolisers based on the CYP1A gene exhibit differences in caffeine metabolism, with poor metabolisers experiencing prolonged caffeine presence in the body, thus increasing the likelihood of adverse effects, including heightened cardiovascular risks, especially with high doses or combinations with other stimulants like taurine (Cornelis et al., 2006). Conversely, rapid metabolisers tend to exhibit positive responses even to higher caffeine doses. Recent findings by Grgic et al. (2021) indicate that regardless of metaboliser status (with focus on the CYP1A9 gene), most individuals derive similar benefits from caffeine supplementation (3 mg/kg) in terms of strength training power, jump ability, and performance in a Wingate test. Furthermore, a study by Grgic et al. (2020) suggests that a significant proportion (at least 84%) of individuals identified as 'non-responders' to caffeine based on the ADORA2A gene exhibit improved performance in tests of jump ability, strength, and anaerobic capacity following caffeine intake (3 mg/kg).

Although physical characteristics are crucial for successful soccer match-play, cognitive functioning, and skill execution and performance represent the major difference between elite and recreational soccer players (Williams, 2000). This observation could be largely attributed to elite players having an enhanced selective attention (Fontani et al., 2006; Kioumourtzoglou et al., 1998), and to a better ability to recognise and perform soccer-specific skills (Williams et al., 2006; Reilly et al., 2000). Fatigue-associated lapses in concentration, decision-making, and skill performance are related to conceding goals in the latter stages of match-play during a soccer game (Reilly, 1997).

Therefore, it could be suggested that the beneficial properties of caffeine supplementation for team sport athletes may not be to increase endurance performance per se, but rather to attenuate any fatigue-related decrements in skilful performance, concentration, or cognitive function (Foskett et al., 2009).

The effect of caffeine on soccer-related measures of soccer skill performance during simulated sport activity was tested by Foskett et al (2009). In their study with 12 first division football players (age: 23.8±4.5 y) it was observed that the consumption of 6 mg/kg of caffeine before exercise increased passing accuracy, and significantly less penalty time was accrued, during two validated tests to assess soccer skill performance (intermittent shuttle-running protocol and Loughborough Soccer Passing Test; LSPT). In addition, their investigation found that caffeine improved the functional power of the lower limb muscles, as measured by a vertical jump, with no detrimental effect on other physiological or performance parameters. Therefore, the authors concluded that caffeine before match-play may be beneficial for skill performance of soccer players. In another study (Jordan et al., 2014) 17 soccer players from the elite youth category (age: 14.1±0.5 y) performed an agility test (reactive agility test) validated for soccer. The authors indicated, based on the results of their investigation that the intake of 6 mg/kg of caffeine 60 min before the test significantly improved the reaction time of the players in their non-dominant leg. Therefore, based on the existing evidence, it appears that acute moderate dose caffeine intake before exercise has the capacity to improve several soccer-related abilities and skills such as vertical jump height, repeated sprint ability, running distances during a game, and passing accuracy (Mielgo-Ayuso et al., 2019). However, more research is needed to determine the optimal dose and timing of caffeine intake for improving motor skill and cognitive performance in soccer players (Baker et al., 2014).

2.6 Polyphenols

Polyphenols are ubiquitous within plants, where they are produced as secondary metabolites and involved in a diverse range of critical processes including growth, pigmentation, pollination and resistance to pathogens and environmental stressors (Duthie et al., 2003). Polyphenols have a common chemical structure with two or more hydroxyl groups attached to one or more benzene rings (Bowtell et al., 2019). Currently, there are over 8000 polyphenols identified that are distinguished into four main groups: flavonoids, stilbenes, lignans and phenolic acids (Somerville et al., 2017). The taste and colour characteristics of fruits and vegetables are strongly influenced by the polyphenol content. Both the quantity and variety of polyphenols present are determined by the plant species, growing conditions sometimes termed terroir (sunlight, water and nutrient availability, temperature), post-harvest processing, and transport and storage conditions (Oracz et al., 2015). A summary of example compounds and dietary sources of polyphenols are listed in Table 2.2.

Polyphenols possess radical scavenging properties related to their chemical structure (Bors et al., 2001) and are also metal chelators (Morel et al., 1994). Phenolics increase endogenous antioxidant capacity through signalling via the nuclear factor-like 2 (Nrf2)/antioxidant response element (ARE) pathway, resulting in increased synthesis of downstream endogenous antioxidants such as superoxide dismutase, catalase and peroxiredoxin (Huang et al., 2015). However, it seems that antioxidant effects of polyphenols arise from their pro-oxidant action after in vivo exposure to reactive oxygen species (ROS). Polyphenols also possess anti-inflammatory properties and have been shown in vitro to reduce signalling via the pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and to inhibit cyclooxygenase enzymes, COX-1 and COX-2 (Esposito et al., 2014), which are also the target of nonsteroidal anti-inflammatory drugs (Bowtell et al., 2019).

There have been observations in resting human studies that have evidenced changes in plasma biomarkers of oxidative damage (Bowtell and Kelly, 2019) which supports the antioxidant and anti-inflammatory effects of fruit-derived polyphenols (Kelley et al., 2006; Karlsen et al., 2010; Kolehmainen et al., 2012; Martin et al., 2016). Also, it has been stated that acute and chronic supplementation with polyphenols improves vascular function (endothelium-dependent vasodilation), specifically nitric oxide (NO) dependent flow mediated dilatation (FMD; Bowtell et al., 2019). Hooper et al., (2012) published a meta-analysis of 42 cocoa studies and pointed out significant increases in FMD following acute (3.2%) and chronic (1.3%) supplementation, respectively. Furthermore, Rodriguez-Mateos et al., (2013) found that a range of blueberry polyphenol doses (0.3-1.8 g polyphenols) also increased FMD. Likewise, another meta-analysis based on 14 studies revealed that acute supplementation with a mixture of flavonoids increased FMD by 2.3% (based on 18 studies) and by 0.7% with chronic supplementation (Kay et al., 2012). The optimal dose identified was 500 mg/day total flavonoids or 300 mg/day of procyanidins (Bowtell and Kelly, 2019).

Table 2.2 Dietary sources of the different polyphenol families and sub-families

Polyphenol	**Example compounds**	**Dietary source**
Stilbenes	Resveratrol	Grapes
Lignans	Enterodiol	Seeds, whole grains, legumes
Phenolic acids	Cinnamic Benzoic	Caffeic acid-coffee Gallic acid-tea
Flavonoids Flavanols Flavonols Flavones Flavanones Isoflavones Anthocyanidins Proanthocyanidins Procyanidins	Epicatechin Catechins Quercitin Luteolin Naringenin and hesperetins Genistein Cyanidin, maldivin, delphinidin B-type dimers Ellagitannins Gallotannins	Cocoa Green tea Onion, apples, deep green vegetables Parsley and other herbs Citrus fruits Soy products, Cherries and berries Cocoa Pomegranate Mango

Adapted from Bowtell and Kelly, 2019.

The increase in NO bioavailability is likely achieved through a variety of mechanisms acting synergistically (Bowtell et al., 2019): to 1) activate endothelial nitric oxide synthase (Chalopin et al., 2010); 2) inhibit superoxide-producing enzymes such as NADPH oxidase (Maraldi, 2013), thus reducing depletion of NO due to peroxynitrite formation from NO and superoxide; and 3) signal through Nrf2 and increase endogenous antioxidant capacity (Ramirez-Sanchez et al., 2013). In addition to the health benefits associated with these antioxidant, anti-inflammatory and vasoactive properties of polyphenols (Bowtell and Kelly, 2019), there is a strong rationale to suggest that polyphenols may improve exercise performance and recovery from intensive exercise (Bowtell et al., 2019).

Studies investigating the performance-enhancing effects of acute polyphenol (single dose < 3h - pre-exercise) supplementation on sports performance (Roelofs et al., 2017; Trexler et al., 2014; Oh et al., 2010; Cases et al., 2017; Deley et al., 2017) have shown that the timing of consumption is a crucial factor as ergogenic effects have only been seen in those studies where polyphenols were consumed within 1 hour of exercise. Effects of intake 1 hour prior to exercise appears due to the coincidence with the peak concentration of plasma phenolic metabolites and, therefore, maximal physiological effects. It is important to note that these studies involved trained athletes, so it is not clear whether training status may also influence efficacy (Bowtell et al., 2019). The observed ergogenic effects in recreationally active subjects are likely to involve vascular mechanisms, with lowered pulse pressure, and increased brachial artery diameter and flow observed in conjunction with performance improvements (Cases et al., 2017; Roelofs et al., 2017; Trexler et al., 2014). Therefore, it has been suggested that the acute consumption of ~ 300 mg polyphenols an hour prior to exercise may enhance endurance and repeated sprint performance, most likely due to improved muscle perfusion (Bowtell et al., 2019).

Likewise, chronic polyphenol consumption seems to produce ergogenic effects for recreationally active participants (Cook et al., 2015; Perkins et al., 2015; Godwin et al., 2017; Willems et al., 2016) and, to a lesser extent, for trained athletes (Murphy et al., 2017; Allgrove et al., 2011). The most likely mechanisms appear to be reduced exposure to, or increased capacity to, detoxify reactive oxygen species (ROS) and reactive nitrogen species (RNS) or increased antioxidant enzyme activity (Bowtell et al., 2019). These responses seem to occur in parallel with enhanced vascular function, possibly resulting in improved muscle perfusion and enhanced oxygen extraction (Richards et al., 2010).

2.6.1 Coffeeberry

Due to the interest on the role of polyphenols in sports performance enhancement, there has been an emergence of research investigating the effects of different fruits on exercise performance in recent years such as pomegranate, blackcurrant, and tart cherries (Bell et al., 2014; Perkins et al., 2015; Trexler et al, 2014). In line with this, some products containing fruit (or vegetable) ingredients have been made commercially available and targeted towards athletes (e.g., tart cherry juice, blackcurrant extract, beetroot juice/shots). The fruit of coffee plants, also referred as either coffeeberry or coffeecherry (Reed et al., 2019) remains less well-studied compared to other more commonly consumed fruits. Coffeeberry contains multiple phenolic constituents (Esquivel and Jimenez, 2012), particularly chlorogenic acids (CGA) and the dominant one within the coffee fruit is 5-0-caffeoylquinic acid (Tajik et al., 2017). Evidence has demonstrated that CGA are known for their antioxidant and anti-inflammatory properties; these health benefits have lately been the focus of many epidemiologic studies. It has been proposed that this compound has benefits on type-2 diabetes mellitus, endothelial function, blood pressure, and neurodegenerative diseases, amongst others (Tajik et al., 2017).

Nutritional supplements that are high in bioavailable antioxidants are particularly popular within physically active individuals (Slater et al., 2003). It has been stated that coffeeberry phenolic acids are substantially more potent than vitamin C or vitamin E in antioxidant and free radical scavenging activity (Halvorsen et al., 2006). Although this could be of interest to athletes, the effects of coffeeberry have not been widely studied in the field of sports nutrition and performance enhancement (Ostojic et al., 2008). Ostojic et al. (2008) provided the first direct analysis of the effects of chronic coffeeberry supplementation on antioxidant status and exercise performance in college athletes and showed that the intake of 800mg/d of coffeeberry for 28 days did not significantly alter endurance and anaerobic performance indicators but had certain effects on the improved total antioxidant capacity.

It has been stated that approximately one-third of coffeeberry CGA becomes bioavailable in plasma and crosses the blood-brain barrier and acts on the central nervous system (Kim et al., 2012). CGA within coffeeberry have been shown to impact upon cognitive function and cerebral blood flow (Reed et al., 2019; Jackson et al., 2020). This makes coffeeberry a potential candidate to impact motor skill performance in soccer, much in the same way as caffeine. Two recent studies have suggested a positive impact of coffeeberry extract on cognitive function. In one study (Jackson et al, 2020), robust effects on mood and cerebral blood flow were seen for apple and coffeeberry beverages with increased subjective energetic arousal and haemodynamic responses. In the other study (Reed et al., 2019), coffeeberry extract (300mg) attenuated both increases in self-reported fatigue and decreases in self-reported alertness resulting from the completion of a series of fatiguing cognitive tasks. These findings support the interest on the impact that coffeeberry in isolation could have on sports performance, especially in those sports which contain a cognitive and motor component such as soccer.

The literature review highlights the following:

- Although excelling in physical abilities is undoubtedly important for succeeding in soccer matches, skill proficiency plays an equally crucial role. As a result, technical capability emerges as a pivotal factor in attaining success during matches.
- Fatigue significantly influences match outcomes, this is particularly evident in the final 10 minutes of 90-minute matches, where an increase in goals conceded is observed. The decrease in movement output and soccer-specific skill execution during matches is largely attributed to acute fatigue stemming from high physiological, technical, and cognitive demands.
- Skill performance, also known as technical performance, is a pivotal element linked to athletic success. The effectiveness of technical proficiency serves as a distinguishing factor for match performance, as successful execution of tasks such as accurate passing, controlled and swift dribbling, and precise shooting significantly influences match outcomes.
- Whilst various mechanisms contribute to match-induced fatigue, peripheral fatigue associated with nutritional factors may play a significant role in its development.
- Evidence suggests that carbohydrate intake may enhance performance in tasks simulating the skills and cognitive demands of team sports. It's been proposed that consuming carbohydrates affects brain serotonin levels, a neurotransmitter linked to central nervous system fatigue during exercise. Consequently, it is reasonable to assume that carbohydrate ingestion during

exercise could mitigate central fatigue. However, the precise role of serotonin in exercise-induced central fatigue remains incompletely understood and merits further investigation.

- Studies examining the immediate effects of carbohydrate ingestion on changes in technical performance in soccer have yielded mixed findings. Carbohydrate supplementation is more likely to positively impact technical performance when soccer players are fatigued. Most research in this area has employed soccer-specific protocols to evaluate the efficacy of carbohydrate intake on skill performance. Several studies involving semi-professional and professional soccer players have reported enhancements in soccer-specific skills such as shot speed, shooting accuracy, and passing accuracy with carbohydrate consumption compared to a placebo. However, certain studies have failed to observe beneficial effects of carbohydrates on specific aspects of soccer technical performance. This discrepancy is likely attributed to differences in assessment protocols for soccer-specific skills or the inclusion of data from real matches rather than simulated match scenarios.
- Evidence suggests that both short-term (acute) and long-term (chronic) intake of polyphenols can enhance vascular function, particularly endothelium-dependent vasodilation, which relies on nitric oxide (NO) for flow-mediated dilatation. The observed performance-enhancing effects of acute polyphenol supplementation likely stem from vascular mechanisms, as indicated by reductions in pulse pressure and increases in brachial artery diameter and flow, alongside improvements in performance. Consequently, it is proposed that consuming polyphenols before exercise may boost endurance and repeated sprint performance, largely attributable to enhanced muscle perfusion.
- Research indicates that approximately one-third of chlorogenic acid (CGA) contained within coffeeberry becomes bioavailable in plasma, crosses the blood-brain barrier, and affects the central nervous system. CGA found in coffeeberry has been demonstrated to influence cognitive function and cerebral blood flow, and to mitigate increases in self-reported fatigue and declines in self-reported alertness following a series of fatiguing cognitive tasks. These

findings underscore the potential impact of coffeeberry, particularly in sports involving both cognitive and motor components like soccer.

- Drawing from current trends in modern soccer, it is expected that the game will increasingly demand more physically from players in the future, amplifying fatigue towards the end of matches. Consequently, factors such as the capacity for quicker recovery between high-intensity periods, energy conservation, and sustaining skill performance throughout the entire 90 minutes are likely to gain significance. Exploring nutritional strategies, including optimising carbohydrate intake pre-match and during the game, along with employing ergogenic aids, could potentially sustain performance levels across the duration of the match.

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CHAPTER 3
INGESTING A 12% CARBOHYDRATE-ELECTROLYTE BEVERAGE BEFORE EACH HALF OF A SOCCER MATCH SIMULATION FACILITATES RETENTION OF PASSING PERFORMANCE AND IMPROVES HIGH-INTENSITY RUNNING IN ACADEMY PLAYERS

3.1 Abstract

This study investigated the influence of ingesting a 12% carbohydrate-electrolyte (CHO-E) solution providing 30 g of carbohydrate before each half of a 90-min soccer match simulation (total carbohydrate intake: 60 g) (SMS) protocol on skill performance, sprint speed and high-intensity running capacity. Eighteen elite academy (age 18±2 y) soccer players ingested two 250 mL doses (pre-exercise and at half-time) of a 12% CHO-E solution or electrolyte placebo administered in a double-blind randomised cross-over design. During an indoor (artificial grass pitch) SMS, dribbling, passing, and sprint performance were assessed, and blood was drawn for glucose and lactate analysis. High intensity running capacity was assessed following the SMS. Dribbling speed/accuracy and sprint speed remained unchanged throughout the SMS. Conversely, passing accuracy for both dominant (mean % difference (95% CI): 9 (3-15)) and non-dominant (mean % difference (95% CI): 13 (6-20)) feet was better maintained during the SMS on CHO-E ($p<0.05$), with passing speed better maintained in the non-dominant foot (mean % difference (95% CI): 5.3 (0.7 to 9.9), $p=0.032$). High-intensity running capacity was greater in CHO-E vs. placebo (mean % difference (95% CI): 13 (6 to 20), $p=0.010$). Capillary blood glucose concentration was higher in CHO-E than placebo at half-time (CHO-E: 5.8±0.5 mM vs. placebo: 4.1±0.4 mM, $p=0.001$) and following the high-intensity running capacity test (CHO-E: 4.9±0.4 mM vs. placebo: 4.3±0.4 mM, $p=0.001$). Ingesting a 12% CHO-E solution before each half of a match can aid in the maintenance of soccer-specific skill performance, particularly on the non-dominant foot, and improves subsequent high-intensity running capacity.

Keywords: sport drink; skill, exercise, metabolism, football

3.2 Introduction

Soccer is characterised by prolonged intermittent activities involving multiple sprints, high intensity actions, and technical motor skills. As a result, fatigue in soccer is a complex phenomenon underpinned by central and physiological mechanisms and is most prominent during the latter stages of a match (Mohr et al., 2005; Reilly, 1997). At the metabolic level, the decline in muscle glycogen content during a soccer match (Krustrup et al., 2006) is associated with a reduced work rate (Ostojic and Mazic, 2002). Since the brain is dependent on a supply of blood glucose (Duelli and Kuschinsky, 2001), enhancing exogenous carbohydrate availability could preserve central nervous system integrity (Meeusen and Decroix, 2018) and help to attenuate the loss of motor skill performance (Russell et al., 2012). Moreover, the benefit of carbohydrate feeding on peripheral fatigue during intermittent exercise is evidenced by the previously reported better maintenance of high intensity running capacity (McGregor et al., 1999).

Several studies have demonstrated that consuming carbohydrate beverages before and at regular intervals during sporting activities improves subsequent high-intensity exercise capacity (Phillips et al., 2012; Nicholas et al., 1995; Davis et al., 2000; Kingsley et al., 2014) and can also aid the retention of skill performance (Harper et al. 2017). This benefit of carbohydrate feeding has been demonstrated in soccer (Ali et al., 2007; Currell et al., 2009; Russell et al., 2012) and in other skill-based sports such as tennis (McRae and Galloway, 2012) and squash (Bottoms et al., 2007) where weaker shots on the backhand side were most affected. In relation to soccer, from a practical perspective, carbohydrate beverages can only be supplied to players during the warm-up, and during scheduled breaks in play (Clarke, 2008). Thus, opportunities available for soccer players to ingest sufficient carbohydrates (~30-60g/h) are limited (Ali and Williams, 2009; Cermak and van Loon, 2013).

A potential strategy to overcome the limited opportunities to consume carbohydrate during soccer match-play is to administer highly concentrated carbohydrate beverages. In this context, the negative consequences of ingesting concentrated carbohydrate solutions on gastro-intestinal comfort

(Clarke, 2008) can be partially alleviated by the ingestion of maltodextrin plus fructose combinations (O'Brien and Rowlands, 2010; Jeukendrup, 2010). Accordingly, a study simulated a real-world soccer context by providing soccer players with a highly concentrated (12%) maltodextrin:fructose formulation at the end of the warm- up and at half-time (Harper et al., 2017). Skill performance and intermittent endurance performance were improved on the 12% carbohydrate trial compared with placebo, as evidenced by the maintenance of dribbling speed, and self-paced soccer-specific exercise performance during the latter stages of the soccer match simulation (Harper et al., 2017). Crucially, players reported minimal gastro-intestinal discomfort despite the provision of a 12% concentrated carbohydrate solution.

While an excellent study, the work of Harper et al. (2017) does raise several applied questions. First, the impact of carbohydrate ingestion *per se* was not isolated since players were also dehydrated by ~2% body mass during both trials. Second, participants ingested only a small, standardised breakfast ~135-min before exercise, and performed the match simulation in the morning rather than in the afternoon when professional academy team games or senior fixtures are normally scheduled in Europe. Third, participants were University standard soccer players rather than young professionals and no assessment of skill performance was performed on dominant and non-dominant feet. To follow-up the preliminary work of Harper et al., (2017) the present study set out to control for many of these additional factors i.e., maintain body mass loss within 1%, adopt pre-match feeding guidelines prior to an afternoon kick-off, assess outcomes in professional academy players, and distinguish between potential effects in dominant and non-dominant feet. Therefore, the primary aim of the present investigation was to provide further practical insight into the influence of ingesting a 12% carbohydrate-electrolyte beverage on soccer skill performance and high-intensity running capacity in professional academy soccer players. It was hypothesised that ingesting 250 mL of a 12% carbohydrate-electrolyte beverage before kick-off and during the half-time period, versus the ingestion of an equivalent volume of a placebo-electrolyte beverage, would improve the retention of soccer-specific skills (dribbling speed and accuracy; passing speed and accuracy), sprint speed, and anaerobic endurance running capacity during and after a 90-min soccer match simulation (SMS) conducted in a cool environment.

3.3 Methods

3.3.1 Participants

Eighteen male well-trained soccer players (7 midfielders, 6 defenders, and 5 strikers) were recruited from local football academies to participate in this investigation. *A priori*, a power calculation was conducted (GPower version 3 software) for appropriate sample size based on previously published data (Harper et al., 2017). This calculation revealed that 14 participants (using a crossover design) are required for 80% power with a mean difference in skill performance score of 0.8×SD and significance set at $p<0.05$. All players had 5 or more years of playing experience, had been training consistently for one year or more and were free from injury at the time of the recruitment and testing (age: 18±2 y, body mass: 73.4±6.0 kg, stature: 177.7±4.8 cm, body mass index: 23.1±1.0 kg/m^2, estimated $\dot{V}O2$ max: 55.9±1.5 mL/kg). The experimental procedures were approved by the University of Stirling Research Ethics Committee.

3.3.2 Study Design

Players attended two preliminary study visits ($\dot{V}O2$ max estimation and full trial familiarisation) before undertaking two main trials (carbohydrate-electrolyte; CHO-E; and placebo) with beverages administered in a double-blind randomised, crossover manner. All visits were separated by 7-14 days. A shuttle running test protocol was used to estimate $\dot{V}O2$ max (Yo-Yo IR1; Bangsbo et al., 2008), with the level obtained used to determine the speed corresponding to 40%, 50%, 85% and 90% of $\dot{V}O2$ max for use during and after the soccer match simulation (SMS) protocol. Players followed 48-h habitual diets (avoiding caffeine and alcohol) and recorded food consumed (analysed retrospectively; Nutritics, Nutritics Ltd., Dublin, Ireland) before the familiarisation visit. The pre-familiarisation trial diet was replicated for both main trials. Players refrained from strenuous exercise 24-h before the familiarisation trial and main trial days. On the familiarisation visit, players undertook a 10-min warm-up (incorporating light aerobic activity, dynamic stretches, 20-m sprints), before completing the 90-min SMS protocol (Russell et al., 2011) followed by a high intensity (90% $\dot{V}O2$ max) running test to exhaustion. All testing sessions were performed on an indoor artificial grass pitch.

3.3.3 Experimental trials

Experimental trials were scheduled to start in the afternoon to reflect times at which this cohort typically engages in soccer matches (Figure **3.1.A**). At the training ground, researchers provided players with a standardised breakfast (2 eggs, 2 slices of bread, 1 medium-sized banana providing 423 kcal, 46g carbohydrate, 26g protein, 14g fat) and then a pre-trial standardised meal 2-h before beginning the main trials providing 1.9±0,4 MJ and 2g carbohydrate per kg of body mass (pasta in a tomato sauce) plus 500 mL of water.

Soccer players performed the 90-min SMS that incorporates six blocks of activity equally split across two 45-min periods (**Figure 3.1.B**). To assess dribbling performance, players dribbled a ball between 6 cones (3-m apart) towards a camera as fast and precisely as possible. For the sprint assessment, players ran as fast as possible through timing gates (Brower, USA) placed 15-m apart, with a 1-m run-in. At the end of each of the 12 blocks of activity, players directed alternate passes towards target zones (2.0-m × 1.0-m) placed to the left and right at a distance of 7.9 m. The passing target was divided into three equally sized areas, with the centre area worth 10 points and the two areas at either side worth 5 points. Passes that missed the areas on the target were scored as zero. The bouts of passing consisted of 8 passes (4 with the dominant foot followed by 4 with the non-dominant foot).

Digitisation (Kinovea version 0.8.15; Kinovea Org., France) yielded dribbling speed, dribbling accuracy, passing accuracy, and passing speed. Dribbling, sprint, and passing performance were expressed as means for each 15-min of the protocol. After the SMS protocol was completed, players performed a fixed high intensity running capacity test to the point of volitional fatigue. The test consisted of 20-m shuttles at a speed corresponding to 90% of their $\dot{V}O2$ max until volitional exhaustion (the duration of the test ranged from 1 min 21 sec to 1 min 42 sec). Exhaustion was defined as the inability to maintain the required pace for two consecutive shuttles. Running capacity performance was expressed as the total running distance completed during the test. Following completion of exercise, body mass loss was calculated from the difference between pre- and post-exercise body mass (SECA Quadra 808, Hamburg, Germany), corrected for fluid intake and urine output. Abdominal discomfort ratings (nausea, fullness, bloatedness) were assessed routinely by asking players to place a vertical line on a 100-mm visual analogue scale (VAS). Rate of perceived exertion (RPE) (Borg, 1973) and heart rate were monitored at various times over the duration of the trials.

Beverages (250 mL) were ingested on two separate occasions. First, 15-min before beginning the SMS and second, immediately upon completing the first half (15 min before beginning the second half). The CHO-E and placebo beverages were ready-to-drink formulations (PepsiCo International Ltd., USA) matched for flavour, colour and texture containing comparable amounts of Na^{+} (41 mg/100 mL). The CHO-E drink was a 12% solution (blend of maltodextrin and sucrose, 60 g/500 mL, Gatorade Football Energy, PepsiCo Inc) delivering 40 g of carbohydrate per hour during the SMS. The placebo drink was non-caloric and taste-matched using artificial sweeteners. Although water was made available *ad libitum* no player consumed any water during either trial.

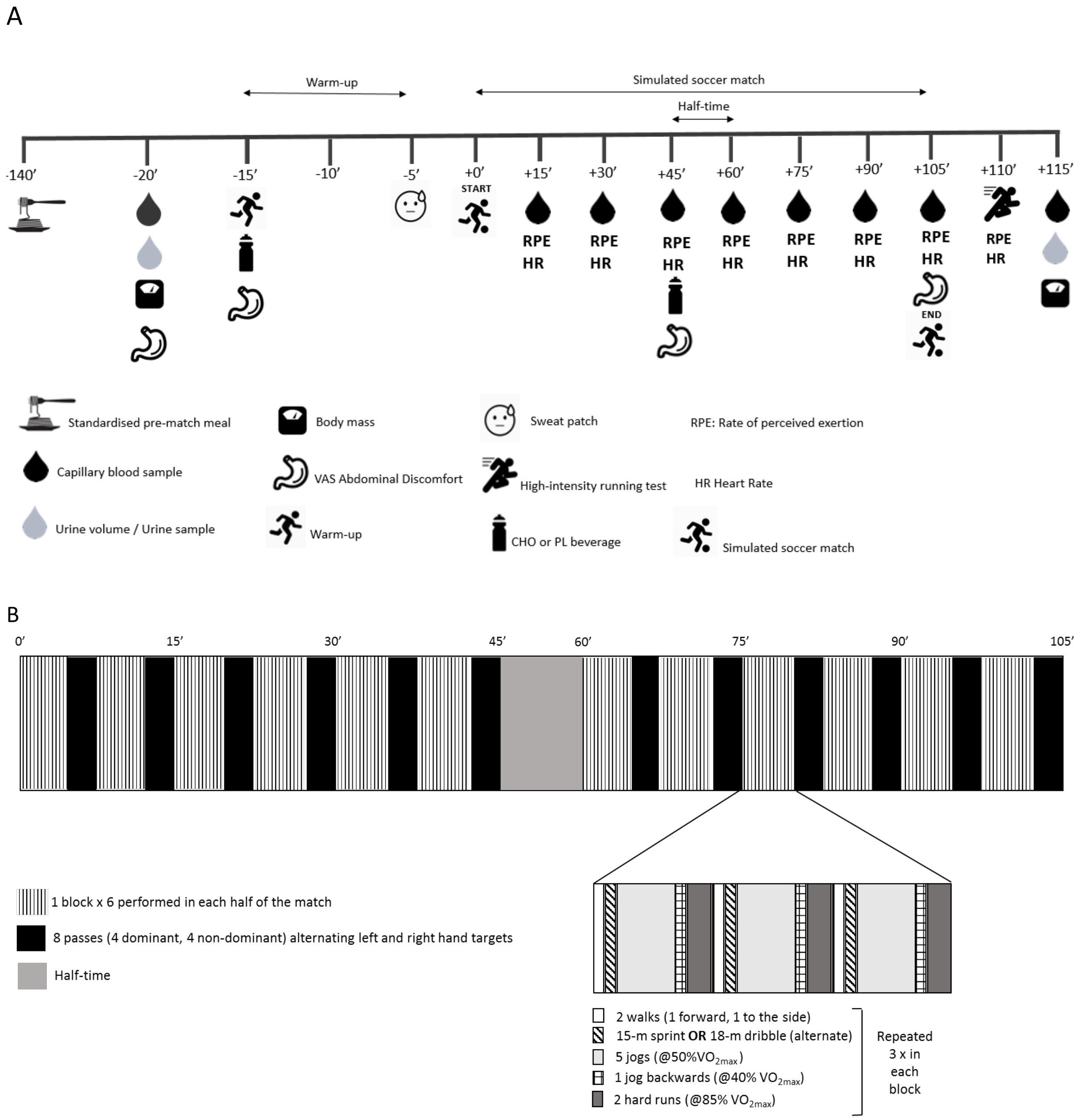

Figure 3.1. Schematic diagram of study protocol (A) and outline of the Soccer Match Simulation protocol (B).

3.3.4 Analytical Procedures

Capillary blood samples (30-µl aliquots) were dispensed into 300 µl of ice cold 0.3-N perchloric acid and shaken vigorously before being placed in an ice bath. On completion of the trial, samples were centrifuged and stored at –70°C until analysis. Analysis of blood glucose and lactate concentrations were completed using the method of Maughan (1982). Urine samples were collected into a 0.5-L plastic container and total mass (to the nearest 0.1 g) assessed to determine urine volume. A 5-mL aliquot was then dispensed into a plain screw- capped tube and stored at 4°C until analysis of osmolality on the evening of the trial days (freezing point depression method, Löser Micro-Digital Osmometer M15).

3.3.5 Statistical Analysis

Data are presented as mean (SD) with 95% confidence intervals for mean differences shown in the text. Statistical significance was set at $p<0.05$. Effect sizes based on using Cohen's d with threshold values for trivial, small, moderate, large, very large, and extremely large effects set at <0.2, 0.2. 0.6, 1.2, 2.0, and 4.0 (Hopkins et al., 2009) were reported along with a written description. Performance variables were analysed using two-way repeated measures analysis of variance (ANOVA) whenever data contained multiple time points. When a significant time × trial interaction was observed, post-hoc pairwise comparisons with Bonferroni confidence-interval adjustment were performed to identify at what time point(s) differences existed between conditions. If no time × trial interaction was observed, main time or trial effects were only reported. Paired samples t-tests were used to examine mean differences between trials when data were collected at one time point only (e.g., high- intensity anaerobic endurance running capacity).

3.4 Results

No significant trial order effect was observed for any performance variable, hence any differences between conditions were considered to be due to treatment effects. Two out of the 18 participants correctly identified the order of the treatments. Dietary analysis revealed no difference in nutritional intake between CHO-E (total energy: 8.9±2.2 MJ/d; carbohydrates: 290±63 g/d; proteins: 101±47 g/d; fats: 70±17 g/d) and placebo (total energy: 9.8±1.8 MJ/d; carbohydrates: 293±62 g/d; proteins: 89±27 g/d; fats: 87±27 g/d; all $p>0.05$) trials in the 48-h period before the main experimental trial days. Ambient temperature (CHO-E: 6±1 °C, placebo: 6±1 °C) and humidity (CHO-E: 59±10 %, placebo: 61±13 %) were similar between trials ($p>0.05$).

3.4.1 Physiological responses to soccer match simulation

There was no significant time × trial interaction for heart rate (HR), ratings of perceived exertion (RPE) and hydration status responses to the SMS ($p>0.05$). HR increased from the 15-min time point (144±9 bpm, CHO-E and placebo trials combined) throughout the SMS protocol (time effect: $p=0.01$), rising to 158±6 bpm (CHO-E and placebo combined) by the end (90 min) of the SMS protocol, with no difference between trials ($p=0.42$). Likewise, RPE scores increased from the 15-min time point (10±2, CHO-E and placebo trials combined) throughout the SMS protocol (time effect: $p=0.01$), rising to 15±3 (CHO-E and placebo combined) by the end (90-min) of the SMS protocol, with no difference between trials ($p=0.17$). No differences were observed for any of the fluid balance and hydration status variables recorded pre and post the SMS protocol (Pre-trial urine osmolality: 513±159 mOsm/kg ; post-trial urine osmolality: 464±137 mOsm/kg; body mass loss: 0.7±0.4%, all $p>0.05$ for CHO-E and placebo trials combined).

3.4.2 ***Dribbling speed and accuracy***

Dribbling speed did not decline throughout the SMS on both CHO-E and placebo trials (**Figure 3.2.A**), with no time × trial interaction ($p=0.42$), time ($p=0.49$) or trial ($p=0.38$) effects. The mean difference (95% CI) in dribbling speed between CHO-E and placebo trials was 0.03 (-0.24 to 0.09) m/s with an effect size (Cohen's d) of 0.2 (small effect). Likewise, dribbling accuracy did not change over time in either CHO-E or placebo trials (**Figure 3.2.B**) and the was no time ($p=0.54$), trial ($p=0.41$) or time × trial interactions ($p=0.38$). The mean difference (95% CI) in dribbling accuracy between trials was -0.03 (-0.23 to 0.16) m with an effect size (Cohen's d) of 0.1 (trivial effect).

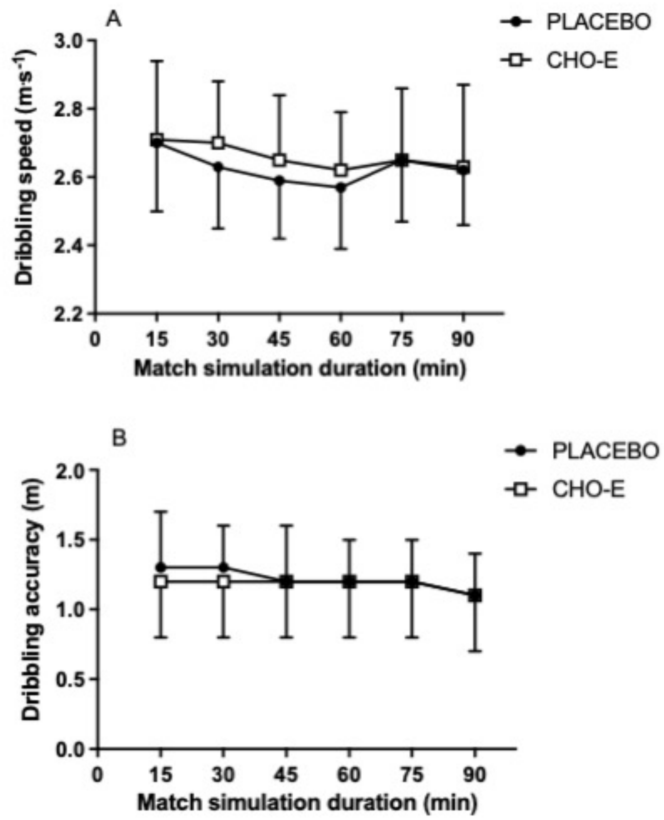

Figure 3.2 Dribbling speed (A) and dribbling accuracy (B) during the soccer match simulation on placebo and CHO-E (carbohydrate-electrolyte) trials. No main effects of trial, time, or trial × time were observed.

3.4.3 *Passing accuracy and speed*

There was a significant time × trial interaction (p=0.02) on passing score (accuracy) for both feet. Passing accuracy was greater in CHO-E than placebo at 15 min (p=0.005) and 90 min (p=0.02) timepoints for the dominant foot (**Figure 3.3.A**) and at 60 min (p=0.012) and 75 min timepoints (p=0.001) for the non-dominant foot (**Figure 3.3.C**). Passing accuracy, calculated as mean scores measured during the SMS, also was greater in CHO-E than placebo when passes were completed with either the dominant (**Figure 3.3.B**) or non-dominant (Figure **3.3.D**) foot. The mean difference (95% CI) in passing score for the dominant foot between CHO-E and placebo trials was 5 (1 to 9) points, with an effect size (Cohen’s d) of 0.7 (moderate effect). The mean difference (95% CI) in passing score for the non-dominant foot between trials was 6 (3 to 10) points with an effect size (Cohen’s d) of 0.8 (moderate effect).

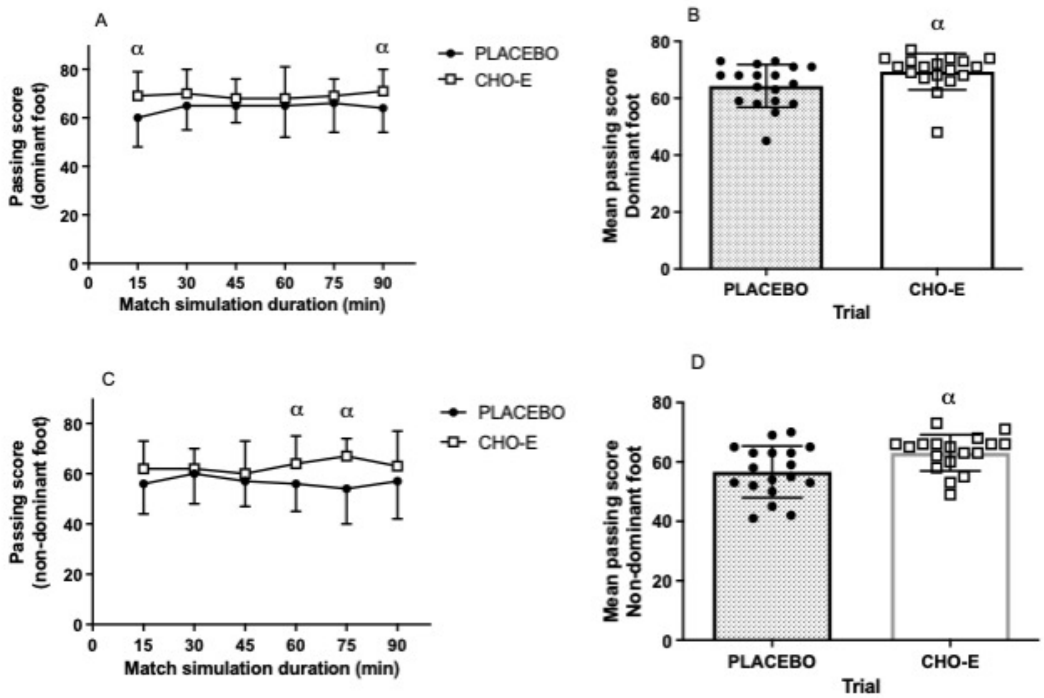

Figure 3.3. Passing score (A) during the soccer match simulation and mean passing score (B) for the dominant foot. Passing score (C) during the soccer match simulation and mean passing score (D) for the non-dominant foot. Significant main effects of trial (p<0.05) and trial x time (p<0.05) were observed. $^{\alpha}$ indicates time points at which significant differences were evident between placebo and CHO-E (carbohydrate-electrolyte) trials. * Indicates significant difference in mean passing score.

Passing speed was similar on both trials for the dominant foot with no time, trial, or time × trial interaction effects observed (all p>0.05) (**Figure 3.4.A**). The mean difference (95% CI) in passing speed between trials for the dominant foot was 0.4 (-0.1 to 0.9) m/s with an effect size (Cohen's d) of 0.2 (trivial effect). In contrast, for the non-dominant foot, a significant time × trial interaction was observed (p=0.03). Post-hoc analyses revealed that passing speed was better maintained from 75-min onwards in CHO-E trial compared with placebo (p=0.001; **Figure 3.4.C**). Mean passing speed was greater in CHO-E than placebo for the non-dominant foot only (p=0.04; **Figure 3.4.B** and **3.4.D**). The mean difference (95% CI) in passing speed for the non- dominant foot between trials was 0.6 (0.1 to 1.2) m/s. The effect size (Cohen's d) was 0.4 (small effect).

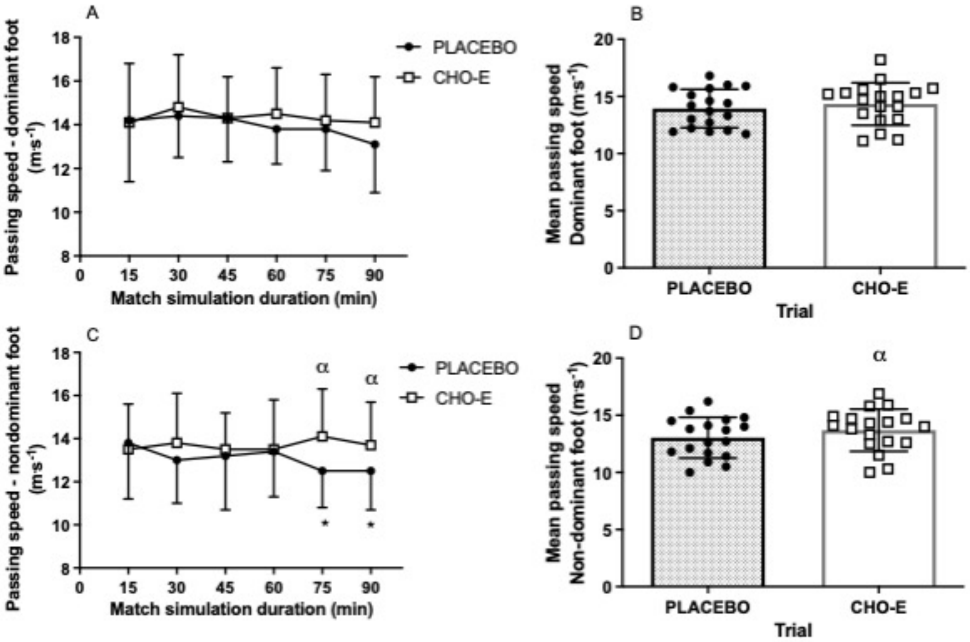

Figure 3.4. Passing speed (A) during the soccer match simulation and mean passing speed (B) for the dominant foot. Passing speed (C) during the soccer match simulation and mean passing speed (D) for the non-dominant foot. Significant main effects of trial ($p<0.05$) and trial x time ($p<0.05$) were observed on non-dominant foot only. $^{\alpha}$ indicates time points at which significant differences in passing speed were evident between placebo and CHO-E (carbohydrate-electrolyte) trials. * Indicates a significant decrease in passing speed compared to 15-minute value on PLACEBO trial only. $^{\beta}$ indicates significant difference in mean passing speed.

3.4.4 Sprint Speed

Sprint speeds did not decline throughout the SMS with no time (p=0.38), trial (p=0.47) or time × trial interactions (p=0.31) detected (**Figure 3.5**). In addition, average sprint speed during the 90- min SMS was similar between trials. The mean difference (95% CI) in sprint speed for the non- dominant foot between trials was 5.9 (5.8 to 6.1) m/s. The effect size (Cohen's d) was 0.2 (small effect).

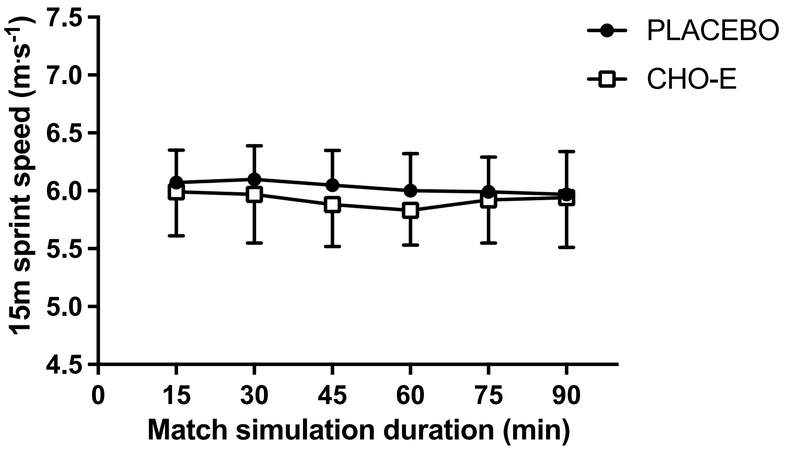

Figure 3.5. Sprint speed during the soccer match simulation on placebo and carbohydrate-electrolyte drink (CHO-E) ingestion trials No main effects of trial, time, or trial × time were observed.

3.4.5 High intensity anaerobic endurance running capacity

Anaerobic endurance capacity, expressed as running distance completed at 90% of the V̇O2 max, was 11.8% better on the CHO-E trial than placebo (p=0.01; **Figure 3.6**). The mean difference (95% CI) in running capacity between trials was 54 (15 to 94) m, with an effect size (Cohen's d) of 0.4 (small to moderate effect).

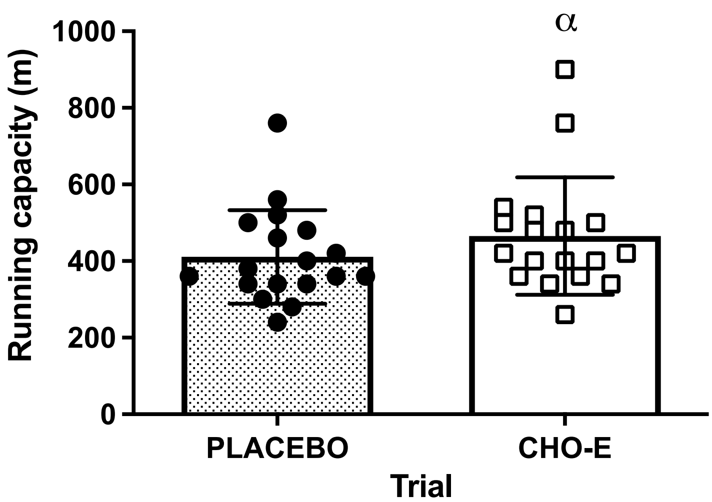

Figure 3.6. High intensity anaerobic endurance running capacity on completion of the soccer match simulation on placebo and carbohydrate-electrolyte drink (CHO-E) ingestion trials. α significant effect (p<0.05) with greater running capacity on CHO-E than PLACEBO.

3.4.6 Gastrointestinal comfort

VAS scores recorded for nausea were low and remained constant (15-min time point: 12±15, 90-min time point: 21±16) throughout the SMS with no time (p=0.54), trial (p=0.11) or time × trial (p=0.37) effects (**Figure 3.7.A**). There was a significant main effect of trial (p =0.014) and a time × trial interaction for fullness (p=0.007), with participants reporting greater fullness scores in the CHO-E condition pre-trial (39±22) and at the end (90 min) of the SMS protocol (37±25; **Figure 3.7.B**). Perceived feelings of bloatedness increased during the SMS, with participants feeling more bloated after ingesting the second drink at half time (45±23), compared to pre-trial feelings (30±22), on both trials. However, there was no time × trial (p=0.78) interaction for bloatedness scores.

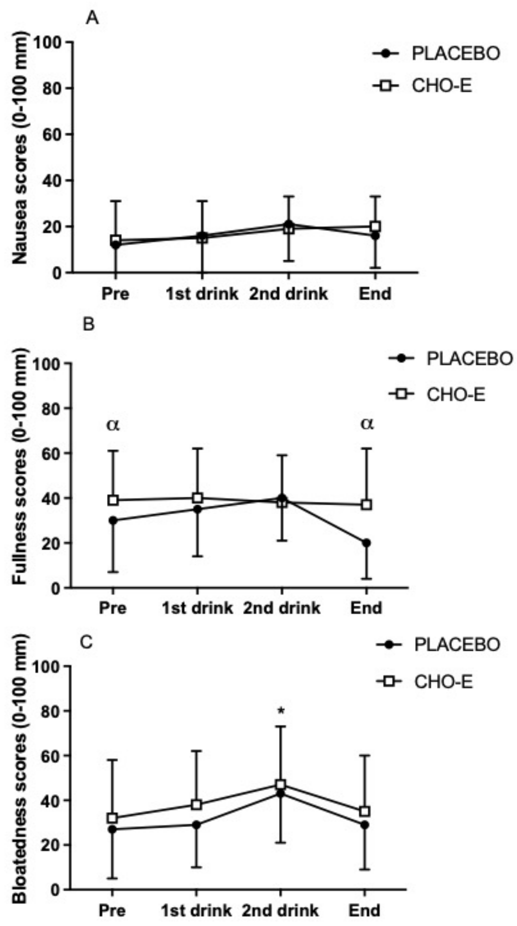

Figure 3.7. Nausea (A), fullness (B), and bloatedness throughout the experimental trials on placebo and carbohydrate-electrolyte drink (CHO-E) ingestion trials. *significant difference (p<0.05) from 0 time point. α-significant difference between trials at half time and final time point.

3.4.7 Blood analytes

There was a significant time × trial interaction (p=0.001), main time effect (p=0.001) and main trial effect (p=0.002) for capillary blood glucose concentration. Whereas a 26% decline in glucose concentration from half-time to the first 15-min of the second half in the CHO-E condition was observed, glucose concentrations remained constant in placebo. Glucose concentrations were greater in CHO-E than placebo at half-time and after completing the high intensity running capacity test (**Figure 3.8.A**). No significant time × trial interaction effect (p=0.12) or main trial effect (p=0.27) was observed for blood lactate concentrations over the course of the SMS (**Figure 3.8.B**). However, lactate values increased above baseline at 15-min and, with the exception of half-time, values remained constant during the 90-min SMS. Lactate concentrations increased after the high-intensity anaerobic endurance running capacity test.

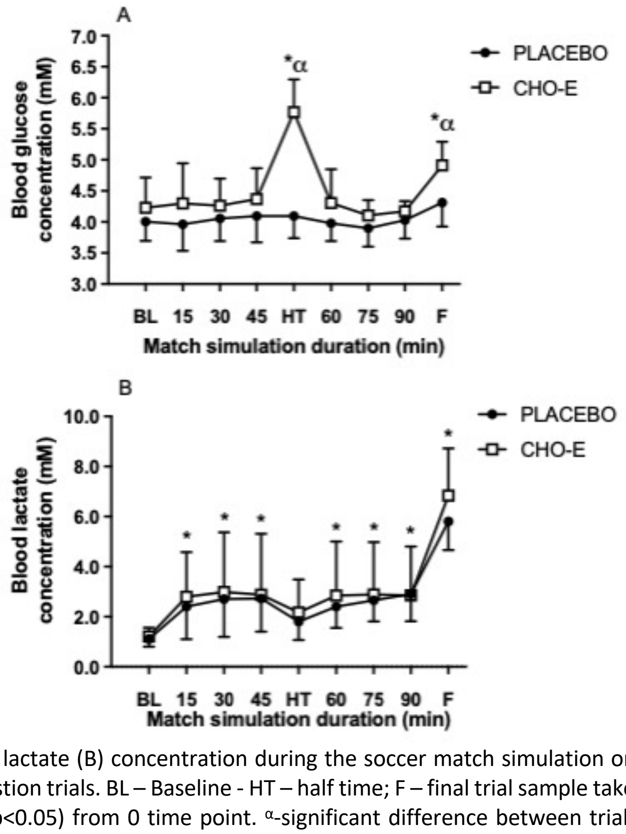

Figure 3.8. Blood glucose (A), lactate (B) concentration during the soccer match simulation on placebo and carbohydrate-electrolyte drink (CHO-E) ingestion trials. BL – Baseline - HT – half time; F – final trial sample taken following running capacity test. *significant difference (p<0.05) from 0 time point. α-significant difference between trials at half time and final time point.

3.5 Discussion

The primary aim of this study was to investigate the influence of ingesting 30g of carbohydrate as a 12% CHO-E solution, prior to and at half-time for a total of 60g during a 90-min SMS, on soccer-specific skill performance, speed and high-intensity running capacity in academy soccer players. The SMS protocol was performed on an indoor artificial grass surface 2-h following intake of a pre-match meal, compliant with recommended carbohydrate guidelines. We demonstrated that ingesting the 12% CHO-E solution versus an electrolyte-matched placebo better maintained passing accuracy in both the early and latter stages of the SMS protocol and passing speed during the latter stages of the SMS protocol with minimal impact on gut comfort. Although there was no benefit of ingesting the 12% CHO-E solution on dribbling speed and accuracy, or sprint speed, compared with placebo, post-match high-intensity running capacity was improved on the CHO-E trial. In terms of practical application, these data suggest that ingesting a 12% CHO-E solution before and during a soccer match may benefit soccer-specific skill performance and anaerobic endurance capacity.

A recent study reported the better maintenance of ball dribbling speed during the final 30-min of a SMS when ingesting a 12% CHO-E solution pre-match and during half-time vs. an electrolyte or water placebo condition (Harper et al., 2017). In contrast, this study demonstrated no influence of ingesting a 12% CHO-E solution on dribbling speed vs. an electrolyte-matched placebo. This discrepant finding may be attributed to several methodological factors. Unlike previous studies, the experimental trials were performed on an artificial grass surface and players wore their own soccer boots, which may have facilitated the better execution of skills. Moreover, trials were performed in the early afternoon instead of the morning with the aim to better reflect competitive practices of this group of players in Scotland. In accordance with published carbohydrate recommendations for exercise (Williams and Rollo, 2015; Thomas et al., 2016), we also provided a standardised breakfast containing 46 g of carbohydrate in addition to a pre-match meal consisting of 2 g carbohydrate per kg of body mass ingested 2-h prior to starting the SMS protocol. In contrast, Harper et al., (2017) provided soccer players with a breakfast containing 10% of daily energy requirement (~35 g of carbohydrate) ingested

~135-min before exercise. Interestingly, whereas Harper et al. (2017) reported a statistically significant decline in dribbling speed over time, in the present study dribbling speed remained constant throughout the 90-min protocol. Although muscle glycogen concentration was not measured in the present study, it was speculated that including a carbohydrate-rich pre-match meal prevented the decline in muscle glycogen content that has previously been associated with impaired performance (Mohr et al., 2005). Hence, the benefit of ingesting a 12% CHO-E solution on ball dribbling performance appears to be context-specific, e.g., when it is not possible to comply with pre-match carbohydrate intake guidelines, when fatigue has a markedly detrimental impact on dribbling performance, or other factors.

In the present study, whereas ingesting the 12% CHO-E solution resulted in the better maintenance of passing accuracy in both dominant and non-dominant feet, passing speed was better maintained with carbohydrate ingestion during the latter stages of the SMS protocol in the non-dominant foot only. It seems that this is the first study to differentiate between dominant and non-dominant feet when measuring soccer passing performance. Previous work demonstrates that if a task is familiar to an individual, then there will be an element of automaticity, and fewer central nervous system (CNS) resources are required for optimal performance (McMorris and Graydon, 1997). Conversely, when a task becomes more complex, the task outcome is more likely to be influenced by arousal (McMorris and Graydon, 1997). Thus, it is intuitive that performing the passing test with the non-dominant foot required a greater allocation of CNS resources than when performing the passing test with the dominant foot. It also has been proposed that fatigue is associated with a decrement in central control (Welsh et al., 2002), thus it can be inferred that the non- dominant side would demand more activity from the CNS and therefore be more susceptible to fatigue. Previous work has demonstrated that carbohydrate ingestion enhances CNS activity and motor control (Liu et al., 2000; Welsh et al., 2002). In this regard, Bottoms et al. (2007) showed that when compared to placebo the ingestion of a carbohydrate solution resulted in skill retention specifically in the backhand drive (weaker shot) in squash players. Hence, it is intuitive that ingesting the 12% CHO-E solution had a more profound effect on passing performance with the non-dominant foot than with the dominant foot. Consistent with this proposed mechanism, it appears from this study data that the impact of carbohydrate feeding is observed primarily with the non-dominant foot, particularly towards the end of the SMS.

Studies showing a deterioration in soccer specific skills (Ostojic and Mazic, 2002; Ali et al., 2007; Ali and Williams, 2009) when players did not consume carbohydrates have argued that lowered glucose concentrations are associated with declines in skill performance. In the present study, blood glucose concentrations at half-time, and at the end of the full protocol were significantly higher when ingesting the 12% CHO-E solution vs. placebo. However, ingesting the 12% CHO-E solution failed to prevent the decline in blood glucose concentration 15-min into the second half of the SMS, likely reflecting increased glucose disposal at the onset of the second half of the SMS. Others (Russell et al., 2012) have reported skill performance decline in placebo condition versus carbohydrate consumption despite mean blood glucose concentration remaining euglycemic. Taken together, it appears that blood glucose concentration *per se* is not a key driver for the changes in skill performance detected between trials.

Blood lactate concentration during the SMS protocol was similar on both trials and reflects typical match intensity responses (Krustrup et al., 2006). Although marginal, the higher lactate concentrations in the CHO-E trial following the high-intensity anaerobic running test likely reflects greater capacity for flux through glycolysis in the face of additional substrate availability on that trial. Although sprint speed over 15-m did not decline throughout the SMS, mean values were comparable to those reported during actual match- play (Krustrup et al., 2006). Interestingly, Balsom et al. (1992) previously demonstrated that 15-m sprints could be performed at 30-s intervals without impaired performance in the absence of carbohydrate supplementation. In the present study, 18 × 15-m sprints were performed over the 90-min SMS protocol separated by a minimum period of 5-min, during which lower intensity exercise intervals were performed. This protocol would suggest there was sufficient time for phosphocreatine resynthesis between sprints, thus preventing a decline in sprint speed over the SMS on both trials. We reported that high-intensity running capacity following the SMS was significantly better in the 12% CHO-E vs placebo trial. Similar results were reported by Alghannam (2011) and Nicholas et al. (1995), supporting the argument that carbohydrate ingestion exhibits an ergogenic benefit on anaerobic endurance capacity after prolonged intermittent exercise.

In conclusion, ingesting a 12% CHO-E solution before a SMS protocol and at half-time aided the retention of soccer-specific skill performance, particularly passing performance towards the end of the SMS protocol, and enhanced high-intensity running capacity after simulated match-play. The current study adds to the evidence base that optimisation of carbohydrate ingestion strategies appears to have a practically relevant benefit on key skill (passing) and physiological (high intensity running capacity) related factors that likely influence performance towards the end of a soccer match.

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CHAPTER 4
A PRELIMINARY STUDY OF THE RELIABILITY OF SOCCER SKILLS WITHIN A MODIFIED SOCCER MATCH SIMULATION PROTOCOL

4.1 Abstract

This study examined test-retest reliability of soccer-specific skills within a modified version of the soccer match simulation (SMS) protocol. Ten professional academy soccer players (18±1 y) from the United Kingdom completed 30 minutes of the modified SMS on two occasions under standardised conditions. During each trial, participants performed 20-m dribbling, short passing (4.2-m), long passing (7.9-m), shooting skills, and 15-m sprints within four blocks of soccer specific activity. Collapsed normative data (mean (SD)) for trial 1 and trial 2 for dribbling speed was 2.7 (0.2) m/s, for sprint speed 5.9 (0.4) m/s, for short pass speed 11.1 (0.5) m/s, for long pass speed was 12.2 (0.5) m/s, and for shooting speed was 13.3 (0.4) m/s. Mean results from trial 1 and trial 2 were not different for all measures evaluated ($P > 0.05$). Good to excellent reliability (ICC 0.76-0.99) was observed for long and short passing speed, shooting speed, sprint speed, and long pass accuracy, with CVs typically < 5-10%. Moderate reliability (ICC 0.50-0.75) was observed for dribbling speed. Poor reliability (ICC <0.50) was observed for dribbling accuracy and shooting accuracy. The reliability of the modified version of the SMS protocol is promising for most of the skills assessed, with the exception of dribbling and shooting accuracy in this group of professional youth soccer players. The modified protocol is easy to implement within professional club settings without specialist equipment, but due to the limited sample size the reliability requires further confirmation in a larger sample.

Keywords: football; reproducibility; accuracy, speed

4.2 Introduction

Soccer simulation protocols aim to replicate movement patterns and physiological demands of match-play (Russell et al., 2011; Drust et al., 2000; Nicholas et al., 2000; Thatcher and Batterham, 2000). Free running intermittent exercise simulation protocols are designed to simulate the activity pattern characteristics of soccer, however, several factors, such as the omission of game-specific skills (Russell et al., 2011), and the use of a non-grass surface might reduce the ecological validity of these protocols (Russell et al., 2011). As such, modified versions of protocols have been implemented to investigate soccer specific skills (Foskett et al., 2009; Ali et al., 2008; Rostgaard et al., 2008).

Russell et al. (2011) developed the Soccer Match Simulation (SMS) protocol. The SMS is a modified version of the Loughborough Intermittent Soccer Test (LIST) which was the first intermittent exercise simulation protocol designed to simulate the activity pattern characteristics of soccer. The LIST includes 75-min of intermittent activity followed by a run to exhaustion (Nicholas et al., 2000), however, it does not include game-specific skills. The SMS includes additional movement components and soccer-specific skills are embedded to enhance the ecological validity of the protocol (Russell et al., 2011). This means the SMS has application to studies investigating interventions on both the physical and skill components of soccer players performance (Russell et al, 2011; Harper et al., 2017). The SMS protocol has been successfully used to evaluate performance, physiological responses, and the efficacy of nutritional interventions (Kingsley et al., 2014; Harper et al., 2017). However, is important to know the reliability of sport-specific tests like the SMS or its modified versions, as day to day variation needs to be understood in order to determine differences between trials in intervention studies. Reliability refers to the reproducibility/precision of values from a test, assay, or other measurement in repeated trials on the same individuals (Hopkins, 2000). Russell et al. (2010) assessed the reliability of the skills contained in the SMS and reported moderate, to moderately strong, relative reliability for passing (speed, accuracy, and success), shooting (accuracy), and dribbling (speed and accuracy). When assessed over 120-min (two 45-min halves plus 2 additional 15-min periods) Harper et al. (2016) reported the physiological and performance responses were reliable but did not include skill outcome measures of passing or shooting.

In the previous chapter, a modified version of the SMS protocol was utilised to assess soccer skill performance. The protocol was modified for ease of use in a professional soccer academy setting, as well as to provide greater insight into potential differences between dominant and non-dominant foot. In order to assess the passing variables, readily available passing targets were utilised (i.e., soccer mannequins) instead of banners with target boxes and an illumination system (Russell et al., 2011). Furthermore, for the first time, the assessment of passing and shooting, discriminated between the dominant and non-dominant foot. This involved increasing the number of passes and shots taken during the match simulation protocol. Discriminating between feet could be of importance as fatigue is associated with a decrement in central control (Welsh et al., 2002) and may be more likely to affect skill performance with the non-dominant foot (Rodriguez-Giustiniani et al., 2019).

Previous studies on skill-based sports like squash (Bottoms et al., 2007) and tennis (McRae and Galloway, 2012) have shown positive effects of nutritional interventions on the maintenance of skill performance, particularly on weaker/non-dominant shots such as backhand drive-in squash. Indeed, in the study presented in Chapter 2 passing speed was better maintained in the non-dominant foot when evaluating the effects of a carbohydrate-electrolyte beverage on soccer skill performance using a simulated soccer match protocol.

Taken together, it seems reasonable to distinguish between the dominant and the non-dominant foot when investigating the reliability of soccer-skill assessments. Due to the changes that we have made in the delivery of the SMS, and the skills within, it is relevant to assess the reliability of this modified version of the protocol. It was theorised that this modified protocol would demonstrate similar reliability to previous studies using the SMS. Specifically, the aim of the present study was to quantify test-retest reliability and compare the magnitude of these statistics to previous investigations on the SMS.

4.3 Methods

4.3.1 Participants

Ten male well-trained professional outfield soccer players (5 midfielders, 3 defenders, 2 strikers) from the United Kingdom, who were accustomed to skill assessments as part of their regular training, were recruited from a local professional football club development squad in order to participate in this investigation. All players had five or more years of playing experience, had been training consistently for 1 year or more, were regularly participating in match-play with their squad, and were free from injury at the time of the recruitment and testing (age: 18±1 y, body mass: 75.0±6.5 kg, stature: 179.2±5.6, body mass index: 23.4±1.0 kg/m^2). The experimental procedures were approved by a local Ethics of Research Committee and the study was conducted in accordance with the declaration of Helsinki.

4.3.2 Study Design

Players attended one preliminary visit for a familiarisation session before undertaking two main trials in which test re-test reliability was assessed. All visits were expected to be separated by a minimum of two days and a maximum of seven days. All the participants completed the two main trials 7 days apart. Players followed 48-hr habitual diets (avoiding caffeine and alcohol) and recorded food consumed before the familiarisation visit. The pre-familiarisation diet was replicated for both main trials. Players refrained from strenuous exercise 48-hr before the familiarisation and main trial days. All testing sessions were performed on an indoor artificial grass pitch (length: 37-m; width: 19-m; ceiling height: 6.5-m). Soccer balls were inflated to a pressure of 14 psi before each trial.

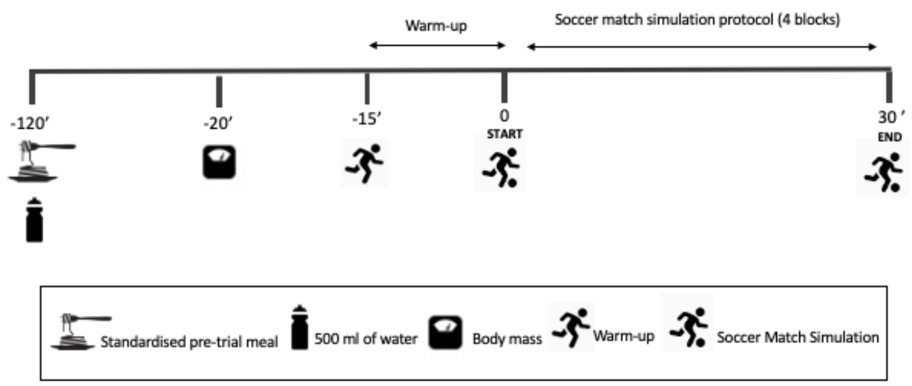
4.3.3 Familiarisation and main trials procedures

All trials started in the afternoon to reflect the time at which this group typically engages in soccer match play. At the training ground, researchers provided players with a standardised breakfast (2 eggs, 2 slices of bread, 1 medium-sized banana providing 1.8 MJ, 46g carbohydrate, 26g protein, 14g fat). A pre-trial standardised meal also was provided 2-h before beginning the main trials, with the meal containing 2g carbohydrate per kg of body mass (pasta in a tomato sauce) plus 500 mL of water. Upon attendance for testing (familiarisation and main trials), body mass (SECA Quadra 808, Hamburg, Germany) was assessed immediately after voiding of bladder and bowels. Familiarisation and main trials commenced after a 15-min standardised warm-up (consisting of running, dynamic stretching, and 20-m sprints) that preceded each trial. During each trial, participants were required to perform soccer dribbling, passing, and shooting skills, and 15-m sprints throughout four blocks of a modified soccer match simulation (SMS) protocol (1) lasting a total of 30 minutes (**Figure 4.1.A**). Each block of the SMS protocol consisted of 3 repeated cycles of three 20-m walks, one walk to the side, five 20-m jogs, one 20-m backwards jog, two 20-m strides and an alternating timed 15-m sprint or a 20-m dribble (**Figure 4.1.B**), followed by passing and shooting assessments (Russell et al., 2011).

4.3.4 Simulated soccer match protocol, skills testing, and analysis

The SMS includes exercise blocks consisting of 3 repeated cycles of three 20-m walks, one walk to the side, an alternated timed 15-m sprint or a 20 m dribble, a 4-s passive recovery period, five 20-m jogs at a speed corresponding to 40% $\dot{V}O2$ max, one 20-m backwards jogs at 40% $\dot{V}O2$ max and two 20-m strides at 85% $\dot{V}O2$ max followed by passing and shooting assessments. So as to assess the reliability of this modified version of the protocol, the participants completed four blocks of the abovementioned cycles. In order to assess dribbling speed and accuracy, players dribbled a ball between 7 cones (cones 2–7 were placed 3-m away from the preceding cone, and cones 1 and 7 were 1 m away from each end of the course; **Figure 4.2.A**). Participants were required to dribble the ball as quickly and accurately as possible from one end to the other over the 20-m total distance. Participants dribbled towards a video camera that was placed directly in line with the cones. For the sprint assessment, players ran as fast as possible through timing gates (Brower®, USA) placed 15-m apart, with a 1-m run-in. At the end of each block of activity, players performed a bout of passing where they directed alternate passes towards target zones placed to the left and right at distances of 4.2-m (short pass) and 7.9-m (long pass).

A



B

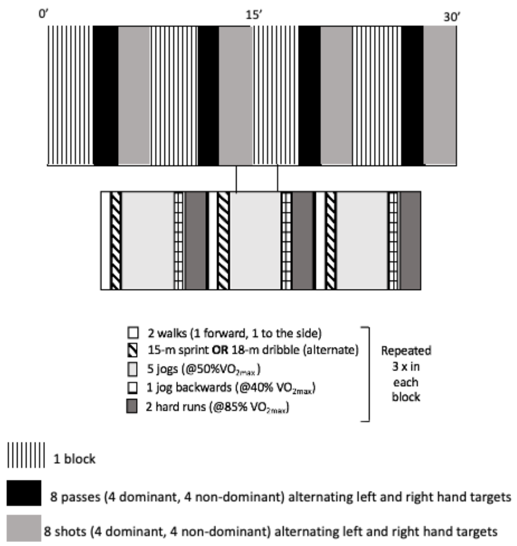


Figure 4.1. Protocol Schematic (A) and Simulated Soccer Match Outline (B)

Soccer mannequins (Diamond Football®, Senior Pro Free Kick) with their bases were used as passing targets. The base (0.5-m wide) was the central zone of the target, with cones at a distance of 0.5-m of each side as the lateral zones of the target. A pass to the centre area was worth 10 points and the two lateral areas were worth 5 points. Passes that missed the target areas were scored as 0. Passing bouts consisted of 8 passes (2 with the dominant and 2 with the non-dominant foot to the short pass target, and 2 with the dominant and 2 with the non-dominant foot to the long pass target; **Figure 4.2.B**). Then, a shooting skill assessment was performed, for this, participants were instructed to kick the ball as firmly and accurately as possible to a shooting target. Shooting target zones were at a distance of 15-m in the four corners of the goal. These target areas have been identified as optimal ball placement to beat a goalkeeper when shooting (Ali et al., 2007). Each shooting target was divided into two areas, a centre area (75 cm x 60 cm) and an extended area (100 cm x 90 cm) with the centre area worth 10 points and the extended area worth 5 points. Shots that missed the areas on the target were scored as 0. The bouts of shooting consisted of 8 shots (4 with the dominant foot and 4 with the non-dominant foot; **Figure 4.2.B**).

Video footage of the skill tests were captured using GoPro cameras (GoPro®, Hero 5), one was placed 1-metre apart from the last cone of the dribbling course and the other 1-metre behind the passing and shooting zone. Manual digitisation (Kinovea® version 0.8.15; Kinovea Org., France) yielded dribbling speed, dribbling accuracy, passing accuracy, and passing speed, as well as shooting accuracy and speed. Passing and shooting speed was calculated from the time interval between ball contact with the foot and subsequent ball contact with the target area.

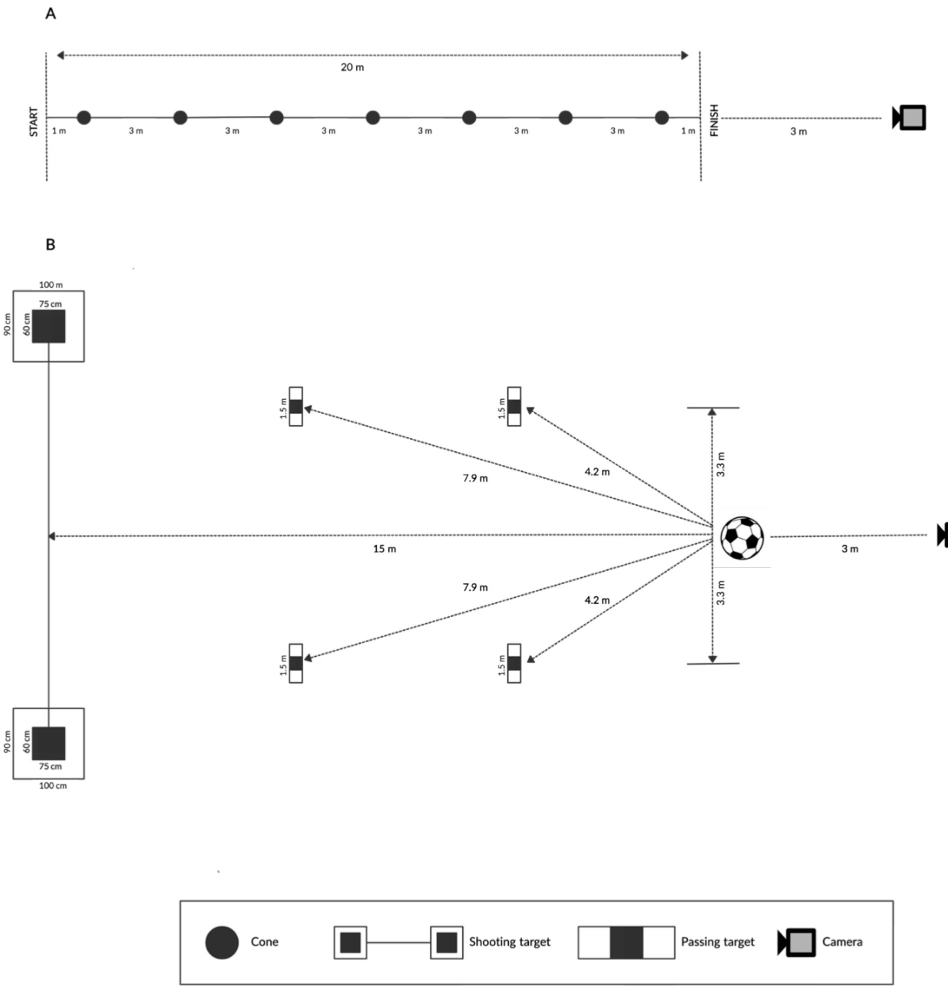


Figure 4.2. Schematic representation of the (A) dribbling assessment and the (B) passing and shooting assessments.

4.3.5 Inter and intra rater reliability

In order to determine inter-rater and intra-rater reliability, data from three participants was selected across the two trials for each one of the variables evaluated through digitisation. For the assessment of inter-rater reliability two experienced video analysts within a professional football club analysed the data which was compared with the data gathered by the investigators of this study. To assess intra-rater reliability, repeated measures from one of the authors of the study and two video analysts for each one of the variables assessed were compared.

4.3.6 Statistical analysis

Statistical analysis was carried out using SPSS software (Version 16.0; SPSS Inc., USA) and a custom-made spreadsheet (Hopkins, 2015). Systematic bias was evaluated as the mean change between trial 2 and trial 1, determined using paired t-tests and statistical significance set at $P < 0.05$. Soccer skills relative and absolute reliability was assessed using the intra-class correlation coefficient (ICC) and the coefficient of variation (CV), respectively. The ICC was derived from the methods of a two-way mixed effects model for consistency in a single measure. Interpretation of the ICC values was based on guidelines provided by Koo and Li (2016): ICC's less than 0.50 were indicative of poor reliability, values between 0.50 and 0.75 indicated moderate reliability, values between 0.76 and 0.90 indicated good reliability, and values greater than 0.90 indicated excellent reliability. The coefficient of variation was calculated from the standard error of measurement. All the aforementioned statistics were presented with 95% confidence intervals (CI).

4.4 Results

Descriptive and reliability statistics along with mean differences (95% CI) for each of the repeated skill assessments are presented in **Table 4.1** and **Table 4.2.** Mean results from trial 1 and trial 2 were not significantly different for all measures evaluated ($p>0.05$). Reliability statistics for the assessed skills are also presented in tables 1 and 2. Long passing speed with both feet, with the dominant foot, and with the non-dominant foot were the most reliable outcomes that were identified since the ICC showed excellent reliability (>0.90).

Short pass speed with both feet and shooting speed with both feet showed good reliability along with a CV <5%, whereas sprint speed also presented good reliability with a CV <10%. Good reliability (ICC=0.76-0.90) and a CV in the 10-20% range was reported for both short and long pass accuracy with both feet. Dribbling speed was moderately reliable with a CV <10% whereas shooting accuracy and dribbling accuracy both showed poor reliability (ICC=0.50). When assessing passing and shooting performance with the dominant and the non-dominant feet separately (**Table 4.2**), we observed that long pass accuracy with the dominant foot showed excellent reliability (ICC>0.90). We reported good reliability (ICC=0.76-0.90) with a CV <5% for short pass and shooting speed with the non-dominant foot, whereas short pass speed with the dominant foot had good reliability (ICC=0.76-0.90) and a CV <10%. Shooting speed with the dominant foot, short pass accuracy with the dominant foot, long pass accuracy with the non-dominant foot, and shooting accuracy with the non-dominant foot were all moderately reliable (ICC=0.50-0.75) while short pass accuracy with the non-dominant foot and shooting accuracy with the dominant foot showed poor reliability (ICC<0.50).

Inter-rater and intra-rater error reliability was good to excellent for almost all variables studied, except for long pass with non-dominant foot which only achieved moderate intra-rater reliability in analyst 2 for trial 1 (**Table 4.3**). The coefficient of variation was good below 10% for most of the variables assessed except in four cases (dribbling accuracy, intra-rater reliability, analyst 2; shooting accuracy with both feet, intra-rater reliability, researcher; shooting accuracy non-dominant foot, intra-rater reliability, analyst 1; shooting speed dominant foot, analyst 1) in which the CV values were between 10 – 20%. We did not observe any of the CV values to be > 20%.

Table 4.1.Descriptive and reliability statistics obtained from two trials assessing soccer-skills performance within a modified SMS protocol.

Skill	Trial 1	Trial 2	Mean change (95% CI) Raw values	Mean change (95% CI) %	t-test (P-value)	ICC (95% CI)	CV (%) (95% CI)
Dribbling Speed (m/s)	2.7 ± 0.2	2.7 ± 0.2	0.0 (-0.1, 0.1)	-0.1 (-5.3, 4.9)	0.99	0.68 (0.14, 0.91)	7.6 (7.5, 7.7)
Dribbling Accuracy (cm)	1.2 ± 0.4	1.1 ± 0.3	-0.1 (-0.4, 0.3)	0.1 (-0.4, 0.3)	0.61	0.15 (-0.49, 0.69)	23.1 (22.9, 23.3)
Sprint Speed (m/s)	5.9 ± 0.4	5.9 ± 0.4	0.0 (-0.1, 0.2)	0.0 (-0.2, 0.1)	0.69	0.87 (0.58, 0.97)	6.9 (6.7, 7.2)
Short Pass Accuracy Both Feet (points)	56 ± 10	54 ± 6	-2 (-6, -2)	2.2 (-1.9, 6.1)	0.25	0.85 (0.43, 0.95)	13.9 (8.9, 18.9)
Long Pass Accuracy Both Feet (points)	44 ± 7	43 ± 5	-1 (-3, 2)	0.1 (-0.2, 0.3)	0.65	0.76 (0.22, 0.92)	15.5 (11.8, 19.2)
Short Pass Speed Both Feet (m/s)	11.1 ± 0.4	11.0 ± 0.5	-0.1 (-0.3, 0.2)	0.6 (2.3, -3.4)	0.58	0.84 (0.44, 0.96)	3.4 (3.2, 3.7)
Long Pass Speed Both Feet (m/s)	12.1 ± 0.6	12.2 ± 0.4	-0.1 (-0.1, 0.2)	0.1 (-0.2, 0.1)	0.33	0.95 (0.81, 0.99)	4.3 (3.9, 4.6)
Shooting Accuracy Both Feet (points)	69 ± 6	71 ± 5	2 (-2, 6)	-1.9 (-0.1, 0.2)	0.33	0.40 (-0.27, 0.81)	6.3 (2.9, 9.7)
Shooting Speed Both Feet (m/s)	13.6 ± 0.3	13.6 ± 0.4	0.1 (-0.2, 0.1)	-0.1 (-0.2, 0.1)	0.27	0.89 (0.62, 0.97)	2.5 (2.3, 2.7)

Data presented as mean ± standard deviation, CI: confidence interval. P-value determined from test re-test data using paired sample t-test for all measurements' outcomes (n=10). SMS: soccer match simulation, CI: confidence interval, ICC: intra-class correlation coefficient, CV: Coefficient of variation.

Table 4.2. Descriptive and reliability statistics obtained from two trials assessing soccer passing and shooting performance with the dominant and non-dominant foot within a modified SMS protocol.

Skill	Trial 1	Trial 2	Mean change (95% CI) Raw values	Mean change (95% CI) %	t-test (P-value)	ICC (95% CI)	CV (%) (95% CI)
Short Pass Accuracy Dominant Foot (points)	31 ± 5	30 ± 3	-1.0 (-2.0, 4.0)	1.0 (-2.0, 3.9)	0.47	0.53 (-0.10, 0.86)	11.7 (9.2, 14.2)
Short Pass Accuracy Non-Dominant Foot (points)	25 ± 5	23 ± 5	-2.2 (-2.5, 6.9)	2.2 (-2.6, 6.7)	0.32	0.22 (-0.34, 0.66)	15.5 (12.4, 18.6)
Long Pass Accuracy Dominant Foot (points)	24 ± 6	24 ± 3	-0.1 (-1.2, 1.4)	0.1 (-1.2, 1.4)	0.86	0.92 (0.72, 0.98)	18.6 (15.8, 21.4)
Long Pass Accuracy Non-Dominant Foot (points)	20 ± 4	19 ± 3	-0.7 (-1.6, 3.0)	0.7 (-1.6, 3.0)	0.51	0.56 (0.05, 0.84)	15.3 (13.1, 17.5)
Short Pass Speed Dominant Foot (m/s)	11.6 ± 0.6	11.4 ± 0.8	-0.2 (-0.2, 0.5)	0.2 (-0.2, 0.5)	0.36	0.76 (0.39, 0.92)	5.6 (5.2, 6.0)
Short Pass Speed Non-Dominant Foot (m/s)	10.7 ± 0.3	10.7 ± 0.3	0.0 (-0.2, 0.14)	0.0 (-0.2, 0.1)	0.63	0.78 (0.33, 0.94)	2.9 (2.2, 3.1)
Long Pass Speed Dominant Foot (m/s)	10.7 ± 0.3	10.8 ± 0.5	0.1 (-0.3, -0.4)	0.2 (-0.3, 0.0)	0.09	0.95 (0.81, 0.99)	3.7 (3.5, 4.0)
Long Pass Speed Non-Dominant Foot (m/s)	11.4 ± 0.5	11.3 ± 0.5	-0.1 (0.0, 0.2)	0.1 (0.2, 0.2)	0.02	0.96 (0.85, 0.99)	3.7 (3.4, 4.0)
Shooting Accuracy Dominant Foot (points)	39 ± 5	38 ± 4	-1.0 (-2.1, 4.1)	1.1 (-1.9, 4.0)	0.43	0.43 (-0.23, 0.82)	8.5 (5.7, 11.3)
Shooting Accuracy Non-Dominant Foot (points)	29 ± 7	32 ± 6	3.5 (-6.7, -0.3)	-3.6 (-7.0, -0.2)	0.04	0.75 (0.26, 0.93)	18.3 (14.3, 22.3)
Shooting Speed Dominant Foot (m/s)	14.1 ± 0.3	14.0 ± 0.3	-0.1 (-0.2, 0.4)	0.1 (-0.2, 0.4)	0.49	0.56 (-0.07, 0.87)	2.4 (2.2, 2.6)
Shooting Speed Non-Dominant Foot (m/s)	13.0 ± 0.5	13.1 ± 0.5	0.1 (-0.1, 0.2)	0.0 (-0.1, 0.2)	0.65	0.87 (0.55, 0.97)	4.1 (3.8, 4.4)

Data presented as mean ± standard deviation, CI: confidence interval. P-value determined from test re-test data using paired sample t-test for all measurements' outcomes (n=10). SMS: soccer match simulation, CI: confidence interval, ICC: intra-class correlation coefficient, CV: Coefficient of variation.

Table 4.3 Inter-rater and intra-rater reliability.

	ICC (95% CI)							
	Inter-rater reliability		Intra-rater reliability: researcher		Intra-rater reliability: analyst 1		Intra-rater reliability: analyst 2	
Skill	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Dribbling Speed (m/s)	0.99 (0.96, 1.00)	0.98 (0.84, 0.99)	0.99 (0.54, 1.00)	0.99 (0.89, 1.00)	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)	0.97 (0.21, 0.99)	0.89 (0.72, 0.92)
Dribbling Accuracy (cm)	0.99 (0.97, 1.00)	0.98 (0.82, 1.00)	0.98 (0.40, 1.00)	0.98 (0.33, 0.99)	0.99 (0.97, 1.00)	0.99 (0.96, 1.00)	1.00	0.94 (0.38, 0.99)
Short Pass Accuracy Both Feet (points)	0.99 (0.96, 1.00)	0.99 (0.90, 1.00)	1.00	0.98 (0.56, 1.00)	0.99 (0.80, 1.00)	0.98 (0.65, 1.00)	0.83 (0.21, 0.98)	0.91 (0.44, 0.99)
Short Pass Accuracy Dominant Foot (points)	0.95 (0.63, 0.99)	0.96 (0.05, 0.99)	1.00	0.99 (-0.01, 0.99)	0.95 (0.30, 0.99)	0.87 (0.31, 0.99)	0.97 (0.22, 0.99)	0.96 (0.41, 0.99)
Short Pass Accuracy Non-Dominant Foot (points)	0.99 (0.96, 1.00)	0.98 (0.83, 0.99)	0.98 (0.62, 0.99)	0.98 (-0.46, 0.99)	0.99 (0.91, 1.00)	0.97 (0.34, 0.99)	0.99 (0.43, 0.99)	0.78 (0.13, 0.90)
Long Pass Accuracy Both Feet (points)	0.99 (0.95, 1.00)	0.97 (0.68, 0.99)	1.00	0.99 (0.93, 1.00)	0.99 (0.79, 1.00)	0.97 (0.03, 0.99)	0.85 (0.23, 0.97)	0.99 (0.82, 1.00)
Long Pass Accuracy Dominant Foot (points)	0.99 (0.86, 0.99)	0.99 (0.92, 1.00)	0.98 (0.77, 1.00)	0.96 (0.41, 0.99)	0.99 (0.81, 1.00)	0.96 (0.20, 0.99)	0.98 (0.89, 0.99)	0.97 (0.74, 1.00)
Long Pass Accuracy Non-Dominant Foot (points)	0.91 (0.14, 0.99)	0.99 (0.36, 1.00)	1.00	1.00	0.98 (0.59, 1.00)	0.98 (0.50, 0.99)	0.91 (0.30, 0.99)	0.95 (0.30, 0.99)
Short Pass Speed Both Feet (m/s)	0.97 (0.36, 0.99)	0.98 (0.83, 0.99)	1.00	1.00	0.99 (0.61, 1.00)	0.96 (0.10, 0.99)	0.99 (0.91, 1.00)	0.99 (0.91, 1.00)
Short Pass Speed Dominant Foot (m/s)	0.99 (0.94, 1.00)	0.98 (0.85, 0.99)	1.00	1.00	0.98 (0.03, 0.99)	0.96 (0.03, 0.99)	0.95 (0.53, 1.00)	0.99 (0.79, 1.00)
Short Pass Speed Non-Dominant Foot (m/s)	0.98 (0.84, 1.00)	0.96 (0.66, 0.99)	1.00	1.00	0.91 (0.22, 0.99)	0.93 (0.37, 0.99)	0.99 (0.81, 1.00)	0.99 (0.81, 1.00)
Long Pass Speed Both Feet (m/s)	0.98 (0.85, 0.99)	0.91 (0.18, 0.99)	0.96 (0.31, 0.99)	0.94 (0.41, 0.99)	0.98 (0.47, 0.99)	0.86 (0.61, 0.99)	0.98 (0.59, 1.00)	0.85 (0.32, 0.97)
Long Pass Speed Dominant Foot (m/s)	0.99 (0.96, 1.00)	0.97 (0.69, 0.99)	0.94 (0.94, 0.99)	0.97 (0.48, 0.99)	0.98 (0.58, 0.99)	0.98 (0.14, 1.00)	1.00	0.89 (0.74, 0.93)
Long Pass Speed Non-Dominant Foot (m/s)	0.97 (0.80, 0.99)	0.95 (0.66, 0.99)	0.96 (0.67, 0.99)	0.88 (0.43, 0.99)	0.93 (0.07, 0.99)	0.92 (0.67, 0.99)	0.73 (0.38, 0.84)	0.97 (0.73, 0.99)
Shooting Accuracy Both Feet (points)	0.95 (0.61, 0.99)	0.97 (0.75, 0.99)	0.96 (0.25, 0.99)	0.97 (0.56, 0.99)	0.93 (0.11, 0.99)	0.87 (0.42, 0.99)	0.91 (0.77, 0.99)	0.98 (0.33, 0.99)
Shooting Accuracy Dominant Foot (points)	0.94 (0.59, 0.99)	0.91 (0.59, 0.99)	1.00	1.00	0.95 (0.12, 0.99)	0.93 (0.10, 0.99)	0.94 (0.59, 0.99)	0.98 (-0.03, 1.00)
Shooting Accuracy Non-Dominant Foot (points)	0.99 (0.90, 1.00)	0.95 (0.61, 0.99)	0.91 (0.58, 0.99)	0.90 (0.67, 0.99)	0.88 (0.30, 0.99)	0.92 (0.48, 0.99)	0.89 (0.81, 0.98)	1.00
Shooting Speed Both Feet (m/s)	0.98 (0.88, 1.00)	0.98 (0.89, 0.99)	0.96 (0.56, 0.99)	0.99 (0.74, 1.00)	0.95 (0.31, 0.99)	0.96 (0.01, 0.99)	0.93 (0.78, 1.00)	0.98 (0.46, 0.99)
Shooting Speed Dominant Foot (m/s)	0.99 (0.88, 1.00)	0.96 (0.64, 0.99)	0.97 (0.73, 0.99)	0.96 (0.79, 0.99)	0.84 (0.12, 0.99)	0.97 (0.46, 0.99)	0.95 (0.64, 0.99)	0.99 (0.93, 1.00)
Shooting Speed Non-Dominant Foot (m/s)	0.97 (0.56, 0.99)	0.98 (0.85, 0.99)	0.96 (0.26, 0.99)	0.90 (0.61, 0.99)	0.97 (0.48, 0.99)	0.98 (0.43, 0.99)	0.98 (0.85, 0.99)	0.96 (0.41, 0.99)

4.5 Discussion

The aim of this investigation was to examine the preliminary reliability of soccer skill tests within a modified version of the SMS protocol (Russell et al., 2011). In particular, it has been observed that when assessing long passing speed (both feet, dominant foot, and non-dominant foot), sprint speed, short pass accuracy with both feet, long pass accuracy (both feet and with the non-dominant foot) short pass speed (both feet, with the dominant foot, and with the non-dominant foot), and shooting speed (both with and with the non-dominant foot) this version of the SMS protocol demonstrated good to excellent reliability. However, the modified protocol revealed poor reliability for dribbling accuracy, short pass accuracy (with the non-dominant foot) and shooting accuracy. This study is the first protocol of this kind that assesses the reliability of soccer specific skills performed with the dominant and non-dominant foot in professional academy soccer players, on an artificial grass surface.

4.5.1 Reliability of dribbling speed and accuracy

The number of successful dribbling tasks has been identified as a key contributor to match success (Zago et al., 2016). The present study found moderate reliability for dribbling speed but poor reliability for dribbling accuracy. This finding is in contrast to good reliability for both dribbling speed and dribbling accuracy reported previously (Russell et al., 2010). The reason for the difference may, in part, be due to how the skills were assessed. Specifically, integrating the dribbling skill assessment within the SMS in the present study may have reduced the reliability, in comparison to when the dribbling assessment was performed in isolation (Russell et al., 2010). Under circumstances where the player performs the dribbling test between efforts, they are likely to take time to focus exclusively on the required skill.

Harper et al. (2016) investigated the reliability of physiological and performance responses to the SMS across 120 minutes of soccer-specific exercise. In order to do this, the authors used an extended version of the SMS in which they included two additional 15-min periods of intermittent exercise and skill testing, on top of the two 45-min halves of the original SMS protocol. All performance variables assessed were expressed as an average per 15-min of exercise. Harper et al. (2016) demonstrated moderate reliability for dribbling speed in both the 0-15 min (r=0.71) and the 16-30 min (r=0.52) time-points within the SMS. Hence dribbling speed over a 20-m seems to be a moderately reliable soccer skill to assess when embedded within the SMS protocol. On the contrary, dribbling accuracy seems to be poorly reliable within the SMS protocol. Harper et al. (2016) demonstrated moderate reliability during the first 15-min of activity only (r=0.64). However, correlation values (using Pearson's correlation) thereafter (30-120 mins) corresponded with poor reliability. As both mental and physical fatigue influence skill performance (Ramsbottom et al., 1988; Smith et al., 2015; Reilly and Holmes, 1983; Rampinini et al., 2009), Harper et al. (2016) attributed the respective lower reliability values to the fact that skill performance was measured in a fatigued state (Foskett et al., 2009). Thus, with respect to previous observations, the data presented in this investigation would suggest that dribbling accuracy over 20-m when assessed within an SMS protocol has poor reliability even when not fatigued, particularly beyond the initial 15 minutes of activity (Harper et al., 2016).

4.5.2 Reliability of passing accuracy and speed

To retain possession of the ball, accurate passing is a frequent and essential skill throughout soccer match-play (Rampinini et al., 2009; Hughes and Franks, 2004). Longer passing sequences and a greater number of successful passes are associated with more goals scored (Hughes and Franks, 2004). Russell et al., (2010) reported moderate reliability (ICC=0.51) when assessing passing accuracy. As previously highlighted by Russell et al. (2010), it is possible to make comparisons between studies that report reliability in different ways. Using the intraclass correlation coefficient, the present data revealed good reliability for passing accuracy using both feet, for both short and long passes, however, the CV revealed that there was only a moderate rating on variability. Regarding passing speed, we observed good reliability for the short pass and excellent reliability for the long pass with both feet. Russell et al. (2010) observed similar reliability for passing speed with an ICC of 0.76. Ali et al. (2007) aimed to assess the reliability of the Loughborough Soccer Passing Test (LSPT) in elite and non-elite soccer players. While the outcomes from the LSPT (time taken, time penalties for incorrect actions and total time) are not aligned to the outcome measures of the SMS passing assessment (i.e., accuracy, speed), the reliability of these outcomes are comparable with those shown by Russell et al. (2010). When comparing reliability for passing accuracy using the ICC, in the current study to the total time for the LSPT (as global marker of accuracy) greater reliability was demonstrated. Thus, passing performance (accuracy, speed) with both feet appears to be a reliable skill to assess within a SMS. We suggest the improvement observed in the present study may be due to players being able to use their routine soccer footwear (boots), instead of trainers (indoor surface). In addition, replacing the passing markers with mannequins may have also helped the players passing performance by providing a more realistic target.

Skill performance declines with fatigue (Mohr et al., 2005), and it appears that the non-dominant side may be more susceptible to this decline (Rodriguez-Giustiniani et al., 2019; Bottoms et al., 2007; McRae and Galloway, 2007). This could be due to the non-dominant side requiring greater central nervous system allocation to perform accurately, and thus fatigue having a greater impact on it (McMorris and Keen, 1994). Therefore, it is important to discriminate between dominant and non-dominant foot when assessing passing performances. As reported in the previous chapter, long passing speed was better maintained with carbohydrate ingestion versus placebo during the latter stages of a SMS protocol in the non-dominant foot only (Rodriguez-Giustiniani et al., 2019). The present data reveal that reliability of passing accuracy for the long pass is good for the dominant foot, and moderate for the non-dominant foot. However, this level of reliability was not evident for the short pass, which was moderate for the dominant foot and poor for the non-dominant foot.

By reducing the passing distance between the player and target, a reasonable assumption would have been improved, or at least similar, reliability to that of the long pass. It was observed that reliability for passing speed was excellent (ICC: > 0.90) for both the dominant and the non-dominant foot for the long pass, whereas it was classified as good for both the dominant and the non-dominant foot for the short pass. Thus, since short pass targets were positioned at a 30° angle from the ball contact point whereas long pass targets were located at a 60°angle from the ball contact point, it was speculated that the more lateral positioning of the short pass targets may have negatively influenced the reliability. The effect of increasing the passing distance further, to >8 m (typical of goal keepers and common for all outfield players), on reliability of passing remains to be established. Nevertheless, dominant, and non-dominant foot passing skills (accuracy, speed) within the SMS seem to be reliable assessments within this group of players, on an artificial grass surface when football mannequin targets are used.

4.5.3 Reliability of shooting accuracy and speed

The aim of soccer is to score more goals than the opposition, thus, shooting is a crucial skill (Stone and Oliver, 2009). When assessing the reliability of soccer-skills, Russell et al., (2010) revealed poor reliability (ICC=0.38) when assessing shooting accuracy. The present data also show poor reliability for shooting accuracy when considering data for both feet combined. As previously stated, the skill assessments in Russell's study were evaluated in isolation and not integrated within the blocks of running *per se* as in the present study. As comparisons are possible where reliability is reported in dimensionless units, both the present data and that presented by Russell et al. (2010) appear to be more reliable than the Loughborough Soccer Shooting Test (Ali et al., 2007). Ali et al. (2007) evaluated the reliability of the Loughborough Soccer Shooting Test (LSST) in elite and non-elite soccer players, they reported poor reliability (ICC=0.26) for shooting success and accuracy in both groups. Moreover, when discriminating between the dominant and the non-dominant foot the present data also demonstrate poor or moderate reliability for shooting accuracy with the dominant and the non-dominant foot, respectively.

There may be several reasons for poor shooting reliability, such as the increased distance versus the long pass (15-m v 8-m). The increased shooting speed generated when performing a shot (Table 2) may also add variation to the skill, as well as technique performed in the execution of shooting (front of foot/laces) versus passing (instep) per se. These results are consistent with data presented by both Russell et al. and Ali et al. (2007) who reported poor shooting reliability, over distances of 15 m and 16.5 m respectively. It should be noted that only two of the ten participants in the present study were classified as strikers. Players adopt playing positions due to their suitability and skill. Therefore, position specific participants may be required to increase the reliability and assessment of specific skills such as shooting.

4.5.4 Intra- and inter-rater reliability

The data showed good to excellent intra-rater reliability for almost all of the variables when assessed by the study researcher and two video analysts within a professional football team. This indicates that the analysts' assessments were on the whole consistent on repeated analysis. When multiple raters assessed the studied variables, excellent inter-rater reliability was observed. This excellent inter-rater reliability indicates that different raters can consistently assess the experimental trials. Therefore, it is recommended that experienced video analysts/researchers are in charge of the digitisation when assessing skill outcomes using this modified version of the SMS protocol. For large scale projects, the ability to use multiple experienced researchers/analysts offers important methodological considerations on data assessment and input

4.5.5 Limitations

It has been stated that proficient skill performance is affected by cognitive factors such as decision making and game intelligence (Williams and Reilly, 2000). In this study, a randomised lighting system for target identification when assessing passing and shooting as used in the original version of the protocol (Russell et al., 2011) was not utilised. This modification was made to increase ease of implementing the protocol in professional club settings. Therefore, it is unknown how inclusion of decision making and visual searching, would have influenced the skill reliability.

It could be that including such perceptual demands would increase the ecological validity. However, the modified version of the SMS used in the present study could be more practically applicable in professional club settings as the equipment used (mannequins) are readily available.

Due to the fact that more than 40 participants are recommended for reliability studies (Hopkins, 2000; Atkinson and Nevill, 1998) these results are only preliminary and further reliability assessment of this version of the SMS is warranted. However, these results are applicable to professional academy football players. Russell et al (2010) also reported construct validity when they assessed the reliability of the skills contained within the SMS and observed that professional football players performed better than recreational players. There are no data regarding this aspect for this modified version of the protocol, but it would be of interest to test for construct validity in a further larger scale study.

In conclusion, this modified version of the SMS protocol showed encouraging reliability, especially for dribbling speed, sprint speed, short and long pass speed, shooting speed, and long pass accuracy. How skill reliability may change as the academy players transition to senior teams, and the reliability of other soccer specific skills such as heading, and ball control remain to be established. This testing protocol has potential application for research settings out of the laboratory when investigating strategies that aim to improve skill performance in professional soccer players.

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CHAPTER 5
INFLUENCE OF COFFEBERRY ON SOCCER SKILL PERFORMANCE UNDER RESTING CONDITIONS

5.1 Abstract

The present study aimed to investigate the potential effects of coffeeberry ingestion on skill performance and subjective measures of mood/arousal, fatigue, and mental/physical energy under non-fatigued resting conditions. Thirty male well-trained professional outfield soccer players (age 18±2 y) ingested a 300 mg dose of coffeeberry administered in a double-blind randomised cross-over design after completing a baseline skill assessment that evaluated dribbling, sprinting, passing, and shooting performance. Following a 60-min rest period to allow for delivery and absorption of coffeeberry, a second skill assessment evaluating soccer-specific skills (dribbling, sprinting, passing, and shooting) was conducted. While no primary interaction effects were detected in the outcome measures, there were significant time effects ($p<0.05$) as shooting accuracy was higher for both the coffeeberry (from 22±11 points to 33±13 points out of 40 possible points) and the placebo (from 18±9 points to 25±10 points out of 40 possible points) trials between first and second skill assessments. Fatigue ratings increased for both the coffeeberry (from 22±14 to 24±10 out of 100) and the placebo (from 19±14 to 31±17 out of 100) trials, between the first and second skill assessments. These data indicate that coffeeberry has no impact upon soccer skill measures under non-fatigued conditions. Since this study was conducted in a rested state, it is reasonable to propose that there is a need to conduct similar investigations following physical and/or mental fatigue-inducing activities where declines in skill may be evident. If wishing to explore the potential impact of coffeeberry in mitigating the adverse effects that fatigue might impose on soccer-specific skill performance then match play and fatigue situations need examined.

Keywords: football; technique; polyphenols; chlorogenic acid

5.2 Introduction

Performance in team sports is heavily based on various cognitive tasks such as decision-making, motor control, timing, and coordination (Baker et al, 2014). In fact, according to Williams (2000), while physical attributes are important for succeeding in soccer, the distinguishing factors between elite and amateur soccer players are their cognitive abilities, high level skill execution, and ability to maintain execution of those skills when fatigued. Soccer skill performance is dependent upon cognitive, perceptual, and motor skills, which interact in rapidly changing environments. Therefore, acute nutritional interventions that can influence cognitive abilities and the quality of technical actions (i.e., skilled performances) might have an important role to play when seeking to optimise performance. Any such nutritional intervention is likely to be of considerable interest to soccer players and those responsible for the technical preparations of these athletes (Russell and Kingsley, 2014). Cognition plays a crucial role in a soccer player's performance as it involves aspects such as motor control, coordination, decision making, timing, and other cognitive processes. Motor skill, which refers to the ability to carry out complex muscle-to-nerve movements, is further divided into fine and gross motor skills, with the latter being particularly important in soccer, as it affects actions such as dribbling, passing, and shooting (Baker et al., 2014).

There is consistent evidence that both acute and chronic supplementation of polyphenols can improve vascular function by enhancing nitric oxide-dependent-endothelium-dependent vasodilation as measured in vivo as flow-mediated dilatation (Hooper et al., 2012). Moreover, ingested phenolic compounds (or their metabolites), have acute physiological effects that plausibly could influence human brain function. These effects include enhancing regional brain blood flow perhaps through actions on nitric oxide (de Mejia and Ramirez, 2014), or binding to adenosine (Karton et al., 1996), GABA (Wawoski and Marder, 2012), nicotinic (Lee et al., 2011), and opioid receptors (Katavic et al., 2007) or receptor tyrosine kinases (Figueira et al., 2017). Increased brain perfusion induced by nitric oxide-dependent vasodilation could specifically exert polyphenols' potential to promote memory, learning, and cognitive function (Vauzour, 2012). Furthermore, an increase in cerebral blood flow could improve cognitive performance on a wide range of tasks via a number of possible mediating mechanisms, including increased motivation, attention, or arousal (Field et al., 2011). Also, an increased blood flow would enhance the supply of metabolic substrates to individual neurons involved in task specific processing which might improve their efficiency (Scholey et al., 2010). Coffeeberry is the fruit of the coffee plant (also known as coffee cherry or coffee fruit) which is typically discarded during the coffee bean harvesting process, this fruit is loaded with antioxidants and polyphenols, especially phenolic components (prominently chlorogenic acids; Mullen et al., 2011). Investigations examining the effects of coffeeberry are limited, but chlorogenic acids from other sources (coffee, green coffee bean) have been more widely documented (Tajik et al., 2017; Sato et al., 2011; Suzuki et al., 2006). Di Lorenzo et al. (2021) reviewed the effects of chlorogenic acid and its metabolites in coffee, or in beverages prepared in order to mimic coffee intake. Phenolic acids were administered to healthy subjects in the range of 139 to 900 mg/day and were tested with both acute and chronic consumption (over a maximum of eight weeks). Vascular function was positively affected by chlorogenic acid (CGA) provided by decaffeinated coffee intake (50 mL) or purified caffeoylquinic acid (5-CQA). Other confirming studies also demonstrate that phenolic compounds, other than caffeine, can contribute to vasoactive efficacy (Mubarak et al., 2012; Mills et al., 2017).

Coffeeberry have been found to have acute physiological effects that plausibly could influence human brain function and therefore affect psychological variables (Reed et al., 2019). Reed et al. (2019) reported that the ingestion of beverages containing low (100mg) and moderate (300 mg) amounts of coffeeberry extract significantly attenuated both increases in self-reported fatigue and decreases in self-reported alertness resulting from the completion of a series of fatiguing cognitive tasks. The authors reported that the effects of coffeeberry extract on alertness and fatigue became statistically significant 2 hours after beverage consumption; however, the beverages began to exert mental energizing effects from 1 to 2 h post-ingestion. A recent study by Robinson et al. (2021) suggested that the ingestion of a single dose of coffeeberry extract is associated with improved reaction time and may protect against cognitive errors on tasks of working memory and response inhibition in an aging population with subjective cognitive decline. It was concluded that coffeeberry extract is associated with acute neurophysiological changes supportive of faster reaction times and increased, sustained attention.

The present study aimed to investigate the potential effects of coffeeberry ingestion on skill performance and subjective measures of mood/arousal and mental/physical energy initially under non-fatigued conditions. To our knowledge no studies have applied a coffeeberry intervention in an exercise study, therefore, we initially set out to determine potential effects of coffeeberry on measures of skill performance and subjective measures under rested conditions rather than during match-play or fatigued conditions. We hypothesized that coffeeberry may alter the subjective measures of mood/arousal and mental/physical energy, but without fatiguing conditions it would likely not have any impact on soccer skill performance.

5.3 Methods

5.3.1 Participants

Thirty male well-trained professional outfield soccer players from the United Kingdom (5 strikers, 16 midfielders, and 9 defenders), who were accustomed to skill assessments as part of their regular training were recruited from a local Professional Football Club development squad in order to participate in this investigation. All players had five or more years of playing experience, had been training consistently for 1 year or more, were regularly participating in match-play with their squad, and were free from injury at the time of the recruitment and testing (age: 18±2 y, body mass: 73.1±7.6 kg, stature: 178.3±5.7 cm, body mass index: 23.4±1.0 kg/m^2). The experimental procedures were approved by a local Ethics of Research Committee and the study was conducted in accordance with the declaration of Helsinki.

5.3.2 Study Design

The study followed a double-blind, randomised, placebo-controlled, crossover design. After receiving the signed consent form, and performing an initial pre-screening participants undertook a first habituation session of the skill performance outcomes (dribbling, sprinting, passing, shooting). On a subsequent visit, participants went through a familiarisation trial that included a run through of the experimental trial protocol. This included warm up and two soccer-specific skill assessments.

Each of the skill assessments required the participants to perform 3 dribbles, 3 sprints, 8 passes (4 with the dominant foot and 4 with the non-dominant foot), and 8 shots (4 with the dominant foot and 4 with the non-dominant foot). In order to assess dribbling speed and accuracy, players dribbled a ball between 7 cones (cones 2–7 were placed 3-m away from the preceding cone, and cones 1 and 7 were 1-m away from each end of the course). Participants were required to dribble the ball as quickly and accurately as possible from one end to the other over the 20-m total distance. For the sprint assessment, players ran as fast as possible through timing gates (Brower®, USA) placed 15-m apart, with a 1-m run-in. Subsequently, players performed a bout of passing where they directed alternate passes towards target zones placed to the left and right at distances of 4.2-m (short pass) and 7.9-m (long pass).

Soccer mannequins (Diamond Football®, Senior Pro Free Kick) with their bases were used as the passing targets. The base (0.5-m wide) was the central zone of the target, with cones at a distance of 0.5-m of each side as the lateral zones of the target. A pass to the centre area was worth 10 points and the two lateral areas were worth 5 points. Passes that missed the target areas were scored as 0. Passing bouts consisted of 8 passes (2 with the dominant and 2 with the non-dominant foot to the short pass target, and 2 with the dominant and 2 with the non-dominant foot to the long pass target). Then, the shooting skill assessment was performed. For this, participants were instructed to kick the ball as firmly and accurately as possible to a shooting target. Shooting target zones were at a distance of 15-m in the four corners of the goal. These target areas have been previously identified as optimal ball placement to beat a goalkeeper when shooting (Ali et al., 2007). Each shooting target was divided into two areas, a centre area (75 cm x 60 cm) and an extended area (100 cm x 90 cm) with the centre area worth 10 points and the extended area worth 5 points. Shots that missed the areas on the target were scored as 0. The bouts of shooting consisted of 8 shots (4 with the dominant foot and 4 with the non-dominant foot). The ball was rolled into the action area for passing and shooting assessments. The schematic representation of the dribbling, passing, and shooting assessments is presented in the previous chapter.

Video footage of the skill assessments was captured using GoPro cameras (GoPro®, Hero 5), one was placed 1-metre from the last cone of the dribbling course and the other 1-metre behind the passing and shooting zone. Manual digitisation (Kinovea® version 0.8.15; Kinovea Org., France) yielded dribbling speed, dribbling accuracy, passing accuracy, and passing speed, as well as shooting accuracy and speed. Passing and shooting speed was calculated from the time interval between ball contact with the foot and subsequent ball contact with the target area factoring in the distance travelled. All testing sessions were performed on an indoor artificial grass pitch (length: 37-m; width: 19-m; ceiling height: 6.5-m) and were completed in the afternoon to reflect the time at which this group typically engages in soccer match play. Soccer balls were inflated to a pressure of 14 psi before each trial.

5.3.3 Experimental Trials

At least 7 days following the familiarisation visit, participants attended the first of their 2 main experimental trial days (**Figure 5.1**). Trials were a placebo trial and a coffeeberry extract trial (300mg dose) administered in a double-blind placebo controlled cross-over design. Trials were separated by 7-14 days. Participants followed their habitual diets but avoiding coffee, caffeinated and decaffeinated products, apples, and berries/cherries 24-h prior to main testing days, and alcohol 48-h before the main trials) and recorded all food consumed for 48-h before the first trial and replicated it 48-h prior to the second trial. Participants were also requested to abstain from strenuous physical activity for 24-h before participation on main testing days. Participants attended the training ground at 8:30 a.m. and were provided with a standardised breakfast (2 eggs, 2 slices of bread, and 1 medium-sized banana, providing 423 kcal, 46g carbohydrate, 26g protein, 14g fat). Later, a pre-trial standardised pre-match meal was also provided 2-h before beginning the main trials, with the meal containing 2g of carbohydrate per kilogram of body mass (pasta in a tomato sauce) plus 500 mL of water. All trials started between 12 and 2:00 p.m.

Upon arrival at the indoor pitch for the main trials, body mass (SECA Quadra 808. Hamburg, Germany) was assessed immediately after players emptied their bladder (with urine volume collected into a container, sleep quantity (hrs.) and quality (1-5 scale) was also evaluated on arrival, and a baseline blood sample was taken (7 mL obtained by venepuncture), for assessment of caffeine and chlorogenic acids (chlorogenic acid data not yet available), then players undertook a 10-min standardized warm-up (consisting of running, dynamic stretching, and 20-m sprints) followed by a baseline assessment of skills (dribbling, passing, and shooting) before ingesting the allocated trial beverage over a 2-3-minute ingestion period. The beverages (300 mL) were ingested on one occasion, just after the players completed the first skill assessment. The coffeeberry and placebo beverages were ready-to-drink formulations (PepsiCo International Ltd., USA) matched for flavour, colour, and texture. The test drink contained water, citric acid, coffeeberry, gum arabic, natural flavour, sucralose, acesulfame potassium, glycerol ester of rosin, yellow 6. The measured content of chlorogenic acid and caffeine was 117.9±0.8 ug/mL and 42.2±0.4 ug/mL respectively. The placebo drink contained all of the abovementioned ingredients with the exception of coffeeberry and no CGA nor caffeine were detected. After a 60-min rest period, to allow for delivery and absorption of the active ingredients in the test beverage, a second venous blood sample (7 mL) was obtained by venepuncture. Next, a second warm-up and skill assessment were conducted. Urine samples from baseline and at the end of the trial obtained prior to body mass measurements, were retained for the assessment of osmolality using a portable osmometer (OsmoCheck, Vitech Scientific Ltd). Fluid (water) was allowed *ad libitum* during the first trial and was replicated for the second trial. Baseline hydration status was assessed using a combination of body mass and urine osmolality. Urine production was assessed and sweat losses determined from change in body mass corrected for fluid intake and urine losses (% Body Mass Loss = Body Mass Loss (kg)*100 / Pre-trial Body Mass). Subjective scores of exertion (RPE, Borg 1973), and visual analogue scales for determination of mental energy, physical energy, feelings of fatigue and gut comfort, alongside assessment of activation/deactivation and heart rate measurements, were obtained at intervals during the protocol. A schematic of the trial protocol is shown is **Figure 5.1.**

5.3.4 Analytical Procedures

Urine samples were collected into a 0.5-L plastic container and total mass (to the nearest 0.1 g) assessed to determine urine volume. A 5-mL aliquot was then dispensed into a plain screw- capped tube and was analysed for urine osmolality after the trial ended using a portable osmometer (OsmoCheck, Vitech Scientific Ltd). Blood samples were sent to Griffith University in Australia where caffeine and chlorogenic acid were assessed through liquid chromatography-mass spectrometry (Appendix 3).

5.3.5 Statistical Analysis

Baseline data for skill performance measures were assessed using a one-way ANOVA. Subsequently, two-way repeated measures ANOVA were used to assess trial (intervention vs placebo), time (first skill assessment to second skill assessment 1 hour after beverage ingestion) and any trial by time interaction effect. Associations were assessed for any variable that was observed to change over time using Pearson correlation analysis, when r (the correlation coefficient) is near 1 or -1, the linear relationship is strong; when it is near 0, the linear relationship is weak. Data are presented as mean (SD) in the text or are presented as median (range) depending upon the outcome of normality testing. Data presented in figures contain median, interquartile range, minimum and maximum values shown in box and whisker plots, this was to provide more insight into the spread of values obtained within each variable. Statistical significance was set at $p<0.05$ for all analyses.

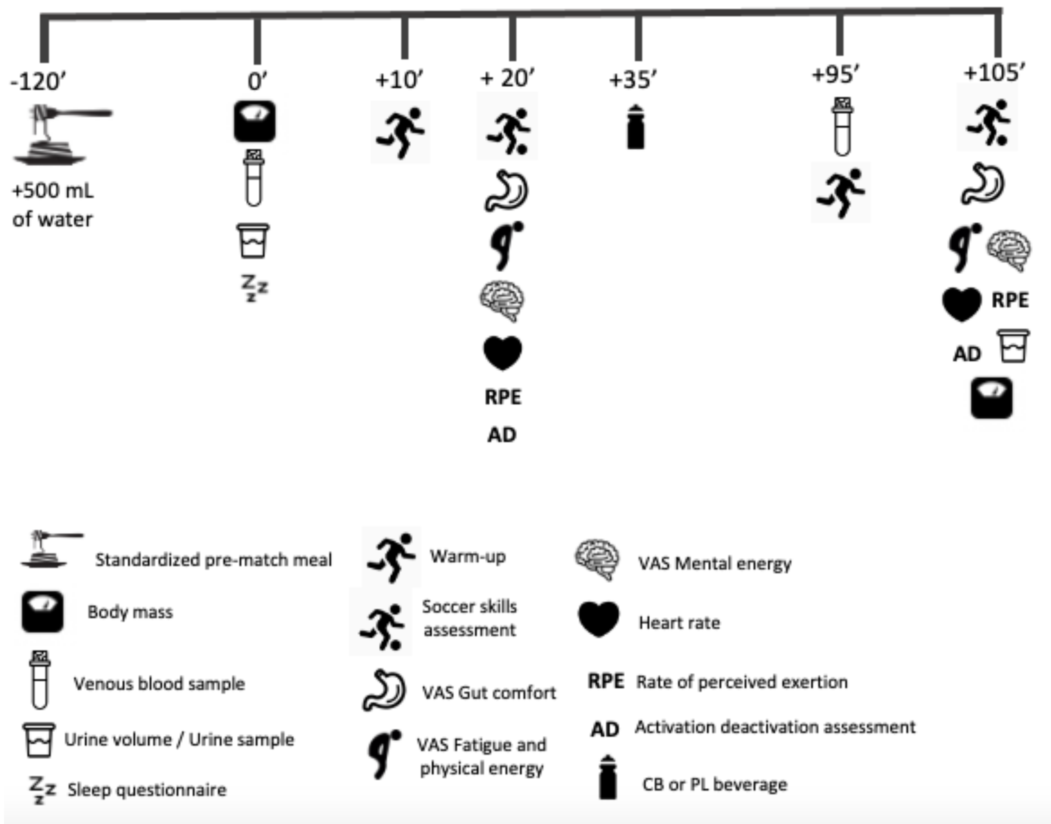

Figure 4.1. Schematic diagram of study protocol

5.4 Results

Data were normally distributed for all variables (p>0.05) and there were no baseline differences between the two trials. There were no differences in nutritional intake between coffeeberry (CB: total energy: 8.4±1.9 MJ/d; carbohydrates: 285±71g/d; proteins: 100±51 g/d; fats: 49±13 g/d) and placebo (PL: total energy: 8.8±2.1 MJ/d; carbohydrates: 287±51 g/d; proteins: 97±17 g/d; fats: 53±17 g/d; all p>0.05) trials in the 48-h period before the main experimental trial days. Ambient temperature (CB: 10±4 °C, PL: 10±3 °C) and relative humidity (CB: 65±10 %, PL: 68±10 %) were similar between trials (p>0.05). Regarding hydration variables there were no significant differences for body mass loss during the trials (CB: 0.23±0.03 %; PL: 0.21±0.03 %), pre-trial urine osmolality (CB: 430±148; PL:433±149 mOsm/kg), and post-trial urine osmolality (CB: 353±177; PL:360±160 mOsm/kg). Likewise, there were no differences noted between trials on pre-trial sleep duration (CB: 8±1 h; PL: 8±1 h, p>0.05).

Caffeine was detectable at low concentration in two participants across all samples collected (Participant 1: placebo trial, baseline sample: 0.4 mg/L, 1-h post-ingestion sample: 0.3 mg/L; coffeeberry trial, baseline sample: 0.3 mg/L, 1-h post-ingestion sample: 0.2 mg/L. Participant 2: placebo trial 1, baseline sample 1: 0.9 mg/L, 1-h post-ingestion sample: 0.9 mg/L; coffeeberry trial, baseline sample 1: 0.6 mg/L, 1-h post-ingestion sample: 0.5 mg/L). No caffeine was detected in samples for all other participants. Statistical comparisons for all variables were examined with and without these two participants and no differences in outcomes were observed. Apart from this, the caffeine values measured were below those reported following caffeine ingestion; thus these participants were included in all outcome analyses reported.

5.4.1. Dribbling

Dribbling speed (m/s) was not different between trials ($p=0.81$) or between initial baseline skill assessment and 1hr post beverage time points ($p=0.10$), and no interaction was evident between trial and time ($p=0.81$; **Figure 5.2.A**). Similarly, there was no trial ($p=0.72$) or time ($p=0.53$) effect, or trial by time interaction ($p=0.79$) for dribbling accuracy (**Figure 5.2.B**).

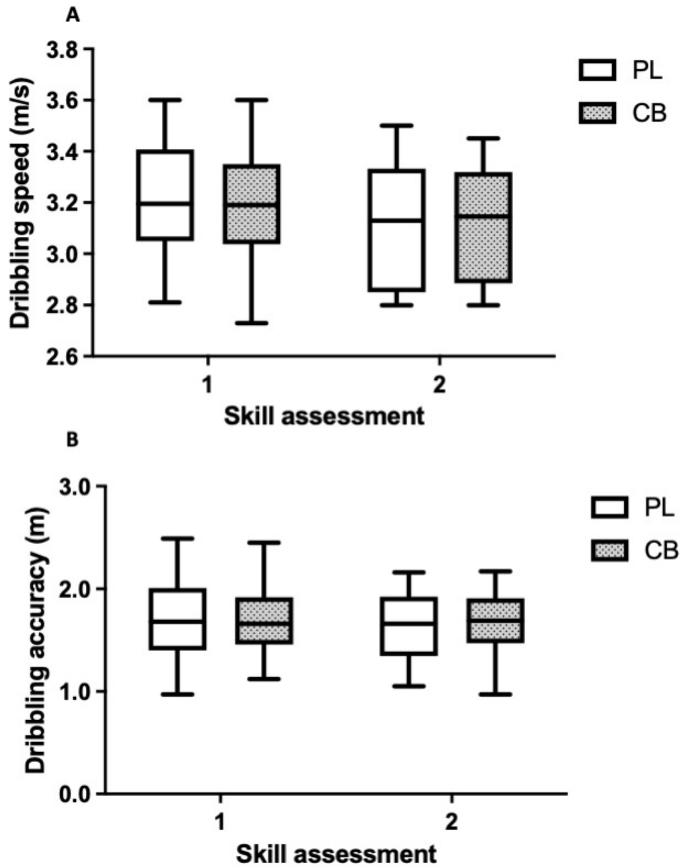

Figure 5.2. Dribbling speed (A) and dribbling accuracy (B) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB). N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot.

5.4.2. Sprinting

There was no trial (p=0.70) effect or a trial by time (p=0.48) interaction. However, there was a significant time effect (p<0.01) with slower sprint times at skill assessment 2 conducted 1 hour after test beverage ingestion (**Figure 5.3**). The mean difference (95% confidence interval) for the change in sprint times when combining two trials together was 0.11 [-0.22, 0.0] s.

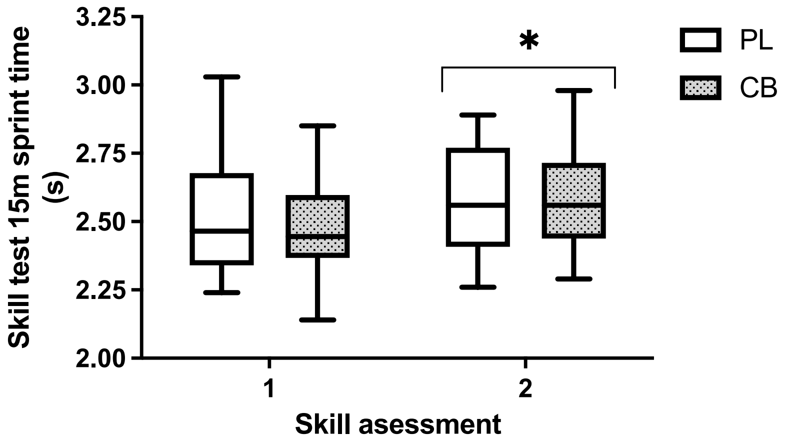

Figure 5.3. Sprint speed during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB). N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot.*represents significant slower sprint times 1 hour after beverage ingestion (p<0.05).

5.4.3. Passing (short pass)

<u>Both Feet:</u>

There were no differences across passing speed in the short pass (m/s) with no trial (p=0.08), time (p=0.48) or trial by time (p=0.61) interaction (**Figure 5.4.A**). Regarding short pass accuracy there was no time (p=0.13) or trial by time interaction (p=0.58), but there was a trial effect (p<0.05; **Figure 5.4.B**), as the values were higher for the coffeeberry trial than the placebo trial when both assessment points were combined.

<u>Dominant Foot:</u>

When assessing passing performance within the short pass with the dominant foot there were no differences on passing speed (m/s) as there was no trial (p=0.14), time (p=0.21) or trial by time interaction (p=0.76) effects (**Figure 5.4.C**). The same scenario occurred with short pass accuracy with no time (p=0.17), trial (p=0.09) or trial by time (p=0.74) interaction effects (**Figure 5.4.D**).

<u>Non-Dominant Foot:</u>

There was no trial (p=0.11), time (p=0.69) and no trial by time interaction (p=0.77) effect for short pass passing speed (m/s) with the non-dominant foot (**Figure 5.4.E**). Similarly, there were no effects of time (p=0.15), trial (p=0.11) or trial by time interaction (p=0.10) for passing accuracy within the short pass on the non-dominant foot (**Figure 5.4.F**).

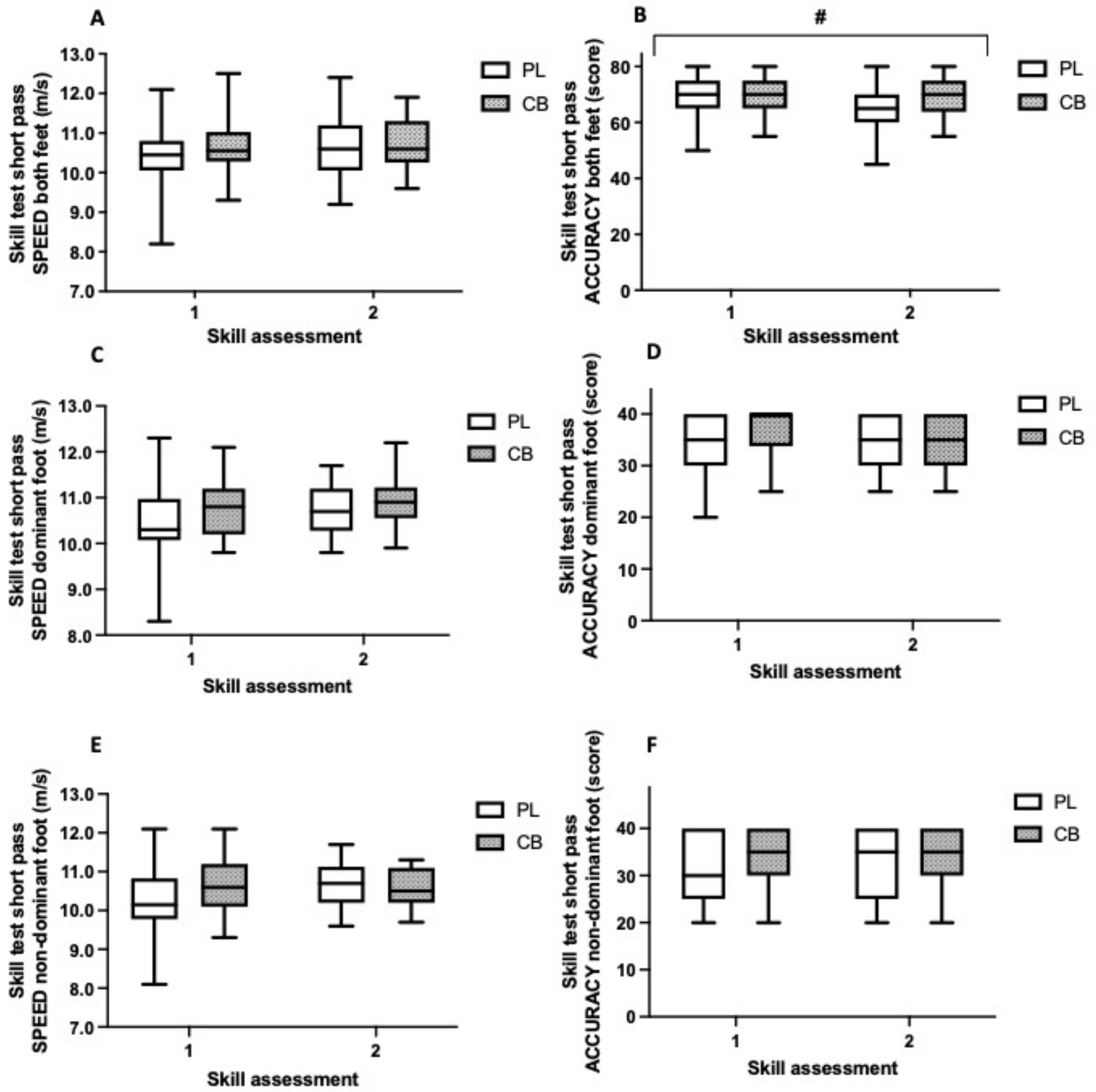

Figure 5.4. Passing speed and passing accuracy on the short pass for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB). N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. # indicates significant differences between PL and CB trials (p<0.05).

4.4.4. Passing (long pass)

Both Feet:

There was no time effect (p=0.61) or trial by time interaction (p=0.81) for long pass speed with both feet (m/s). However, there was a main trial effect (p<0.01; **Figure 5.5.A**). There were also no differences in long pass accuracy with both feet, with no trial (p=0.80) or time (p=0.52) effects nor trial by time interaction (p=0.37) observed between the skills assessments (**Figure 5.5.B**).

Dominant Foot:

There was no significant difference between the trials for passing speed (m/s) with the dominant foot within the long pass as trial (p=0.10) or time (p=0.08) effects nor trial by time (p=0.31) interaction were not observed (**Figure 5.5.C**). The same results were found for long pass passing accuracy as there were no trial (p=0.93) or time (p=0.22) effects and no trial by time (p=0.45) interaction (**Figure 5.5.D**).

Non-Dominant Foot:

There were no trial (p=0.14), time (0.10) or trial by time (p=0.59) effects for long pass speed (m/s) with the non-dominant foot (**Figure 5.5.E**). Similarly, there was no significant difference between trials for passing accuracy with the non-dominant foot as there were no time (p=0.38) and trial (p=0.30) effects, nor a trial by time interaction (p=0.64; **Figure 5.5.F**).

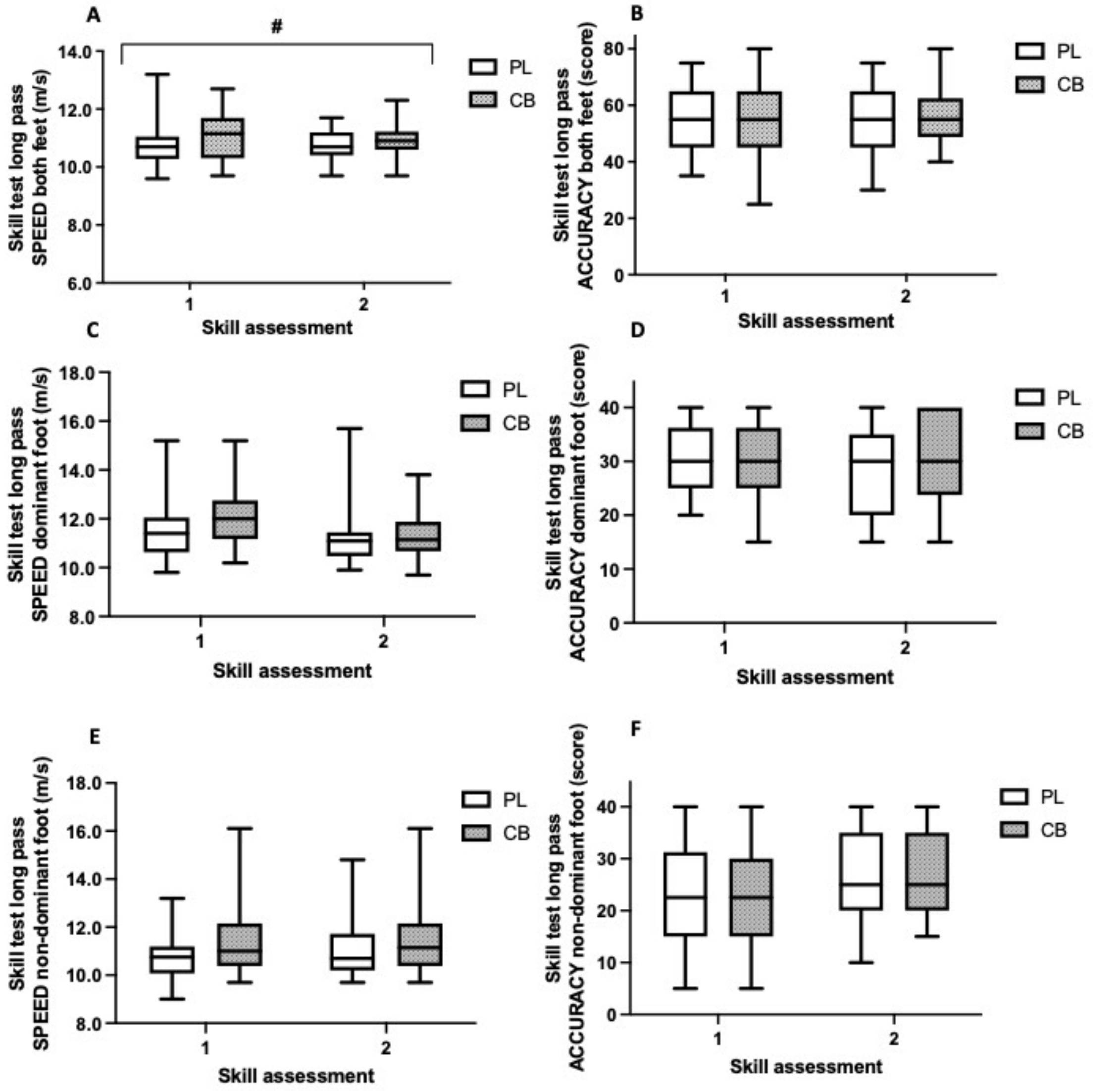

Figure 5.5. Passing speed and passing accuracy on the long pass for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB).N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. # indicates significant differences between PL and CB trials (p<0.05).

4.4.5. Shooting

<u>Both Feet:</u>

There was no trial (p=0.21), or time (p=0.29) effect, nor a trial by time interaction for shooting speed (p=0.05; **Figure 5.6.A**). However, there was a time effect (p<0.01) and a trial effect (p<0.01) but no interaction (p=0.14) for shooting accuracy. The time effect revealed that shooting accuracy improved from skill assessment 1 to skill assessment 2 on both trials, with the trial effect indicating higher scores on CB trial (**Figure 5.6.B**).

Dominant Foot:

When assessing shooting speed with the dominant foot we did not find time (p=0.18) or trial (p=0.08) effects, nor a trial by time interaction (p=0.75; **Figure 5.6.C**). In regard to shooting accuracy there was a time effect (p<0.001). Shooting accuracy improved from pre to post drink ingestion on both trials, however, no trial (p=0.38) or trial by time interaction (p=0.103) effects were encountered (**Figure 5.6.D**).

Non-Dominant Foot:

Regarding the non-dominant foot, we did not find trial (p=0.52) or time (p=0.48) effects and also no trial by time interaction for shooting speed (p=0.21) (**Figure 5.6.E**). Likewise, there was no trial (p=0.07) or time (p=0.23) effects nor a trial by time interaction (p=0.83) for shooting accuracy with the non-dominant foot (**Figure 5.6.F**).

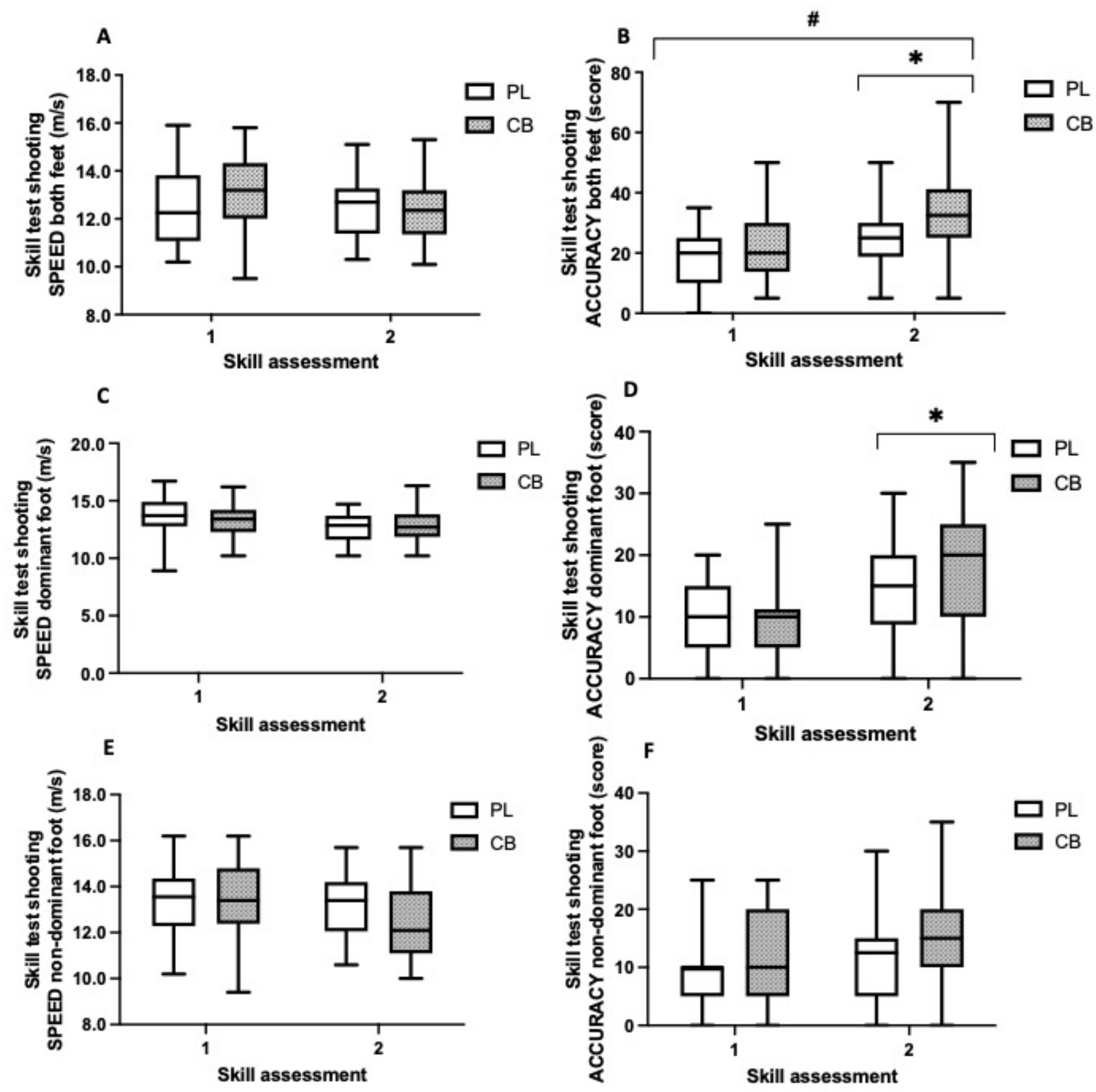

Figure 5.6. Shooting speed and shooting accuracy for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB). N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. # indicates significant differences between PL and CB trials (p<0.05). # indicates significant differences between PL and CB trials (p<0.05). .*represents significant higher scores on skill assessment 2 (p<0.05).

5.4.6. Fatigue, physical energy, mental energy

Regarding fatigue there was a time effect (p<0.05) with higher fatigue values found on skill assessment 2 conducted 1 hour after beverage ingestion. However, no trial (p=0.36) or trial by time interaction effects were present (p=0.29; **Figure 5.7.A)**. There were no differences between trials for physical energy as there were no trial (p=0.21) or time (p=0.16) effects nor a trial by time interaction

(**Figure 5.7.B**). There was no time effect (p=0.24) or trial by time interaction (p=0.64), but there was a trial effect (p<0.001) for mental energy, with higher values on the CB trial (**Figure 5.7.C**).

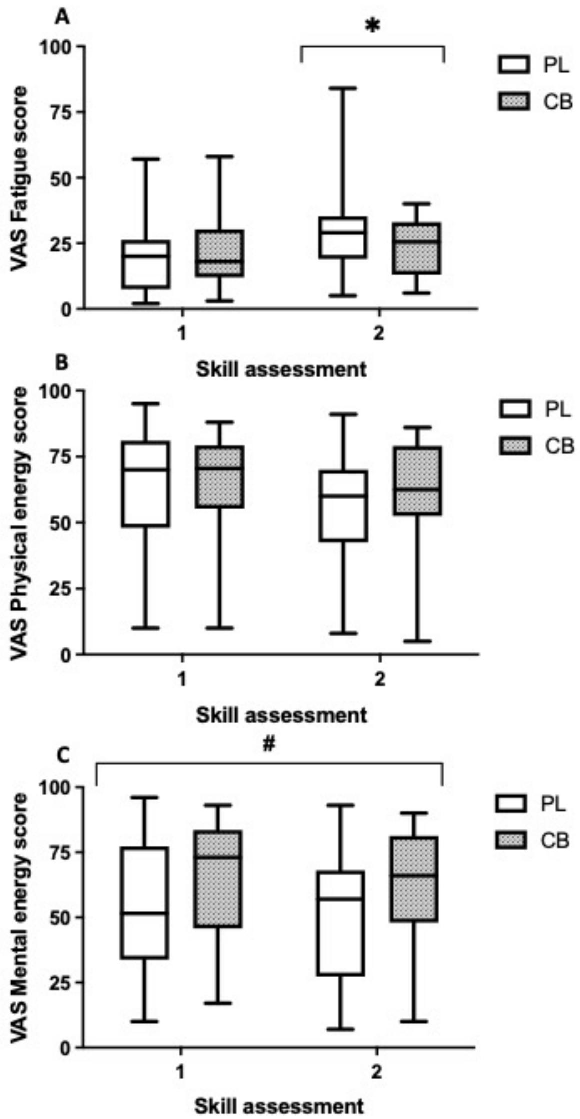

Figure 5.7. VAS (Visual Analogue Scale) for fatigue (A), physical energy (B) and mental energy (C) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB). N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. # indicates significant differences between PL and CB trials (p<0.05). *represents significant higher fatigue values on skill assessment 2 (p<0.05).

5.4.7. Perceived activation-deactivation

Regarding the assessment of activation-deactivation there was a time effect for calmness as the scores were lower for both trials on skill assessment 2 (p<0.001) but no trial (p=0.68) or time by trial interaction (p=0.13) were found (**Figure 5.8.A**). There were no trial (p=0.34), time (p=0.22) or trial by time (p=0.32) effects for tiredness (**Figure 5.7.B**). The energetic score was not different with no trial (p=0.56), time (p=0.50) or trial by time interaction effects (p=0.72; **Figure 5.8.C**). Neither did we observe trial (p=0.68), time (p=0.33) or trial by time interaction effects (p=0.15) for tension (**Figure 5.8.D**).

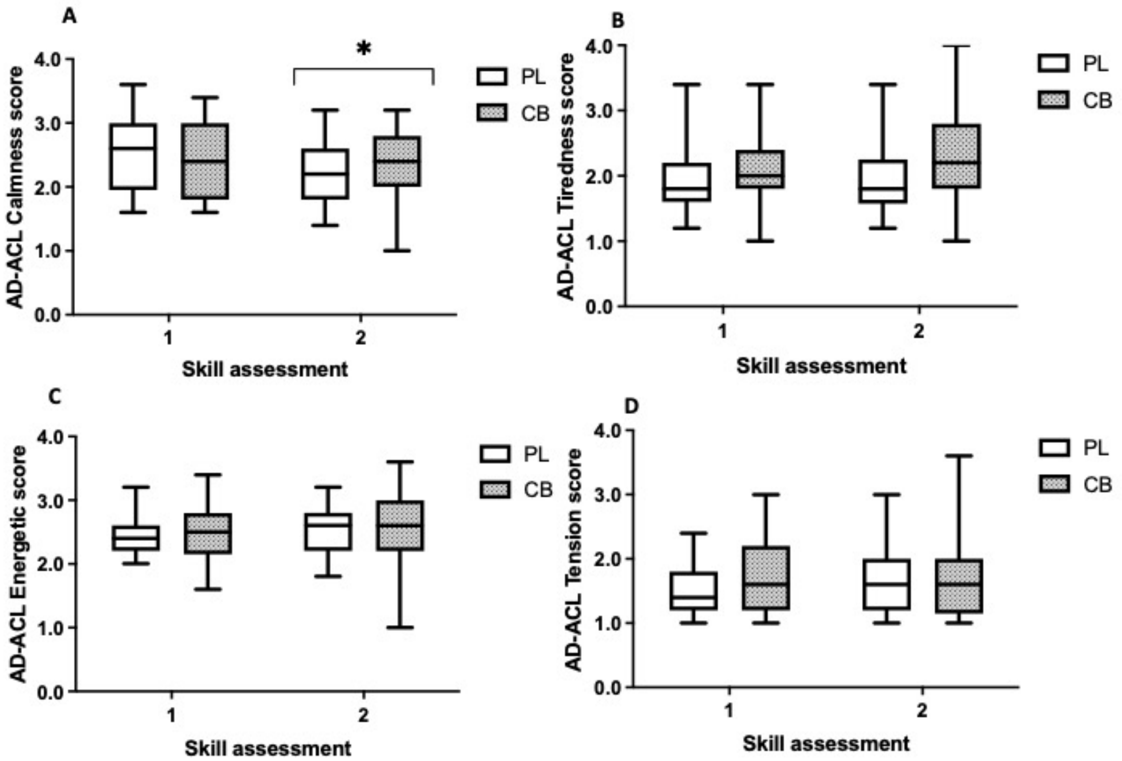

Figure 5.8. Perceived activation-deactivation for calmness (A), tiredness (B), energetic (C), and tension (D) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB). N=30.Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. # indicates significant differences between PL and CB trials (p<0.05). *represents significant reduced calmness on skill assessment 2 (p<0.05).

5.4.8. Bloatedness, fullness, and nausea

Bloatedness, fullness, and nausea were assessed through a visual analogue scale. There was no trial (p=0.44), time (p=0.23) or trial by time interaction for bloatedness (**Figure 5.9.A**). Regarding fullness there was a time effect (p<0.05) with reduced fullness on skill assessment 2, but no trial (p=0.36) or trial by time interaction effects were observed (p=0.72; **Figure 5.9.B**). Likewise, there was a time effect (p<0.001) but no trial (p=0.95) or trial by time interaction noted (p=0.22; **Figure 5.9.C**) for nausea with increased scores after the second skill assessment 1 hour after beverage ingestion.

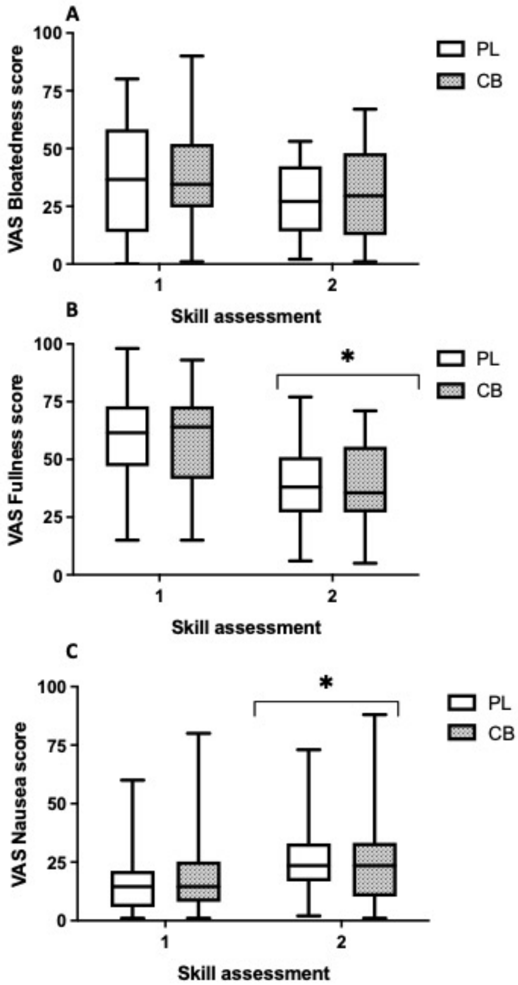

Figure 5.9. VAS (Visual Analogue Scale) for bloatedness (A), fullness (B), and nausea (C) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB). N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. # indicates significant differences between PL and CB trials (p<0.05). *represents significant effect of time with lower values for fullness and higher values of nausea on skill assessment 2. (p<0.05).

5.4.9. Heart rate, rate of perceived exertion (RPE)

There were no differences observed for heart rate with no trial (p=0.78), time (p=0.05), or trial by time interaction (p=0.56) effects noted (**Figure 5.10.A**). Regarding RPE, there was no time (p=0.26) or trial by time interaction (p=1.00), but there was a trial effect (p<0.01) with higher RPE on PL (**Figure 5.10.B**).

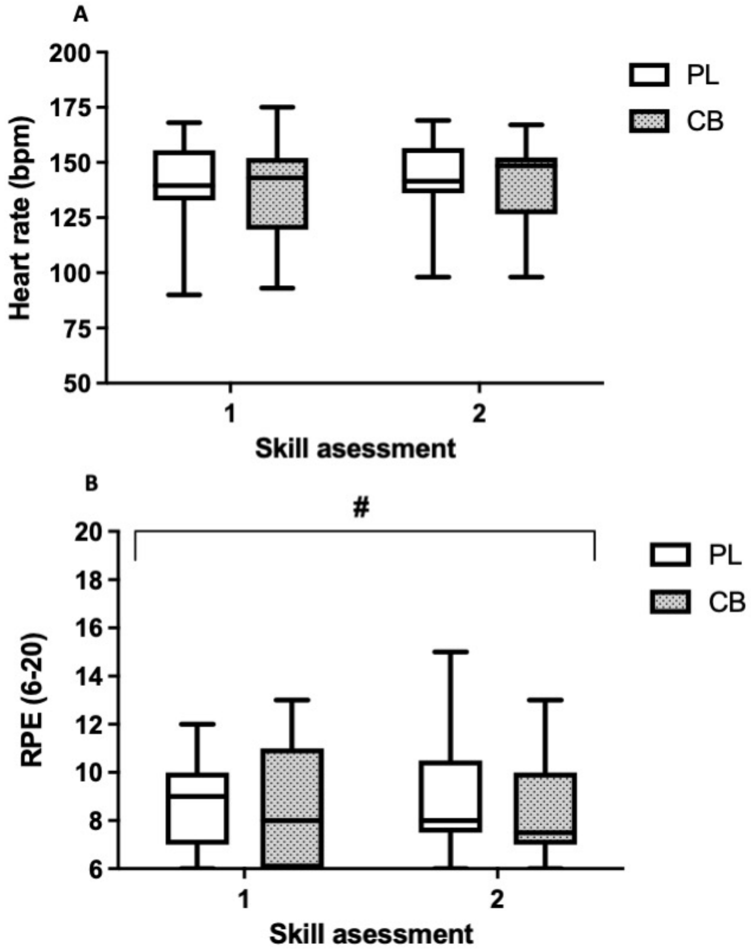

Figure 5.10. Heart rate (A), and RPE (B) during skills assessments 1 (baseline) and 2 (1 hour after beverage ingestion) on placebo (PL) and coffeeberry trials (CB).N=30. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. # indicates significant differences between PL and CB trials (p<0.05).

5.4.10. Association between variables that changed over time

Pearson correlations were performed for the variables in which a significant time effect was found. **Table 5.1** shows the correlation values for sprint and subjective measures, however, there were no significant associations between any of the variables analysed (p>0.05).

Table 5.1. Correlation matrix for sprint time and subjective measures.

Sprint time	1.00				
VAS nausea	-0.25	1.00			
VAS fullness	-0.14	0.05	1.00		
VAS fatigue	-0.15	0.24	-0.03	1.00	
Calmness	-0.16	-0.03	-0.06	-0.15	1.00
	Sprint Time	**VAS nausea**	**VAS fullness**	**VAS fatigue**	**Calmness**

5.5. Discussion

The primary aim of this study was to compare the effects of coffeeberry and placebo ingestion on soccer-specific skills when assessed in a non-fatigued state 1 hour after beverage ingestion. The approach of the present study was to observe whether any changes could be detected under rested conditions after completing a standardised warm-up. It was considered important to assess the potential effects at rest prior to doing any subsequent determination of effects during exercise or under fatigue situations.

The absence of baseline differences between trials for potential confounding variables such as sleep duration the night before trials and the 48-h pre-trial diet indicate that participants adhered to the guidance and standardisation of pre-trial criteria. Conditions during the trials were also well controlled with mean ambient temperature and humidity, and fluid balance and hydration status matched. Having effectively controlled for potential confounding variables, no main interaction effects were observed in the outcome variables. The lack of interaction effects indicates that the coffeeberry beverage ingestion did not elicit any different responses over the time period examined when compared with placebo, under the conditions of the present study. However, time effects were observed, and sprinting speed was slower, shooting accuracy was improved, fatigue was higher, calmness scores were lower, fullness scores decreased, and nausea scores increased, between the first and second skill assessments. In addition, short pass accuracy with both feet, shooting accuracy with both feet, long pass speed with both feet, and mental energy, were all higher on the coffeeberry trial. RPE was lower on the coffeeberry trial, across the two skill assessments conducted before and after test beverage ingestion.

There was some concern about caffeine being detectable in two participants across all blood samples taken. However, the caffeine values observed represent baseline values below usual lower limits of detection. From analysis of the literature, it could be inferred that the plasma caffeine values observed were likely to be due to carry-over from the previous day rather than caffeine ingestion on the morning of the trials. In a study evaluating plasma caffeine concentrations in a US population (de Leon et al., 2003), those participants who ingested less than one cup of brewed coffee over the 24-h period before the sample was taken had a plasma caffeine concentration of 0.6 mg/L. Moreover, participants that reported no intake of caffeine for 24-h prior to the sample being taken were found to have plasma caffeine concentrations of 0.3-0.4 mg/L. The plasma caffeine values for the two participants in the present study were all 0.9 mg/L or less, so it could be inferred that these values were due to residual caffeine intake during the 48-h period before the trials. Also, when ingesting low, moderate, and high doses of caffeine, plasma caffeine levels rise to 3, 8, and 14 mg/L respectively (peak values reached 40-80 min post-ingestion) (Spriet, 2014). Taken together, these observations suggest that the caffeine values observed in two participants in the present study were very low and represent baseline values in studies examining caffeine ingestion (de Leon et al., 2003).

The findings from the present study showed slower sprint times for both trials on the second skill assessment when compared with the first one. Interestingly, there was also a significant time effect on subjective measures of fatigue. This slower sprint time could reflect a fatigue element possibly due to diurnal variation in relation to meal timing, or reflect the effectiveness of the warm-up. Since a standardised warm-up was performed before the two assessments, it is unlikely that this factor played a role. Also, given that there were only about 70 minutes between the skill assessments, diurnal variation might be expected to have a limited effect. It has been stated that a high-carbohydrate meal could increase perceived feelings of central and peripheral fatigue two to four hours after eating (Cunliffe et al., 1997). Hence, the pre-trial meal and its high carbohydrate content might have had an impact on feelings of fatigue over the study period. However, it should be noted that there was no significant association between sprint times and perceived feelings of fatigue. Significant time effects of perceived feelings of nausea and fullness and also calmness were found. Nevertheless, correlation analyses did not reveal any evident associations between these variables and the slower sprint times on the second skill assessment. Furthermore, it is important to note that the participants were resting during the hour between baseline and final measurement time points. So, it is challenging to pinpoint a direct cause of this time effect on sprint time. Taken together with the abovementioned data, there does not seem to be clear evidence that gastrointestinal variables such as nausea or fullness, or fatigue and calmness had an impact on sprint times in the second skill assessment. It is also worth noting that the mean difference in sprint time, while significant, was not greater than 0.12 seconds between the time points on both trials.

Shooting accuracy for both feet seemed to improve from the first skill assessment to the second one in both conditions, and shooting accuracy with both feet was significantly associated with shooting accuracy of the dominant foot, but not of the non-dominant foot. The study on reliability of soccer-skill tests presented in Chapter 3 indicated that shooting reliability for both feet was poor (ICC=0.40; CV: 6.3%). Even though the skills were assessed within a simulated soccer match protocol in Chapter 3, and evaluated on their own in the present study, the actual assessment of the skill was similar. The change in shooting accuracy over time for placebo and the coffeeberry conditions was 7.5 % and 13.8 %, respectively. These values exceed the test to retest variation, so it seems that there could have been a meaningful change over time on this variable. However, another study examining the reliability of a shooting test (Russell et al. 2010) has found poorer reliability for shooting (Russell et al. 2010, shooting accuracy: ICC=0.38;CV:14.4%) so it is possible that the results in the present study fall within test re-test variation of this task. However, a greater sample size or reduced variance in a data set might lead to an overall effect. It does not seem likely that the difference observed over time is a learning effect, as there was sufficient familiarisation prior to data collection, and randomization of trial order.

A trial effect was evident for short pass passing accuracy with both feet. It seems that the participants were more accurate on the coffeeberry trial than in the placebo at both time points. However, the change of 4% and 5% for the placebo and the coffeeberry conditions, respectively, probably just occurred by chance. In Chapter 3 it was established that the coefficient of variation for this variable was of 13.9%. Also, the total sample size of 30 in the present study should provide strong statistical power, and this could lead to more chance of observing a false positive outcome, especially when there are only two time points.

Although no main interaction effects were found when comparing the effects of coffeeberry and placebo ingestion on soccer-specific skills, some differences from the first skill assessment to the second one were encountered irrespective of what beverage was ingested. These observations could reflect small diurnal changes in people's abilities to perform a task, and potential false positive outcomes due to a large sample size, which are important factors to know when considering overall effects of interventions. The present study was performed under resting conditions; therefore, it seems plausible to suggest that conducting similar studies that include physical and/or mental fatiguing tasks are required to potentially evaluate any effects of coffeeberry in prevention of detrimental effects that fatigue could cause to soccer-specific skill performance.

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CHAPTER 6
INFLUENCE OF COFFEBERRY ON SOCCER SPECIFIC SKILL PERFORMANCE DURING SIMULATED SOCCER MATCH PLAY AND FOLLOWING FATIGUING EXERCISE IN ACADEMY PLAYERS

6.1 Abstract

This study investigated the influence of coffeeberry extract ingestion on soccer specific skill performance during simulated soccer match-play and following exercise to volitional exhaustion. Twenty male well-trained professional outfield soccer players (age 18±2 y) ingested a 300 mg dose of coffeeberry administered in a double-blind randomised cross-over design after a baseline skill assessment that evaluated dribbling, sprinting, passing, and shooting performance. After a 60-min rest period, participants undertook a 45-min half soccer match simulation, followed by a shuttle running test at 80% of $\dot{V}O2$ max until the point of volitional exhaustion. Lastly, the participants completed one further assessment of soccer-specific skills. During the simulated soccer match-play a significant trial by time interaction for dribbling accuracy ($p<0.05$) was detected. Post-hoc analyses revealed that dribbling accuracy score was higher in coffeeberry (1.8±0.2 m) than placebo (1.7±0.3 m) at 15 min ($p<0.05$), but lower in coffeeberry (1.6±0.3 m) than placebo (1.8±0.3 m) at 30 min ($p<0.01$). Similarly, a significant time by trial interaction was observed for short pass speed with both feet ($p<0.05$) with passing speed decreasing over time in the placebo (from 10.7±0.3 m/s to 10.2±0.3 m/s) trial but maintained in the coffeeberry trial (10.7±0.4 m/s to 10.6±0.3 m/s). Main time and trial effects ($p<0.05$) were also observed for short pass accuracy with both feet. There were no main interaction effects when comparing only the baseline and final skill assessments (post-high intensity running to exhaustion). Ingesting coffeeberry extract appears to aid in the maintenance of soccer-specific skill performance, particularly passing performance during a simulated soccer match.

Keywords: football; technical; polyphenols; chlorogenic acid

6.2 Introduction

In the previous chapter it was observed that coffeeberry ingestion did not have any significant effects on soccer skill performance under resting conditions. It was suggested that chlorogenic acids within coffeeberry could be more likely to aid in maintenance of soccer skill performance under metabolic stress such as during simulated match-play or following fatiguing exercise. Metabolically demanding conditions may be required to observe any effects of coffeeberry on soccer performance. The physiological effects of chlorogenic acids are multifaceted including: the release of vasoactive molecules such as nitric oxide (Kanegae et al., 2007; Sato et al., 2007) through the stimulation of its production in an endothelial-dependent pathway (Taguchi et al., 2014); an increase in fatty acid utilisation in the liver (Wan et al., 2013); metabolic effects through improved glucose tolerance (Bassoli et al., 2008; Van Dijk et al., 2009) and insulin sensitivity (Tousch et al., 2008); enhancement of regional brain blood flow (de Mejia and Ramirez-Mares, 2014); and increased serum concentrations of exosomal brain derived neurotrophic factor (Reyes-Izquierdo et al., 2013). Of these potential actions it is most likely that the effects of coffeeberry on skeletal muscle metabolism or brain blood flow could impact upon skill performance or high-intensity running capacity. Coffeeberry has also been reported to have positive effects on cognition and mood (Camfield et al., 2013; Reed et al., 2019; Jackson et al., 2023). Since soccer is a physically challenging sport that also has cognitive and motor skill demands, it seems interesting to investigate how coffeeberry could impact the maintenance of technical performance under metabolically demanding situations (e.g., fatigue during a soccer match).

Previous research suggests that coffee related chlorogenic acid derivatives reach their highest concentration in the bloodstream within 40-120 minutes of consumption (Renouf et al., 2010). It is known that within those derivatives, caffeoylquinic acids, in particular 5-0-caffeoylquinic acid represents the most abundant phenolic constituent within chlorogenic acids (Tajik et al., 2017). Studies examining the bioavailability and pharmacokinetics of chlorogenic acids in humans (Stalmach et al., 2009; Stalmach et al., 2014) have shown that the maximum plasma concentration of 5-0-caffeoylquinic acid occurs within ~1 hour of consumption with return to basal plasma concentration within ~3 hours. Furthermore, ingestion of coffeeberry extract demonstrated mental energising effects between 1 to 2 hours after ingestion and pronounced effects on aspects such as subjective ratings of fatigue and alertness for up to 2 to 3 hours after consumption (Reed et al., 2018). Consequently, to assess the potential impact of coffeeberry extract ingestion on maintenance of skill performance during soccer match play, and on high-intensity running to fatigue and skill performance in a fatigued state, careful consideration is needing in timing of intake, and duration of tasks assessed. It would seem from previous data that any effect should be explored within a window of 1-3 hours post ingestion. Therefore, the aim of the present study was to assess the influence of coffeeberry extract on soccer specific skill performance during simulated soccer match-play and following fatiguing exercise. We adopted a half soccer match simulation protocol followed by a fatiguing high-intensity running task that could all be completed within 2-2.5 hours. In line with previous studies, this period would not surpass a time frame within which an acute dose of coffeeberry could be exerting a potential effect.

6.3 Methods

6.3.1 Participants

Twenty male well-trained professional outfield soccer players (10 midfielders, 6 defenders, 4 strikers) from the United Kingdom, who were accustomed to skill assessments as part of their regular training were recruited from a local Professional Football Club development squad in order to participate in this investigation. All players had five or more years of playing experience, had been training consistently for 1 year or more, were regularly participating in match-play with their squad, and were free from injury at the time of the recruitment and testing (age: 18±2 y, body mass: 73.9±8.2 kg, stature: 178.1±6.4, body mass index: 23.2±1.9 kg/m^2, $\dot{V}O2$ max: 56.1±1.7 mL/kg/min). The experimental procedures were approved by a local Ethics of Research Committee and the study was conducted in accordance with the declaration of Helsinki.

6.3.2 Study Design

The study followed a double-blind, randomized, placebo-controlled, crossover design. After receiving the signed consent form, and performing an initial pre-screening, participants undertook a cardiovascular screening visit to a local Sports Medicine Clinic which included a stress test electrocardiogram (ECG) and cardiology consultation that served to confirm the absence of any ECG abnormalities. This was followed by a $\dot{V}O2$ max test using a shuttle running protocol (Bangsbo et al., 2008), and after a short recovery period, a first habituation session of the skill performance outcome assessments (dribbling, sprinting, passing, shooting) was conducted. On a subsequent visit, participants went through a familiarisation trial that included a run through the full experimental trial protocol. This included baseline skill assessment, a 45-minute simulated half soccer match protocol with embedded skill assessment, a high-intensity running to fatigue protocol, and a final skill assessment. The skill assessments required the participants to perform 3 dribbles, 3 sprints, 8 passes (4 with the dominant foot and 4 with the non-dominant foot), and 8 shots (4 with the dominant foot and 4 with the non-dominant foot) whereas the soccer match simulation (SMS) protocol was performed according to a modified version of the protocol described by Russell et al (2011) which according to the results reported in Chapter 3 is reliable for most of the skills assessed. In the SMS, participants were required to perform soccer dribbling, passing, and shooting skills, and 15-m sprints throughout six blocks of the modified protocol lasting a total of 45 minutes. Each block of the SMS protocol consisted of 3 repeated cycles of three 20-m walks, one 20-m walk to the side, five 20-m jogs, one 20-m backwards jog, two 20-m strides and an alternating timed 15-m sprint or a 20-m dribble followed by passing and shooting assessments.

In order to assess dribbling speed and accuracy, players dribbled a ball between 7 cones (cones 2–7 were placed 3-m away from the preceding cone, and cones 1 and 7 were 1 m away from each end of the course). Participants were required to dribble the ball as quickly and accurately as possible from one end to the other over the 20-m total distance. Participants dribbled towards a video camera that was placed directly in line with the cones. For the sprint assessment, players ran as fast as possible through timing gates (Brower®, USA) placed 15-m apart, with a 1-m run-in. At the end of each block of activity, players performed a bout of passing where they directed alternate passes towards target zones placed to the left and right at distances of 4.2-m (short pass) and 7.9-m (long pass).

Soccer mannequins (Diamond Football®, Senior Pro Free Kick) with their bases were used as passing targets. The base (0.5-m wide) was the central zone of the target, with cones at a distance of 0.5-m of each side as the lateral zones of the target. A pass to the centre area was worth 10 points and the two lateral areas were worth 5 points. Passes that missed the target areas were scored as 0. Passing bouts consisted of 8 passes (2 with the dominant and 2 with the non-dominant foot to the short pass target, and 2 with the dominant and 2 with the non-dominant foot to the long pass target). Then, the shooting skill assessment was performed, for this, participants were instructed to kick the ball as firmly and accurately as possible to a shooting target. Shooting target zones were at a distance of 15-m in the four corners of the goal. These target areas have been identified as optimal ball placement to beat a goalkeeper when shooting (Ali et al., 2007). Each shooting target was divided into two areas, a centre area (75 cm x 60 cm) and an extended area (100 cm x 90 cm) with the centre area worth 10 points and the extended area worth 5 points. Shots that missed the areas on the target were scored as 0. The bouts of shooting consisted of 8 shots (4 with the dominant foot and 4 with the non-dominant foot).

Video footage of the skill tests were captured using GoPro cameras (GoPro®, Hero 5), one was placed 1-metre apart from the last cone of the dribbling course and the other 1-metre behind the passing and shooting zone. Manual digitisation (Kinovea® version 0.8.15; Kinovea Org., France) yielded dribbling speed, dribbling accuracy, passing accuracy, and passing speed, as well as shooting accuracy and speed. Passing and shooting speed was calculated from the time interval between ball contact with the foot and subsequent ball contact with the target area.

The fatigue protocol required the players to run at 80% of their $\dot{V}O2$ max until the point of volitional exhaustion, this was included in order to evaluate endurance running capacity, but also to induce fatigue prior to a final skill assessment. All testing sessions were performed on an indoor artificial grass pitch (length: 37-m; width: 19-m; ceiling height: 6.5-m) and were completed in the afternoon to reflect the time at which this group typically engages in soccer match play. Soccer balls were inflated to a pressure of 14 psi before each trial.

6.3.3 Experimental Trials

At least 7 days following the familiarisation visit, participants attended the first of their 2 main experimental trial days. Trials were a placebo trial and a coffeeberry extract trial (300mg dose) administered in a double-blind placebo controlled cross-over design. Trials were separated by 7-14 days. Participants followed their habitual diets but avoided caffeine, caffeinated and decaffeinated products, apples, and berries/cherries for 24-h prior to main testing days and alcohol 48-h before the main trials. Participants were asked to record all food consumed for 48-h before the first trial and replicate it 48-h prior to the second trial. Participants were also requested to abstain from strenuous physical activity for 24-h before participation on main testing days. Participants attended the training ground at 8:30 a.m. and were provided with a standardised breakfast (2 eggs, 2 slices of bread, and 1 medium-sized banana, providing 423 kcal, 46g carbohydrate, 26g protein, 14g fat). Later, a pre-trial standardised pre-match meal was also provided 2-h before beginning the main trials, with the meal containing 2g carbohydrate $\cdot kg^{-1}$ of body mass (pasta in a tomato sauce) plus 500 mL of water. All trials started between 12 and 2:00 p.m.

Upon arrival at the indoor pitch for the main trials, body mass (SECA Quadra 808. Hamburg, Germany) was assessed immediately after players emptied their bladder (with urine volume collected into a container, sleep quantity (hrs.) and quality (1-5 scale) was evaluated on arrival, and a baseline blood sample was taken (7 mL obtained by venepuncture from an antecubital vein, taken into a KEDTA plasma tube, placed on ice, spun at 4 ºC and separated immediately afterwards), for assessment of caffeine and chlorogenic acid (chlorogenic acid data not yet available) and then players undertook a 10-min standardized warm-up (consisting of running, dynamic stretching, and 20-m sprints) followed by a baseline assessment of skills (dribbling, passing, and shooting) before ingesting the allocated trial beverage over a 2-3-minute ingestion period. The beverages (300 mL) were ingested on one occasion, just after the players completed the first skill assessment. The coffeeberry and placebo beverages were ready-to-drink formulations (PepsiCo International Ltd., USA) matched for flavour, colour, and texture. The test drink contained water, citric acid, coffeeberry, gum arabic, natural flavour, sucralose, acesulfame potassium, glycerol ester of rosin, yellow 6. The measured content of chlorogenic acid and caffeine was 117.9 ± 0.8 ug/mL and 42.2 ± 0.4 ug/mL respectively. The placebo drink contained all of the abovementioned ingredients with the exception of coffeeberry and no CGA or caffeine were detected. After a 60-min rest period, to allow for delivery and absorption of the active ingredients in the test beverage, a second venous blood sample (7 mL) was obtained by venepuncture, then players repeated the standardized 10-min warm-up and the assessment of skills. Next, players undertook the 45-min half soccer match simulation. Participants then undertook the shuttle running test at 80% of $\dot{V}O_2$ max until the point of exhaustion. Exhaustion was defined as the inability to maintain the required pace for two consecutive shuttles. Running capacity performance was expressed as the total running distance completed during the test. Finally, the participants then completed one further assessment of the soccer-specific skills.

Fingertip capillary blood samples (30μL) were drawn prior to simulated match play, at the end of the 45-min match play, and at the point of volitional fatigue, for assessment of glucose and lactate concentration. Urine samples from baseline and at the end of the trial obtained prior to body mass measurements, were retained for the assessment of osmolality. Fluid (water) was allowed *ad libitum* during the first trial and was replicated for the second one. Baseline hydration status was assessed using a combination of body mass and urine osmolality. Urine production was assessed and sweat losses determined from change in body mass corrected for fluid intake and urine losses (% body mass loss = body mass loss (kg)*100 / Pre-trial body mass). Subjective scores of effort (RPE, Borg 1973), and visual analogue scales for determination of mental energy, physical energy, feelings of fatigue and gut comfort, alongside assessments of arousal states (calmness, tiredness, energy, tension) and heart rate measurements, were obtained at intervals during the protocol. A schematic of the trial protocol is shown in **Figure 6.1.**

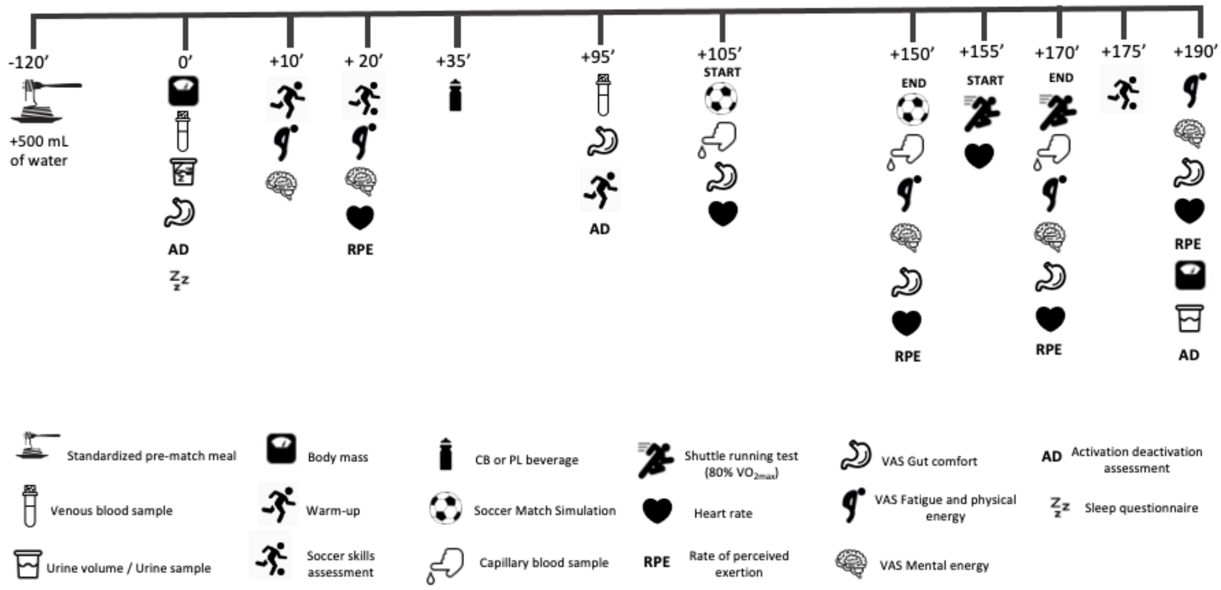


Figure 6.1. Schematic diagram of study protocol

6.3.4 Analytical Procedures

Capillary blood samples (30-µl aliquots) were dispensed into 300 µl of ice cold 0.3-N perchloric acid and shaken vigorously before being placed in an ice bath. On completion of the trial, samples were centrifuged and stored at –70°C until analysis. Analysis of blood glucose and lactate concentrations were completed using the method of Maughan (1982). Urine samples were collected into a 0.5-L plastic container and total mass (to the nearest 0.1 g) assessed to determine urine volume. A 5-mL aliquot was then dispensed into a plain screw- capped tube and was analysed for urine osmolality after the trial ended using a portable osmometer (OsmoCheck, Vitech Scientific Ltd). Blood samples were sent to Griffith University in Australia (Gold Coast Campus, QLD 4222) where caffeine and chlorogenic acid were assessed through liquid chromatography-mass spectrometry (Appendix 1).

6.3.5 Statistical Analysis

Data were first analysed for trial order effects to determine whether order was required as a covariate in subsequent analyses. Baseline data for skill performance measures was assessed using a one-way ANOVA. Subsequently, two-way repeated measures ANOVA was used to assess trial (intervention versus placebo), time (throughout SMS), and trial by time interactions. Likewise, two-way repeated measures ANOVA were used to assess differences between the baseline and the final skill assessments. For any main effects observed follow-up analyses using paired comparisons with Bonferroni correction were performed to determine where differences occurred. Data are presented as mean (SD) in the text or are presented as median (range) depending upon the outcome of normality testing. Data presented in figures contain median, interquartile range, minimum and maximum values shown in box and whisker plots, this was to provide more insight into the spread and values obtained within each variable. Statistical significance was set at $p<0.05$ for all analyses.

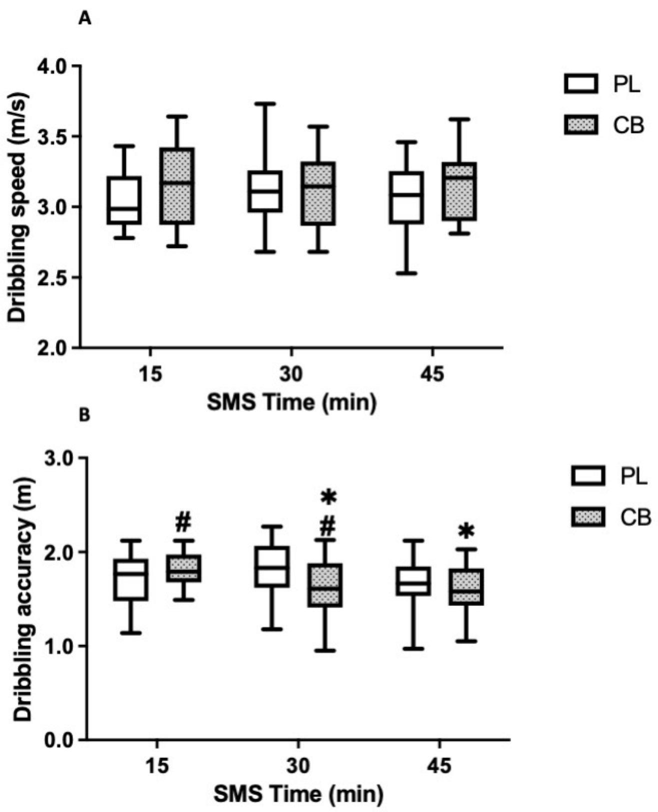
6.4 Results

Data were normally distributed for all variables ($p>0.05$) and there were no baseline differences between the two trials. Dietary analysis revealed no difference in nutritional intake between coffeeberry (total energy: 8.3±2.1 MJ/d; carbohydrates: 279±68 g/d; proteins: 99±50 g/d; fats: 47±12 g/d) and placebo (total energy: 8.6±1.8 MJ/d; carbohydrates: 290±49 g/d; proteins: 98±20 g/d; fats: 50±17 g/d; all $p>0.05$) trials in the 48-h period before the main experimental trial days. Ambient temperature (CB: 9±3 °C, PL: 9±2 °C) and humidity (CB: 67±12 %, PL: 67±11 %) were similar between trials ($p>0.05$). There were no differences noted between trials on pre-trial sleep duration (CB: 8±1 h; PL: 8±1 h) ($p>0.05$).

6.4.1 Soccer Match Simulation skill performance assessment

6.4.1.1 Dribbling

Dribbling speed (m/s) was not different over time ($p=0.83$), or between trials ($p=0.11$) and no time by trial interaction was evident ($p=0.15$; **Figure 6.2.A**). Regarding dribbling accuracy there was a trial by time interaction ($p=0.01$) and a time effect ($p=0.03$) but no trial effect ($p=0.21$; **Figure 6.2.B**). Post-hoc analyses revealed that dribbling accuracy score was higher in CB than PL at 15 min ($p=0.04$), but lower in CB than PL at 30 min ($p=0.01$), and a better maintained dribbling accuracy on the CB trial at 30 and 45 minutes ($p<0.01$) but not evident on the PL trial.



F**igure 6.2.** Dribbling speed (A) and dribbling accuracy (B) during soccer match simulation in placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot.**#** indicates significant differences between PL and CB trials (p<0.05). ***** indicates significant difference in dribbling accuracy compared to 15 min in corresponding trial (p<0.05).

6.4.1.2 Sprinting

There were no trial (p=0.70) or time effects (p=0.11) nor a trial by time interaction (p=0.47) for 15m sprint times during the soccer match simulation (**Figure 5.3**).

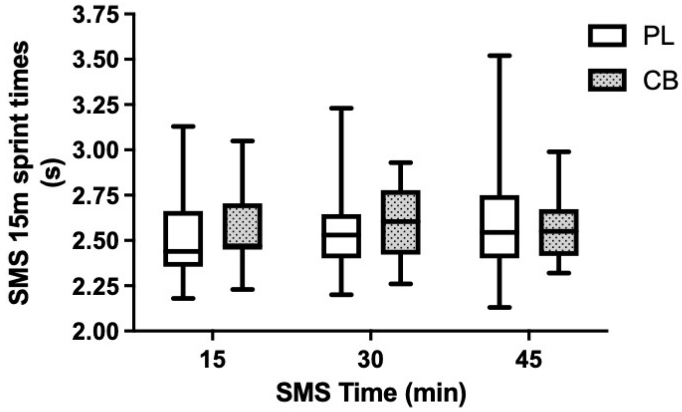


Figure 6.3. Sprint speed during soccer match simulation in placebo (PL) and coffeeberry trials (CB). No main effects of trial, time or trial by time were observed. N=20. Data represents interquartile range and range. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot.

6.4.1.3 Passing (short pass)

Both Feet

There were time (p<0.01) and trial (p<0.01) effects and a trial by time interaction for passing speed (m/s; p=0.007; Figure **6.4.A**). Passing speed decreased over time in PL trial (15 min: 10.7±0.3 m/s vs 45 min: 10.2±0.3 m/s, p<0.001) but did not decline in CB trial resulting in higher passing speed in CB than PL. Regarding passing accuracy, there were trial (p=0.001) and time effects (p<0.001) but no trial by time interaction (p=0.73; **Figure 6.4.B**). Post-hoc analyses revealed that accuracy decreased significantly (p<0.001) from 15 min to 30 min and that accuracy score was higher in the CB trial than in the PL trial (p<0.001).

Dominant Foot

Passing speed with the dominant foot decreased over time (p=0.001), however no trial (p=0.14) or trial by time interaction (p=0.18) was observed (**Figure 6.4.C**). Post hoc analysis revealed a decline in passing speed between 15 min and 45 min. Regarding passing accuracy, there were no time (p=0.41) and trial (p=0.24) effects nor time by trial interaction (p=0.89) (**Figure 6.4.D**).

Non-dominant Foot

There was no trial (p=0.29), time (p=0.49), or trial by time interaction (p=0.47) for passing speed (m/s) with the non-dominant foot (**Figure 6.4.E**). Regarding passing accuracy with the non-dominant foot, there was not a trial by time interaction (p=0.79) nor trial (p=0.14) or time effect (p=0.22; **Figure 6.4.F**).

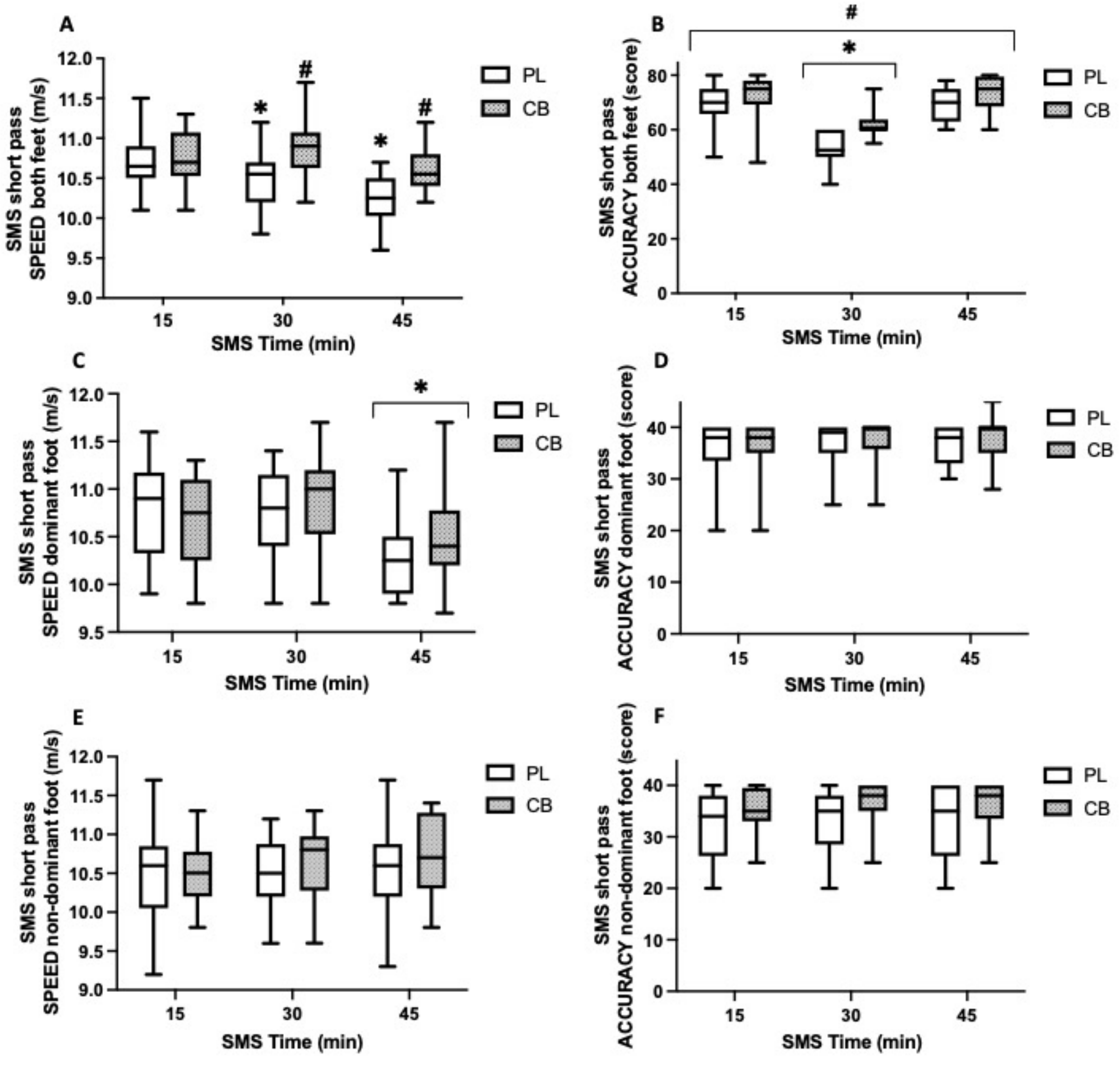


Figure 6.4. Passing speed and passing accuracy on the short pass for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during soccer match simulation in placebo (PL) and coffeeberry trials (CB). N=20. Data represents interquartile range and range. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. **#** indicates significant differences between PL and CB trials (p<0.05). ***** indicates significant difference in dribbling accuracy compared to 15 min in corresponding trial (p<0.05).

6.4.1.4 Passing (long pass)

Both Feet

There were no trial (p=0.30), time (p=0.82) or trial by time interaction (p=0.16) effects for passing speed (m/s; **Figure 6.5.A**). There was no trial by time interaction (p=0.76) nor time effect (0.17), but there was a trial effect (p=0.001) for passing accuracy with higher scores on CB than PL (**Figure 6.5.B**).

Dominant Foot

There was no trial (p=0.29), time (p=0.88) or time by trial interaction (p=0.19) effects for passing speed (m/s) (**Figure 6.5.C**). Regarding passing accuracy, there was a trial effect (p=0.02) with accuracy scores higher for the coffeeberry condition, but no time (p=0.08) effect nor trial by time interaction was encountered (p=0.46) (**Figure 6.5.D**).

Non-dominant Foot

When assessing passing speed (m/s) with the non-dominant foot, there was no time effect (p=0.46) or trial by time interaction (p=0.32), nevertheless a trial effect (p=0.03) was observed with higher passing speed on CB than PL (**Figure 6.5.E**). There was no significant difference between the trials for passing accuracy with the non-dominant foot as no trial (p=0.27) and time (p=0.40) effects nor time by trial interaction (p=0.62) was observed (**Figure 6.5.F**).

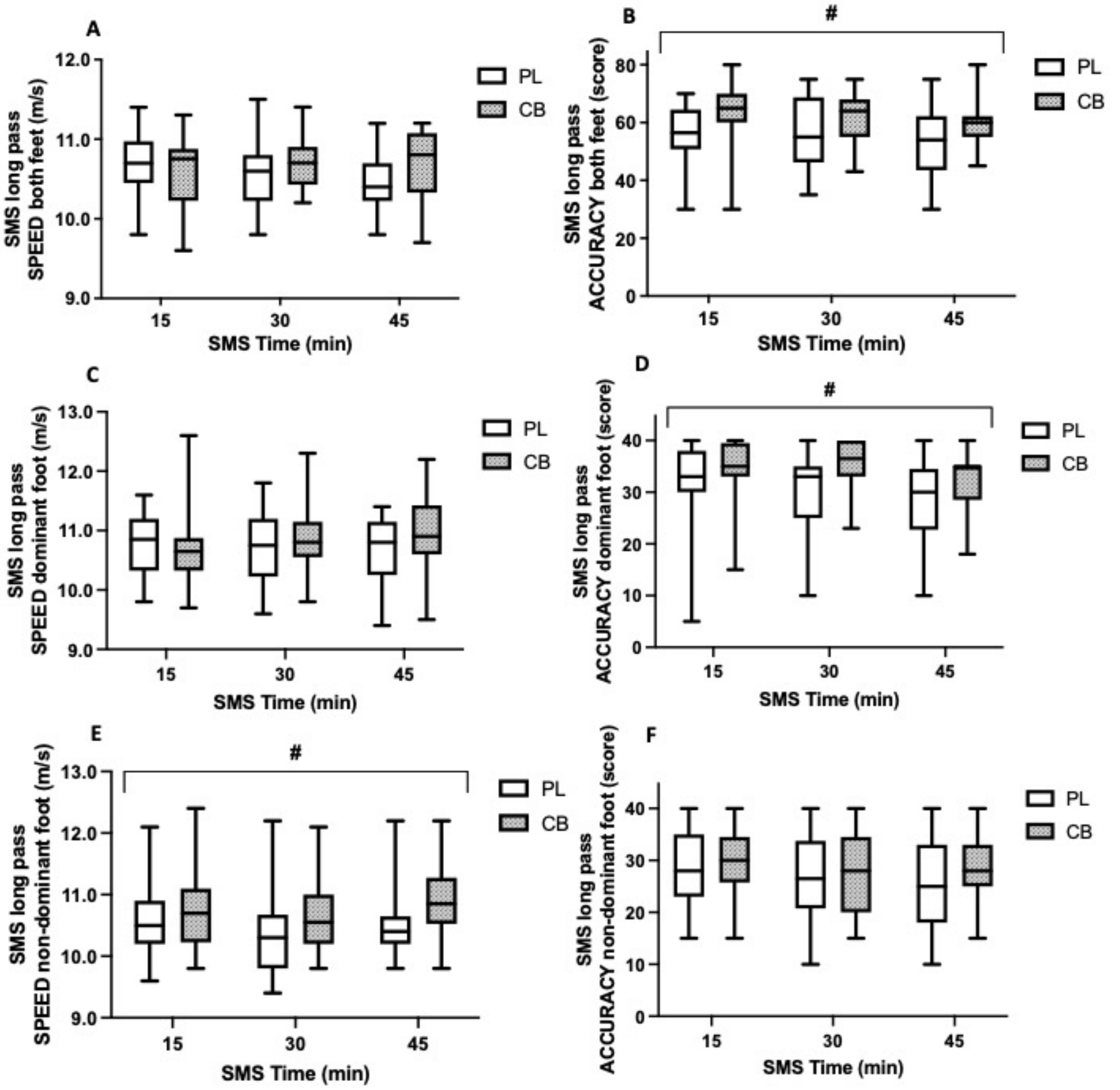


Figure 6.5. Passing speed and passing accuracy on the long pass for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during soccer match simulation in placebo (PL) and coffeeberry trials (CB). N=20. Data represents interquartile range and range. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. **#** indicates significant differences between PL and CB trials (p<0.05).

6.4.1.5 Shooting

Both Feet

There was no trial by time interaction (p=0.50) or time effect (p=0.11), but a trial effect was found (p<0.001) for shooting speed (m/s) with higher speed on CB than PL (**Figure 6.6.A**). In regard to shooting accuracy no time effect (p=0.14) or trial by time interaction (p=0.87) were found but a trial effect (p=0.004) was encountered, with higher scores on CB than PL (**Figure 6.6.B**).

Dominant Foot

There was no trial effect (p=0.24) or trial by time interaction (p=0.32) for shooting speed (m/s) with the non-dominant foot but there was a time effect (p=0.02) with lower shooting speed at 45 min compared with 15 min (**Figure 6.6.C**). Similarly, for shooting accuracy there was no trial (p=0.06) or trial by time interaction (p=0.14) but there was a time effect (p<0.001) with higher scores at 45 min than 15 min **(Figure 6.6.D**).

Non-Dominant Foot

There was no significant difference between the trials for shooting speed with the non-dominant foot as there were no trial (p=0.40) or time (p=0.33) effects, nor trial by time interaction (p=0.13; **Figure 6.6.E**). The same results were found for shooting accuracy, as there were no trial (p=0.92) or time effects (p=0.61), nor trial by time interactions (p=0.08; **Figure 6.6.F**).

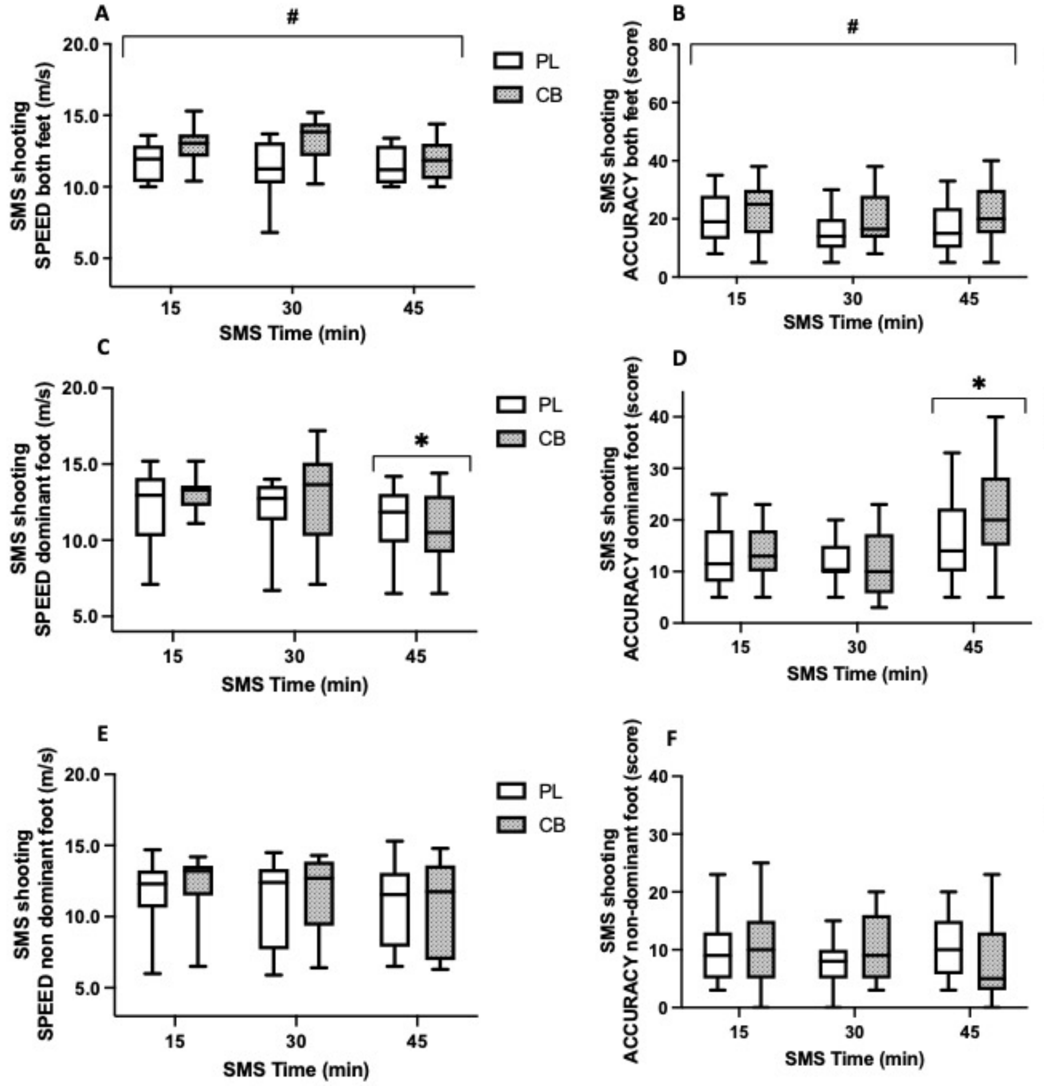


Figure 6.6. Shooting speed and passing for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during soccer match simulation in placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. **#** indicates significant differences between PL and CB trials (p<0.05). ***** indicates significant difference in dribbling accuracy compared to 15 min in corresponding trial (p<0.05).

6.4.2 High intensity running capacity

There was no significant difference between trials in high intensity running to fatigue (p=0.30; **Figure 6.7**). Mean distance on the placebo trial was 1744±1370 m versus 1976±1328 m on the coffeeberry trial. Although this corresponds to a 12% mean difference in running capacity between trials it was not statistically significant.

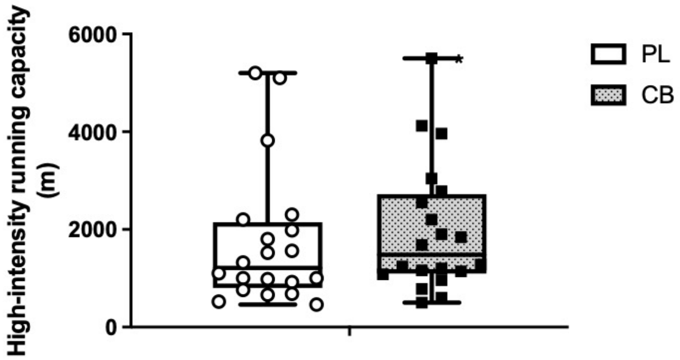


Figure 6.7. High intensity running capacity on placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot.

6.4.3 Baseline skill assessment versus final skill assessment

The following results correspond to the comparison of the effects of coffeeberry and placebo ingestion on dribbling speed and accuracy, sprint speed, and passing and shooting speed and accuracy from baseline to after completion of the 45 minutes of soccer match simulation and high intensity run to exhaustion. When assessing for baseline differences only there were no significant differences between trials.

6.4.3.1 Dribbling

There was no trial effect ($p=0.72$) or time by trial interaction ($p=0.55$) for dribbling speed, however, a time effect ($p<0.001$) was encountered with lower speed at the end of the trial protocol (**Figure 6.8.A**). Regarding dribbling accuracy, there was a trial effect ($p=0.04$) but no time ($p=0.55$) effect or a trial by time interaction ($p=0.90$) with poorer accuracy on CB than PL across both time points examined (**Figure 6.8.B**).

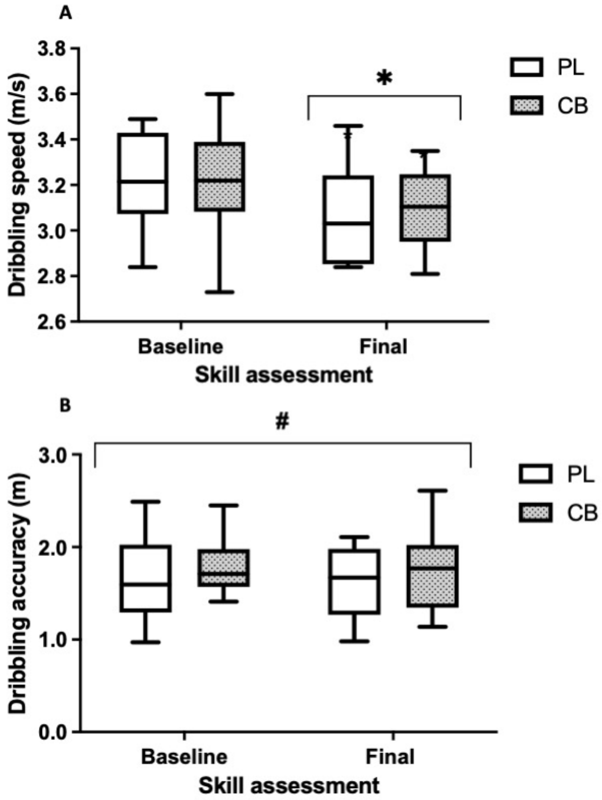


Figure 6.8. Dribbling speed (A) and dribbling accuracy (B) during baseline in placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. **#** indicates significant differences between PL and CB trials (p<0.05). * indicates significant difference from baseline values (p<0.05).

6.4.3.2 Sprinting

A time effect (p<0.001) was found for sprinting performance, with slower sprint time at final compared to baseline assessment, with no trial effect (p=0.11) or a trial by time interaction (p=0.96; **Figure 6.9**).

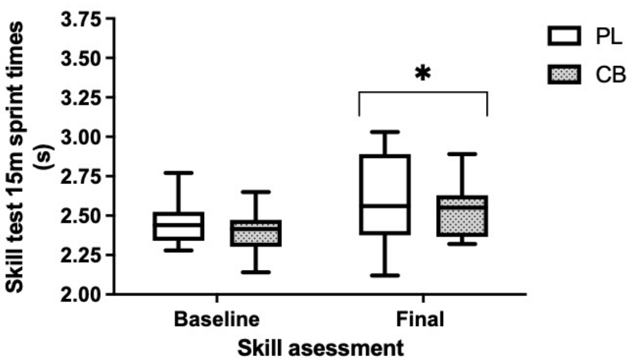


Figure 6.9. Sprint speed during baseline and final soccer skill assessments in placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot.* indicates significant difference from baseline values (p<0.05).

6.4.3.3 Passing (short pass)

Both Feet

There were no differences across passing speed (m/s) as there was no trial (p=0.26) or time (p=0.17) effect nor trial by time (p=0.19) interaction (**Figure 6.10.A**). Regarding passing accuracy, there was no trial effect (p=0.33) or trial by time interaction (p=0.54), but a time effect (p<0.001) was observed with lower accuracy scores at the end of the trial (**Figure 6.10.B**).

Dominant Foot

There was no time effect (p=0.08) or trial by time interaction (p=0.98) for passing speed (m/s), but a trial effect (p=0.01) was observed with higher passing speed on CB than PL (**Figure 6.10.C**). In regard to passing accuracy there was no trial effect (p=0.25) or trial by time interaction (p=0.81), nevertheless, there was a time effect (p=0.01; **Figure 6.10.D**) with lower passing accuracy in the final skill assessment than at baseline.

Non-Dominant Foot

There was no trial (p=0.06), time (p=0.19) effects or a trial by time interaction (p=0.12) for passing speed (m/s) with the non-dominant foot (**Figure 6.10.E**). Similarly, no trial (p=0.34) or time (p=0.83) effects nor a trial by time interaction (p=0.19) was found regarding passing accuracy (**Figure 6.10.F**).

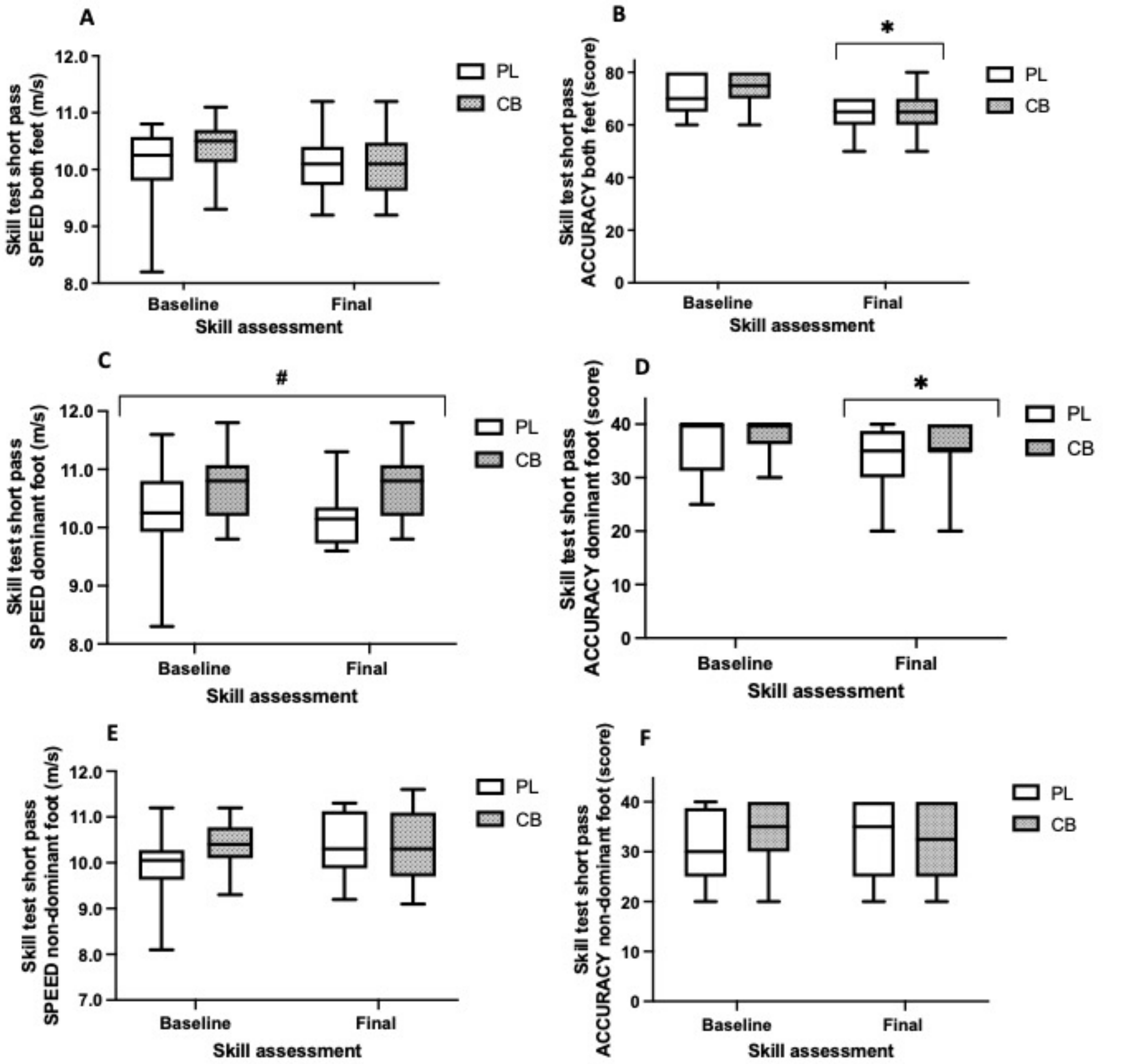


Figure 6.10. Passing speed and passing accuracy on the short pass for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during baseline and final soccer skill assessments in placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. **#** indicates significant differences between PL and CB trials (p<0.05). ***** indicates significant difference from baseline values (p<0.05).

6.4.3.4 Passing (long pass)

Both Feet

There was a trial (p<0.001) and a time effect (p=0.04) but no trial by time interaction (p=0.98) when assessing passing speed in the long pass (m/s; **Figure 6.11.A**). Passing speed was higher on CB than PL, and a significant decrease in speed was observed from the baseline to the final skill assessment. Regarding passing accuracy, there were no time (p=0.08) and trial effects (p=0.12) as well as no time by trial interaction (p=0.76; **Figure 6.11.B**).

Dominant Foot

Trial ($p=0.05$) and time ($p<0.05$) effects were observed, however no trial by time interaction was encountered ($p=0.61$) for passing speed (m/s), with higher values on CB than PL and a decline observed between baseline and final skill assessments (**Figure 6.11.C**). There were no trial ($p=0.25$), time ($p=0.22$) effects or a time by trial interaction ($p=0.81$) for passing accuracy (**Figure 6.11.D**).

Non-Dominant Foot

There was no trial ($p=0.25$), time ($p=0.15$) effects or a trial by time interaction ($p=0.56$) for passing speed (m/s) with the non-dominant foot (**Figure 6.11.E**). Similarly, no trial ($p=0.79$) or time ($p=0.45$) effects nor a trial by time interaction ($p=0.56$) was found for passing accuracy (**Figure 6.11.F**).

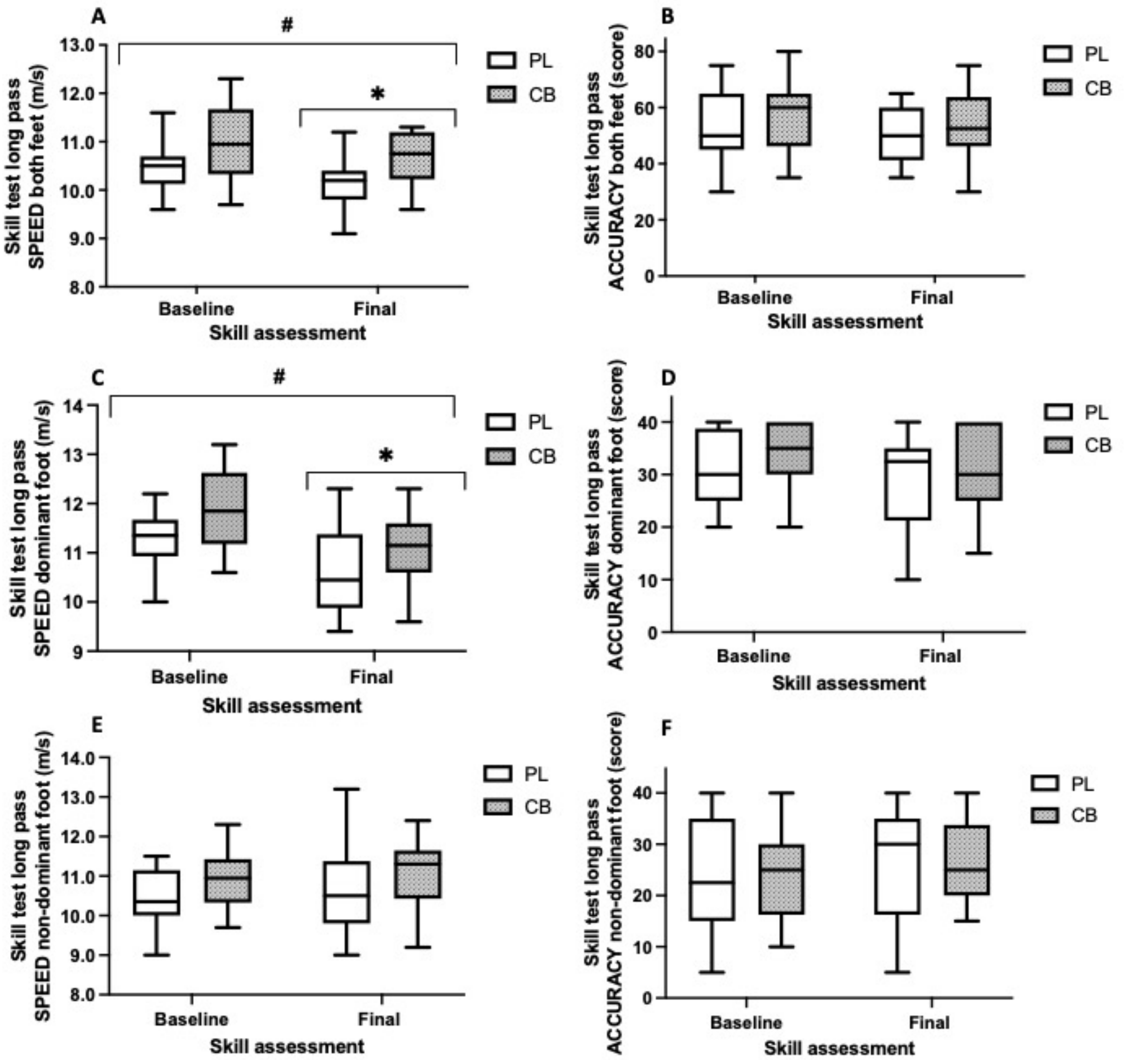


Figure 6.11. Passing speed and passing accuracy on the long pass for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during baseline and final soccer skill assessments in placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. **#** indicates significant differences between PL and CB trials (p<0.05). ***** indicates significant difference from baseline values (p<0.05).

6.4.3.5 Shooting

Both Feet

There were no trial (p=0.69), time (p=0.11) or trial by time interaction (p=0.60) effects for shooting speed (m/s) with both feet (**Figure 6.12.A**). Likewise, for shooting accuracy, there were no trial (p=0.13), time (p=0.73), or time by trial interaction (p=0.29) (**Figure 6.12.B**).

Dominant Foot

On the dominant foot no trial (p=0.47) and time (p=0.97) effects nor a trial by time interaction (p=0.55) was encountered for shooting speed (m/s; **Figure 6.12.C**). Similarly, there were no trial (p=0.80), time (p=0.41), or time by trial interaction (p=0.47) effects for shooting accuracy (**Figure 6.12.D**).

Non-Dominant Foot

There were no trial (p=0.64), time (p=0.16) or trial by time interaction (p=0.94) effects for shooting speed (m/s) with the non-dominant foot (**Figure 6.12.E**). Similarly, no trial (p=0.23) or time (p=0.24) effects nor a trial by time interaction (p=0.07) was found for shooting accuracy (**Figure 6.12.F**).

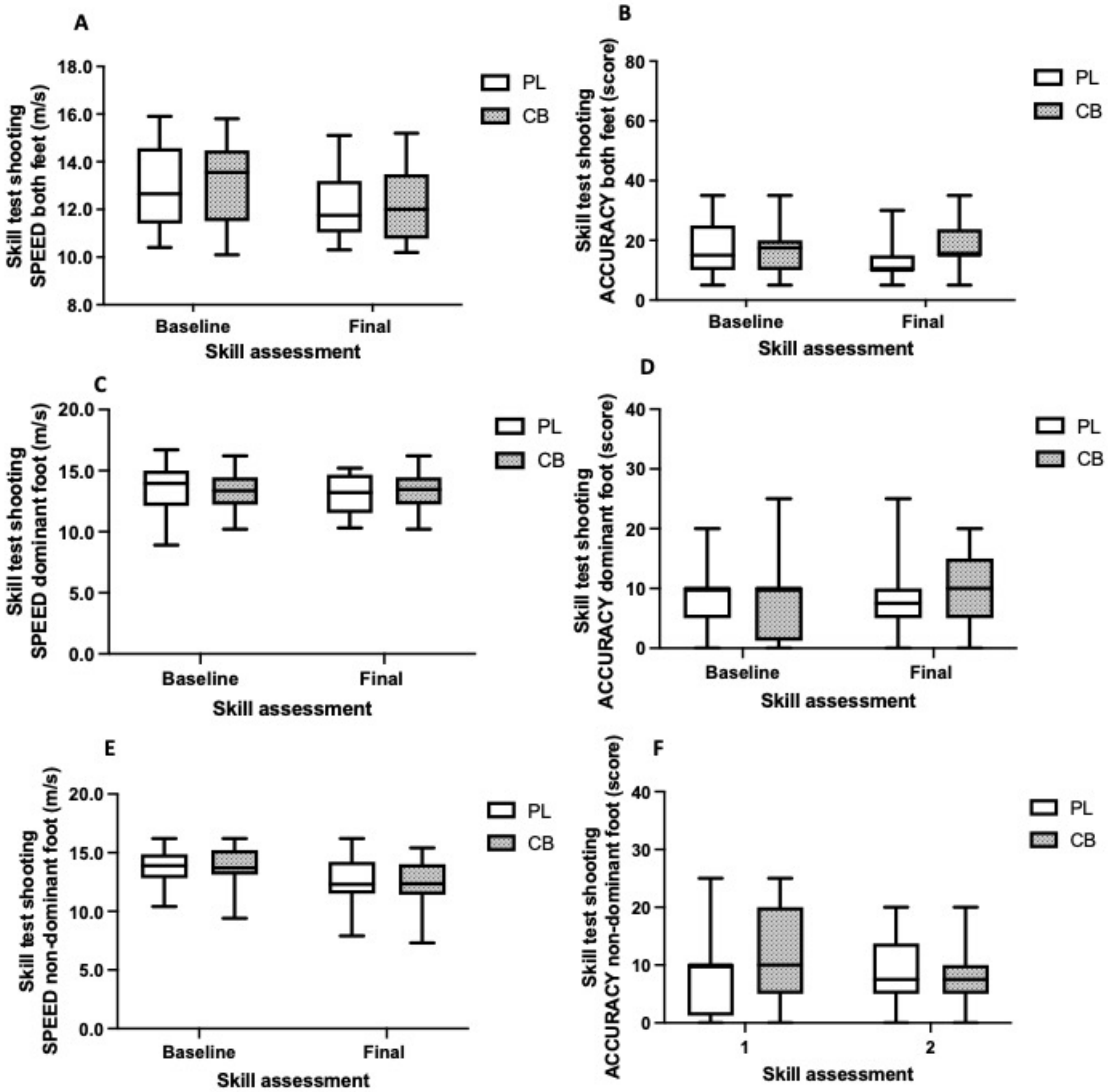


Figure 6.12. Shooting speed and shooting accuracy for both feet (A,B), dominant foot (C,D), and non-dominant foot (E,F) during baseline and final soccer skill assessments in placebo (PL) and coffeeberry trials (CB). N=20. Data represents median, interquartile range, minimum and maximum values shown in the box and whisker plot. **#** indicates significant differences between PL and CB trials (p<0.05). ***** indicates significant difference from baseline values (p<0.05).

6.4.5 Fatigue, physical energy, mental energy

Fatigue scores were low during the early stages of the trial then elevated at all time points after the SMS (time effect, p<0.001), however, there was no trial effect (p=0.40) nor time by trial interaction (p=0.80, **Figure 6.13.A**). Regarding physical energy, there was no trial by time interaction (p=0.99) and no trial effect (p=0.08), however, a time effect was observed (p=0.01) with a decrease in physical energy at all time points after the SMS (**Figure 6.13.B**). Likewise, there was a time effect (p<0.001) for mental energy evidenced by a decline at all time points after the SMS. No trial effect (p=0.26) or trial by time interaction was observed (p=0.53, **Figure 6.13.C**).

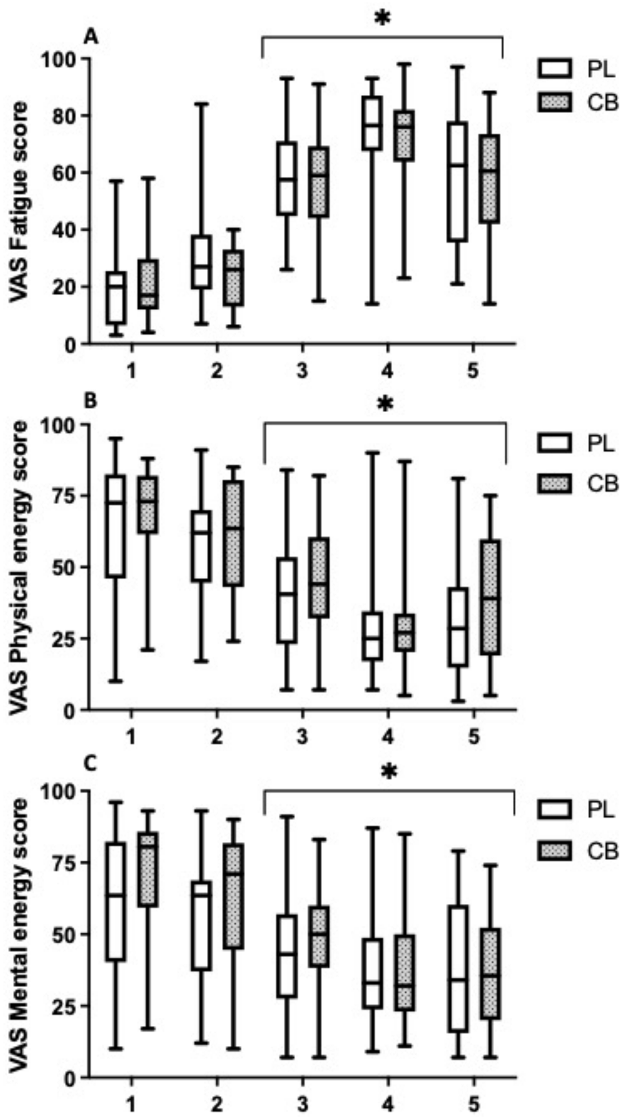


Figure 6.13. VAS (Visual Analogue Scale) for fatigue (A), physical energy (B) and mental energy (C) during the trial in placebo (PL) and coffeeberry trials (CB). Data represents interquartile range and range. 1: before baseline skill assessment, 2: after baseline skill assessment, 3: after SMS, 4: after high-intensity running, 5: after final skill assessment.***** indicates significant difference from baseline values (p<0.05).

6.4.6 Perceived activation-deactivation

There was no trial effect (p=0.51) nor trial by time interaction (p=0.34) for perceived calmness, however, a time effect was observed (p=0.02, **Figure 6.14.A**) with lower calmness rating after drink ingestion and at the end of the trials. Regarding perceived tiredness, there was a time effect (p=0.03) but no trial effect (p=0.32) or trial by time interaction (p=0.62, **Figure 6.14.B**) with higher tiredness at the end of the trials compared with values obtained 1 hour after the coffeeberry ingestion. For perceived energetic state there was no trial (p=0.71) or time (p=0.13) or trial by time interaction effects (p=0.40, **Figure 6.14.C**). There was no trial by time interaction (p=0.30) or time effect (p=0.19) for tension, but there was a trial effect (p=0.01) with a decline in tension on CB across the trial, but an increase in tension on PL at 1h post beverage ingestion (**Figure 6.13.D**).

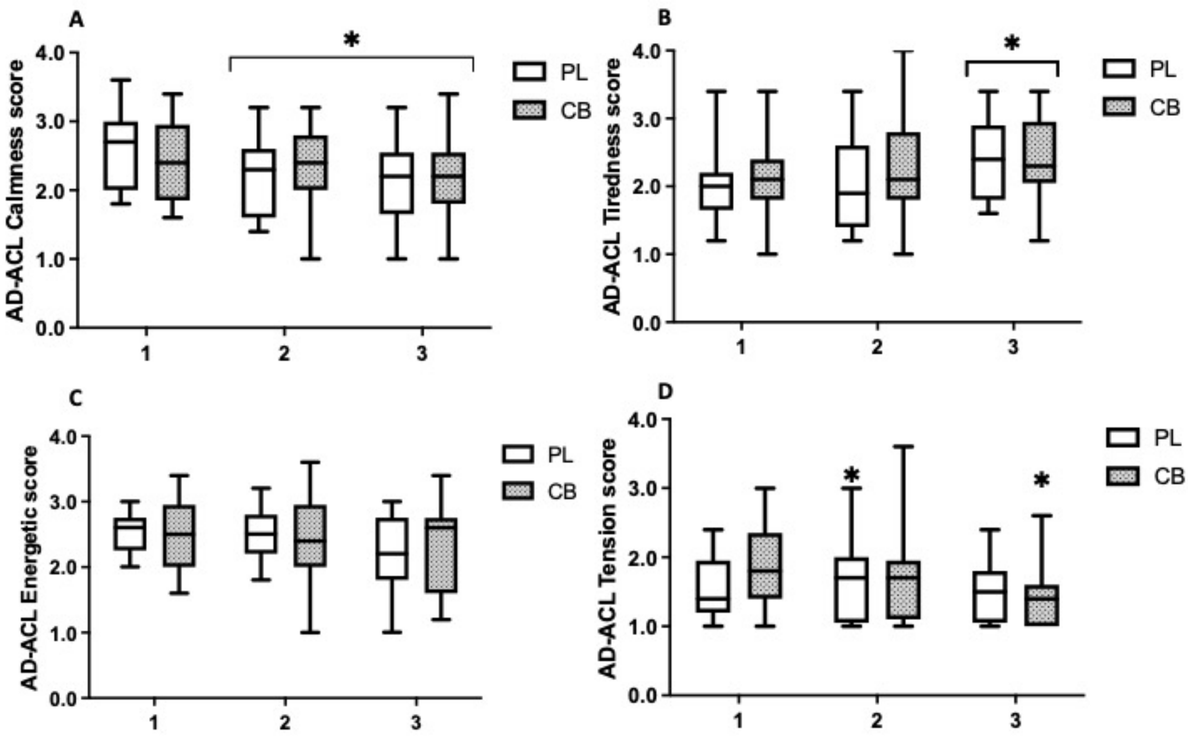


Figure 6.14. Perceived activation-deactivation for calmness (A), tiredness (B), energetic (C), and tension (D) during the trial in placebo (PL) and coffeeberry trials (CB). N=20. Data represents interquartile range and range. 1: baseline, 2: 1-h after ingestion, 3: end of trial. **#** indicates significant differences between PL and CB trials (p<0.05). *****indicates significant difference from baseline values (p<0.05).

6.4.7 Bloatedness, fullness, nausea

Visual analogue scale scores were recorded for bloatedness, fullness, and nausea. There was no trial effect (p=0.79) or trial by time interaction (p=0.59) for bloatedness, however a time effect was observed (p<0.001) with a decrease in bloatedness 1h after drink ingestion and for the remainder of the trial (**Figure 6.15.A**). Likewise, there was a time effect (p<0.001) for fullness, with a decrease 1h after drink ingestion and for the remainder of the trial, with no trial effect (p=0.31) nor trial by time interaction (p=0.24; **Figure 6.15.B**). Regarding nausea, there were no trial (p=0.79), time (p=0.76) or trial by time interaction (p=0.84) effects (**Figure 6.15.C**).

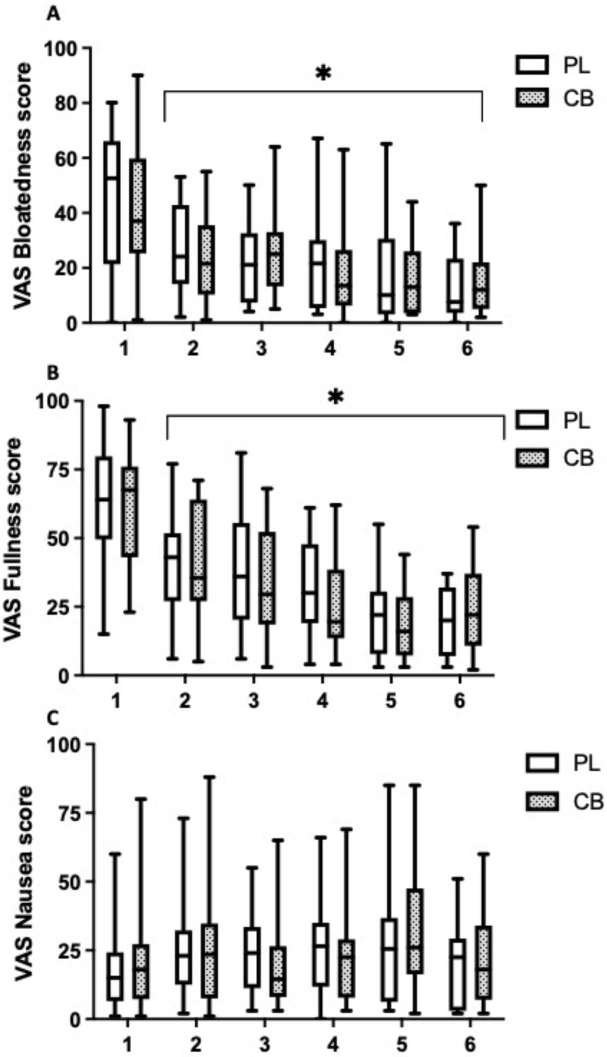


Figure 6.15. VAS (Visual Analogue Scale) for bloatedness (A), fullness (B), and nausea (C) during the trial in placebo (PL) and coffeeberry trials (CB). N=20. Data represents interquartile range and range. 1: start of trial, 2: 1-h after ingestion, 3: before SMS, 4: after SMS, 5: after high-intensity running ,6: after final skill assessment (end of trial). * indicates significant difference from baseline values (p<0.05).

6.4.8 Heart rate, rate of perceived exertion

There was no trial effect (p=0.61) or trial by time interaction (p=0.77) but there was a time effect (p<0.001) for heart rate with an increase above baseline skill assessment values during more intense periods of activity, and reduction below baseline skill assessment values during rest periods over the course of the trial (**Figure 6.16.A**). Similarly, there was a time effect (p<0.001) but no trial effect (p=0.93) or trial by time interaction (p=0.57) for RPE over the course of the trial, with increased RPE during exercise with ratings peaking after high intensity running (**Figure 6.16.B**).

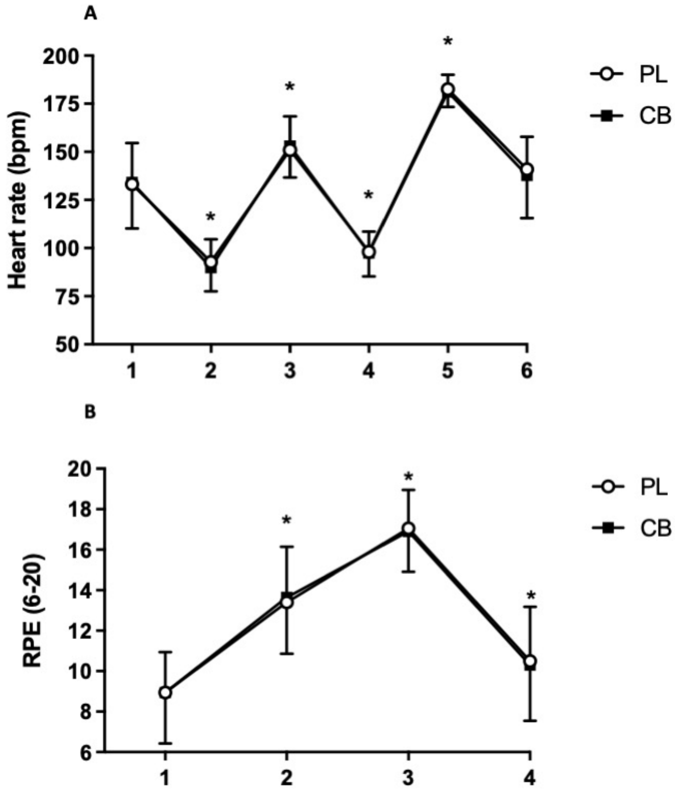


Figure 6.16. Heart rate (A), and RPE (B) during the trial in placebo (PL) and coffeeberry trials (CB). N=20. For heart rate: 1: after baseline assessment, 2: before SMS, 3: after SMS, 4: before high-intensity running,5: after high-intensity running ,6: after final skill assessment (end of trial). For RPE: 1: after baseline assessment, 2: after SMS, 3: after high-intensity running, 4: after final assessment (end of trial). ***** indicates significant difference from baseline values ($p<0.05$).

6.4.8 Blood glucose and lactate concentrations

There was no trial (p=0.30) or time (p=0.46) effect nor trial by time interaction (p=0.32) for blood glucose concentrations over the course of the trial (**Figure 6.17.A**). There was a time effect (p<0.001) but no trial effect (p=0.48) or trial by time interaction (p=0.92) for blood lactate concentrations, with increased lactate concentrations with exercise and peaking after high intensity running (**Figure 6.17.B**).

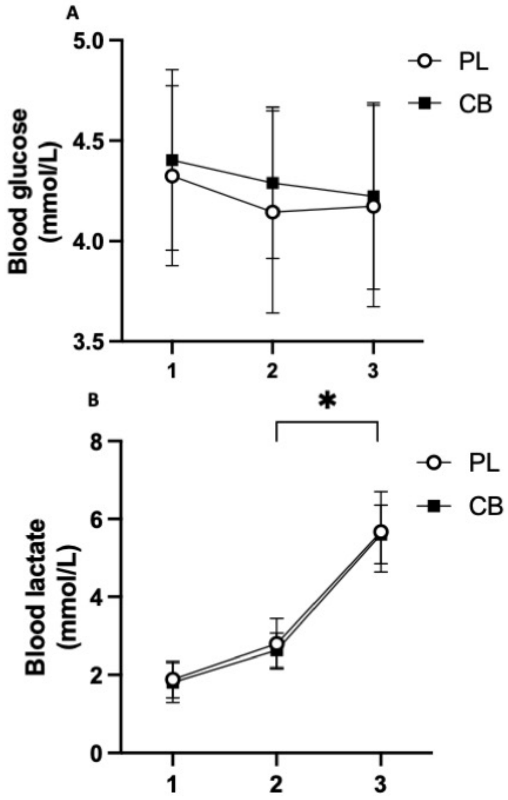


Figure 6.17. Blood glucose (A), and blood lactate (B) concentrations during the trial in placebo (PL) and coffeeberry trials (CB). N=20. For heart rate: 1: before SMS, 2: after SMS, 3: after high-intensity running. * indicates significant difference from baseline values (p<0.05).

6.5 Discussion

The primary aim of this study was to compare the effects of coffeeberry and placebo ingestion on soccer-specific skills during 45 min of simulated soccer match play and following high intensity running to the point of volitional fatigue. The approach of the present study was to explore the potential effects of coffeeberry during exercise (a half soccer match simulation) and under fatigue situations.

A main interaction was observed for dribbling accuracy during soccer match-play. These data indicate that coffeeberry beverage ingestion elicited different responses in this soccer-specific skill performance when compared to placebo. Dribbling performance is part of the fundamental skillset of soccer players (Reilly and Holmes, 1983). In fact, executing a dribble with control and speed is essential for retaining possession and will likely contribute to the outcome of a match (Stone and Oliver, 2009). Previous research has demonstrated that dribbling performance can be influenced by fatigue, as it was shown to deteriorate after 45-min of soccer match play (Stone and Oliver, 2009) and improved and/or maintained by carbohydrate ingestion (Ostojic and Mazic, 2002; Currell et al., 2009; Harper et al., 2006; Harper et al, 2017). The present study shows that dribbling accuracy improved over the 45-min soccer match simulation on the coffeeberry trial, while dribbling speed did not change. At 30 minutes in the match simulation coffeeberry dribbling accuracy was better than placebo. However, the change over time in dribbling accuracy was ~12%. When comparing this value to the variation in data over the first 45-min of the protocol in each visit from the reliability study presented in Chapter 3, it could be suggested that the improvement observed in the present study may not verge on being a meaningful one. For instance, variation over 45-min in the reliability study was ~10%, therefore, it seems that the identified impact of coffeeberry ingestion 1 hour prior to a half soccer match simulation on dribbling accuracy was only just beyond test-retest variation. Therefore, stating an impact upon dribbling skill performance by assisting with improved accuracy without compromising dribbling speed might be a step too far without further replication of these observations.

There was also a main interaction effect detected for short pass accuracy with both feet, as well as time and trial effects. Short pass speed with both feet was maintained over 45-min of soccer match play in the coffeeberry trial, but declined on the placebo trial. It has been stated that the ability to maintain passing performance differentiates successful and unsuccessful soccer teams (Rampinini et al., 2019; Adams et al., 2013) and research has established that passing performance declines as match-play progresses (Rampinini et al., 2009; Carling and Dupont, 2010; Russell et al., 2013). The same decline has been observed during soccer match simulations (Russell et al., 2010; Russell et al., 2011). Moreover, successful short passing was found to be a contributing factor to winning matches in the group stage of the 2014 FIFA World Cup (Liu et al., 2015). However, it has been stated that passing is greatly influenced by extrinsic factors including quality of the opponent (Taylor et., 2008), playing formation (Bradley et al., 2011), and score line (Paixao et al., 2015) meaning that controlled conditions are required to evaluate intervention effects on passing skill. The controlled intervention study presented in Chapter 2 showed that passing performance was positively affected by carbohydrate ingestion, with greater passing accuracy and retained passing speed during the match simulation, particularly on the non-dominant foot. In the present study, the effects were similar, with higher passing accuracy, and better retention of passing speed, on coffeeberry than placebo. These findings if translated to a real soccer match may likely result in a better pass execution following coffeeberry ingestion, and probably less chance of pass interception from the opposition. Therefore, it seems that coffeeberry may have negated the effect of fatigue development on passing performance that was evident in the placebo condition. It could be speculated that there might be a cognitive and/or neuromuscular impact of coffeeberry that enables the retention of passing speed alongside greater passing accuracy. Theoretically, coffeeberry ingestion could have positively impacted cognitive pre-motor processes like visual and spatial processing as the ball was rolled out to players prior to the execution of the pass (Jansen et al., 2012). Also, on the neuromuscular component, coffeeberry ingestion might have induced a faster central processing time, or greater activation of the involved muscle groups to enable greater motor recruitment when striking the ball to perform the pass (Cerrah et al., 2011). However, as these are speculative explanations, further laboratory-based studies should be completed in order to specifically explore them. Interestingly, metabolic markers of glucose and lactate response appear to indicate no differences in glucose metabolism / glycolytic flux between trials thus suggesting no major metabolic effects of coffeeberry and so possibly indicating an impact on cognitive and/or neuromuscular components.

When comparing only the baseline and final skill assessments (post-high intensity running to volitional fatigue) there were significant time effects observed for dribbling speed, sprint time, short pass accuracy with both feet and with the dominant foot, and for long pass speed with both feet and long pass speed on the dominant foot. Unsurprisingly, there were also significant time effects on subjective measures of fatigue (fatigue, mental energy, and physical energy) and on the arousal state (decreased calmness and increased tiredness throughout the trial, with a trial specific response for tension). All of these time effects are most likely to reflect either fatigue or diminishing impact of the coffeeberry beverage as time progressed. When fatigued after completing a 45-min match simulation followed by high-intensity running to volitional exhaustion there were no differences in soccer skill performance detectable between the coffeeberry and placebo trials. Interestingly, although there was no significant difference when evaluating high intensity running capacity prior to the final assessment of skills, when players ingested coffeeberry they ran on average 12% further than when on placebo. This means that overall, under this condition these players accumulated similar levels of fatigue in both trials, but it took slightly longer to achieve that fatigued state in the coffeeberry trial. These observations suggest that the significant time effects observed were likely reflective of fatigue-related responses. Any potential effects of coffeeberry were possibly overridden by fatigue itself. Since interaction effects were observed for soccer skill performance measures during the 45-min soccer match simulation but not after exposure to volitional fatigue, this could support the speculation that fatigue is swamping any potential effects of coffeeberry. However, since the final skill assessment was performed about two and a half hours after the beverage was ingested, and it has been reported that coffeeberry blood concentrations peak within 40-60 min of consumption (Renouf et al., 2010) and that mental energising effects are seen from 1 to 2 hours post-ingestion (Reed et al., 2018), it could also be inferred that by the time the participants completed the fatiguing exercise the effects of coffeeberry were diminishing and it was unable to have any impact.

In summary, it appears that cognitive or neuromuscular factors may be playing a role in the effects of coffeeberry during the 45-min soccer match simulation. It would be interesting to explore potential effects of coffeeberry on visual processing, signal transduction from the motor cortex to the muscle, or on motor unit recruitment itself. Future studies could integrate visual or cognitive function assessments, motor cortex stimulation, transcranial magnetic stimulation (TMS), and femoral nerve stimulation in order to determine which aspect(s) might be impacted by coffeeberry to alter recruitment of muscle groups involved in determining passing speed and accuracy. It would also be prudent to explore the time course of responses to a full match simulation following acute coffeeberry ingestion as this would enable determination of specific time intervals for repeated beverage ingestion.

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CHAPTER 7
GENERAL DISCUSSION

All sports require varying degrees of cognitive, perceptual, or motor skills. Given the dynamic nature of soccer, it can be inferred that it encompasses all three skill aspects (Bate, 1996). The essence of soccer performance lies in executing skilful movement patterns with efficiency and effectiveness, necessitating players to employ cognitive, perceptual, and motor abilities in response to rapidly evolving situations. As a match progresses, players may experience a decline in skill mastery and the frequency of specific technical manoeuvres (Rampinini et al., 2009; Harper et al., 2014). Even highly skilled players are susceptible to fatigue, encompassing both physical and mental aspects. Due to the duration and intensity of matches, players often face fatigue impacts on both physical (running, sprinting, jumping) and mental (concentration, decision-making) levels. This frequently results in a diminished ability to execute skills effectively during crucial moments, as observed by Mohr et al. (2005) and Smith et al. (2016). Consequently, it can be inferred that soccer players encounter physical and mental fatigue, which can negatively affect specific skill performance (Rollo and Williams, 2023).

While physical and mental fatigue remain the primary factors influencing performance of soccer-specific skills, additional factors (neuromuscular, biomechanical, extrinsic) contribute to the decline in skill effectiveness over the course of a soccer match. It has been established that due to the dynamic and unpredictable nature of soccer match-play, which necessitates continuous vigilance and focus (Coutts, 2016), team sports like soccer might place substantial stress on the brain (Walsh, 2014). Consequently, there exists a significant need for proficient technical performance in soccer. Technical performance hinges upon optimal neuromuscular functionality, which is characterised by optimal motor control and coordination involving the intricate interplay between the brain, nervous system, and the engaged muscles. Technical performance is also influenced by the impact of fatigue on biomechanics, as changes throughout a soccer match have been shown to cause reductions in soccer specific skill performance, specifically reductions in shooting performance (Kellis et al., 2006). Soccer matches exhibit stochastic and dynamic characteristics (Liu et al., 2015), therefore, extrinsic factors such as league level, match location, quality of opposition, score line, and ball possession, collectively impact the execution of technical skills (Taylor et al., 2008; Dellal et al., 2011; Lago-Peñas and Lago-Ballesteros, 2011; Bradley et al., 2013). To prevent the possible influence of such extrinsic factors on soccer skill performance, the studies in the present thesis were all completed under controlled conditions using a soccer match simulation protocol to test soccer-specific skill performance. Factors such as ambient temperature, hydration status, pre-match nutrition, and ergogenic aid use were controlled. Hence, with good experimental controls in place the variance in outcomes would be potentially reduced to specifically enable assessment of responses to the study interventions.

6.1 Synthesis of experimental study outcomes

The first study (Chapter 3) aimed to examine the impact that the ingestion of 250 mL of a 12% carbohydrate-electrolyte beverage, before kick-off and during the half-time period (60g total carbohydrate intake), had on the retention of soccer-specific skills (dribbling and passing performance), sprint speed, and anaerobic endurance running capacity during and after a 90-min soccer match simulation. An interesting finding was that higher passing accuracy was maintained by both dominant and non-dominant feet from 60-min onwards (Table 7.1). Notably, maintained passing skill performance was achieved without loss of passing speed, which was better maintained in the non-dominant foot with carbohydrate ingestion.

In the second study (Chapter 4), the test-rest reliability of the soccer-specific skills within a modified version of a soccer match simulation protocol was evaluated. It was determined that the reliability of the modified version of the SMS protocol is promising for most of the skills assessed, with the exception of dribbling and shooting accuracy (Table 7.2). It was also established that the modified protocol is easy to implement within professional club settings without specialist equipment, but due to the limited sample size the reliability requires further confirmation in a larger sample.

Table 7.1. Main findings from Chapter 3 – carbohydrate ingestion and soccer skill performance study on n=18 male academy soccer players.

Chapter	Aim	Sample	Main findings	Key Outcomes Variables
Chapter 3. Ingesting a 12% carbohydrate-electrolyte (CHO-E) beverage before each half of a soccer match simulation facilitates retention of passing performance and improves high-intensity running in academy players.	Investigate the influence of ingesting 60 g of carbohydrate as a 12% CHO-E solution, prior to and at half-time during a 90-min SMS, on soccer-specific skill performance, speed and high-intensity running capacity in academy soccer players.	18 male soccer academy players (18±2 y).	Significant time by trial interaction (p=0.02) on passing accuracy for both feet. Passing accuracy was greater in CHO-E than placebo at 15 min (p=0.005) and 90-min (p=0.02) timepoints for the dominant foot and at 60-min (p=0.012) and 75-min timepoints (p=0.001) for the non-dominant foot. Passing accuracy, calculated as mean scores measured during the SMS, was greater in CHO-E than placebo when passes were completed with either the dominant or non-dominant foot. Anaerobic endurance capacity, expressed as running distance completed at 90% of the V̇O2 max, was 11.8% better on the CHO-E trial than placebo (p=0.01).	Dribbling speed: 🔴 Dribbling accuracy: 🔴 Passing accuracy dominant foot: 🟢 Passing accuracy non-dominant foot: 🟢 Passing speed dominant foot: 🔴 Passing speed non-dominant foot:: 🟡 Sprint speed: 🔴 High-intensity anaerobic capacity: 🟢 Nausea: 🔴 Fullness: 🟢 Bloatedness: 🟡 Glucose: 🟢 Lactate: 🟡 Heart rate: 🟡 RPE: 🟡

🟢 represents time by trial interaction effect 🟡 represents main time and/or trial effects only 🔴 represents no main effects or interaction.

Table 7.2. Main findings from Chapter 4 – reliability of soccer skills on n=10 male academy soccer players.

Chapter	Aim	Sample	Main findings	Statistics Outcomes
Chapter 4. A preliminary study of the reliability of soccer skills tests within a modified soccer match simulation (SMS) protocol.	Quantify the absolute and relative test-retest reliability and compare the magnitude of these statistics to previously investigations on the SMS.	10 male soccer academy players (18±1 y).	Dribbling Speed: moderate reliability (ICC=0.68) / CV: 7.6% Dribbling accuracy: poor reliability (ICC=0.15) / CV: 23.1% Sprint speed: good reliability (ICC=0.87) / CV: 6.9% Short pass accuracy both feet: good reliability (ICC=0.85) / CV: 13.9% Short pass accuracy dominant foot: moderate reliability (ICC=0.53) / CV: 11.7% Short pass accuracy non-dominant foot: poor reliability (ICC=0.22) / CV: 15.5% Long pass accuracy both feet: good reliability (ICC=0.76) / CV: 15.5% Long pass accuracy dominant foot: excellent reliability (ICC=0.92) / CV: 18.6% Long pass accuracy non-dominant foot: moderate reliability (ICC=0.56) / CV: 15.3% Short pass speed both feet: good reliability (ICC=0.84) / CV: 3.4% Short pass speed dominant foot: good reliability (ICC=0.76), CV: 5.6% Short pass speed non-dominant foot: good reliability (ICC=0.78), CV: 2.9% Long pass speed both feet: excellent reliability (ICC=0.95) / CV: 4.3% Long pass speed dominant foot: excellent reliability (ICC=0.95), CV: 3.7%	Dribbling speed: 🟡 Dribbling accuracy: 🔴 Sprint speed: 🟢 Short pass accuracy 🟢 Long pass accuracy 🟢 Short pass speed 🟢 Long pass speed: 🟢 Shooting accuracy: 🔴 Shooting speed: 🟢

			Long pass speed non-dominant foot: excellent reliability (ICC=0.96), CV: 3.7% Shooting accuracy both feet: poor reliability (ICC=0.40) / CV: 6.3% Shooting accuracy dominant foot: poor reliability (ICC=0.43) / CV: 8.5% Shooting accuracy non-dominant foot: moderate reliability (ICC=0.75) / CV: 18.3% Shooting speed both feet: good reliability (ICC=0.89) / CV: 2.5% Shooting speed dominant foot: moderate reliability (ICC=0.56), CV: 2.4% Shooting speed non-dominant foot: good reliability (ICC=0.87) / CV: 4.1%	

🟢represents good/excellent reliability (ICC>0.76) 🟡represents moderate reliability (ICC=0.50-0.75) 🔴represents poor reliability (ICC<50).

The third study (Chapter 5) determined that a single dose of coffeeberry taken one hour before soccer specific skill assessment, under resting conditions, showed no significant effects on soccer skill performance (Table 7.3). While this outcome was not unexpected due to the resting nature of the design, it was important to establish before going on to explore potential effects during exercise or following exercise-induced fatigue. It was concluded that coffeeberry beverage ingestion had no main effects under resting conditions and that it would be advisable to consider conducting comparable studies involving activities that induce physical and/or mental fatigue.

Finally, the fourth study (Chapter 6) aimed to define the effects of an acute dose of coffeeberry ingested pre-exercise on soccer skill performance during simulated soccer match-play and on soccer skills assessed following exercise-induced fatigue. It was established that the ingestion of a coffeeberry extract beverage appeared to benefit the maintenance of soccer-specific skill performance, particularly passing performance during a 45-min soccer match simulation (Table 7.4).

Table 7.3. Main findings from Chapter 5 – coffeeberry on soccer skill performance under resting conditions on n=30 male academy soccer players.

Chapter	Aim	Sample	Main findings	Statistics Outcomes
Chapter 5. Influence of coffeeberry on soccer skill performance under resting conditions	Investigate the potential effect of coffeeberry ingestion on soccer skill performance under resting conditions.	30 male soccer academy players (18±2 y).	No main interaction effects were observed in the outcome variables. However, time effects were observed as sprinting speed was slower, shooting accuracy was improved, fatigue was higher, calmness scores were lower, fullness scores decreased, and nausea scores increased, between the first and second skill assessments.	Dribbling speed: 🔴 Dribbling accuracy: 🔴 Sprint speed: 🟡 Short pass passing speed: 🔴 Short pass accuracy: 🟡 Long pass passing speed: 🟡 Long pass accuracy: 🔴 Shooting speed: 🔴 Shooting accuracy: 🟡 Fatigue: 🟡 Physical energy: 🔴 Mental energy: 🟡

			In addition, short pass accuracy with both feet, shooting accuracy with both feet, long pass speed with both feet, and mental energy, were all higher on the coffeeberry trial, while RPE was lower on the coffeeberry trial, across the two skill assessments conducted before and after test beverage ingestion.	Nausea: 🟡 Fullness: 🟡 Bloatedness: 🔴 Calmness: 🟡 Tiredness: 🔴 Energetic: 🔴 Tension: 🔴 Heart rate: 🔴 RPE: 🟡

🟢represents time by trial interaction effect 🟡represents main time and/or trial effects only 🔴represents no main effects or interaction.

Table 7.4. Main findings from Chapter 6 – coffeeberry on soccer skill performance on n=20 male academy soccer players.

Chapter	Aim	Sample	Main findings	Statistics Outcomes
Chapter 6. Influence of coffeeberry eon soccer specific skill performance during simulated soccer match-play, and following fatiguing exercise in academy football players	Assess the influence of coffeeberry extract on soccer specific skill performance during simulated soccer match-play and following fatiguing exercise	20 male soccer academy players (18±2 y).	Soccer match simulation: -Significant trial by time interaction for dribbling accuracy (p=0.01) and a time effect (p=0.03) but no trial effect (p=0.21). Post-hoc analyses revealed that dribbling accuracy score was higher in CB than PL at 15 min (p=0.04), but lower in CB than PL at 30 min (p=0.01). -Significant time by trial interaction for short pass speed (p=0.007), as well as time (p<0.01) and trial (p<0.001) effects. Passing speed decreased over time in PL trial (15 min: 10.7±0.3 m/s vs 45 min: 10.2±0.3 m/s, p<0.001) but did not decline in CB trial resulting in higher passing speed in CB than PL. Skill performance assessment after fatiguing exercise: No main interaction effects were observed in the outcome variables. However, there were significant time effects observed for dribbling speed, sprint time, short pass accuracy with both feet and with the dominant foot, and for long pass speed with both feet and long pass speed on the dominant foot	Soccer match simulation: Dribbling speed: 🔴 Dribbling accuracy: 🟢 Sprint speed: 🔴 Short pass speed: 🟢 Short pass accuracy: 🟡 Long pass speed: 🔴 Long pass accuracy: 🟡 Shooting speed: 🟡 Shooting accuracy: 🟡 Skill performance assessment after fatiguing exercise: Dribbling speed: 🟡 Dribbling accuracy: 🟡 Sprint speed: 🟡 Short pass speed: 🔴 Short pass accuracy: 🟡 Long pass speed: 🟡 Long pass accuracy: 🔴 Shooting speed: 🔴 Shooting accuracy: 🔴 High-intensity running capacity: 🔴 Fatigue: 🟡 Physical energy: 🟡 Mental energy: 🟡 Nausea: 🔴 Fullness: 🟡 Bloatedness: 🟡 Calmness: 🟡 Tiredness: 🟡 Energetic: 🔴 Tension: 🟡 Heart rate: 🟡 RPE: 🟡 Glucose: 🟡 Lactate: 🟡

🟢represents time by trial interaction effect 🟡represents main time and/or trial effects only 🔴represents no main effects or interaction.

In conclusion, the present thesis intended to investigate how novel nutritional strategies could impact soccer-specific skill performance, from the data gathered, it seems that ingesting a total of 60g of carbohydrates distributed as 30g before starting a soccer match and 30g at half-time represents a nutritional protocol that would help to limit the impact of fatigue on skill performance and high-intensity running capacity, especially during the latter stages of the game. Likewise, the consumption of beverage providing 300mg of coffeeberry might constitute a promising supplementation strategy to help maintaining skill performance throughout a soccer match. Since it has been stated that passing performance could discriminate between successful and unsuccessful soccer teams (Adams et al., 2013) and considering that both the carbohydrate and the coffeeberry interventions had positive effects mainly on passing outcomes, it seems plausible to recommend both of these strategies as part of the nutritional match-day protocols in soccer players.

6.2 Future directions

Since the studies reported in this thesis on the impact of nutritional protocols (e.g., carbohydrate, caffeine, polyphenols) on soccer skill performance have been done in male players, future studies should be done in women in order to determine potential sex differences in the outcomes. There is a need to evaluate whether the menstrual cycle or hormonal contraception might affect the outcomes arising from interventions of this kind. Although hard to control, another interesting approach would be to test match-day nutritional strategies on the outcomes of live matches instead of through the use of soccer match simulation protocols. Detailed notational analyses could potentially assess factors such as passing accuracy and passing speed, as well as player movement patterns, within match situations. It would be interesting to have a follow-up of the study presented in Chapter 6 using a complete 90-min soccer match simulation protocol in order to observe if positive effects of coffeeberry on aspects of passing skill performance are observed when the exercise protocol is extended to 90 minutes, this would help determining whether there is a need for a re-dosing at half time or whether any positive effects are sustained.

Also, as was speculated in Chapter 6 that there could be a relationship between coffeeberry ingestion and neuromuscular function. Future investigations using motor cortex stimulation, transcranial magnetic stimulation (TMS), and femoral nerve stimulation would help to elucidate potential mechanisms of action. Likewise, it would be of interest to test the effects of coffeeberry on cognitive effects related to team sports performance. Finally, it would be of interest to evaluate the potential combined effects of carbohydrates and coffeeberry in a beverage on soccer skill performance outcomes.

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APPENDICES

APPENDIX 1

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Human Kinetics
ORIGINAL RESEARCH

Ingesting a 12% Carbohydrate-Electrolyte Beverage Before Each Half of a Soccer Match Simulation Facilitates Retention of Passing Performance and Improves High-Intensity Running Capacity in Academy Players

Paola Rodriguez-Giustiniani
University of Stirling

Ian Rollo
Gatorade Sports Science Institute

Oliver C. Witard and Stuart D. R. Galloway
University of Stirling

This study investigated the influence of ingesting a 12% carbohydrate plus electrolyte (CHO-E) solution providing 60 g of carbohydrate before each half of a 90-min soccer match simulation (SMS) protocol on skill performance, sprint speed, and high-intensity running capacity. Eighteen elite academy (age: 18 ± 2 years) soccer players ingested two 250-ml doses (pre-exercise and at halftime) of a 12% CHO-E solution or electrolyte placebo administered in a double-blind randomized cross-over design. During an indoor (artificial grass pitch) SMS, dribbling, passing, and sprint performance were assessed, and blood was drawn for glucose and lactate analysis. High-intensity running capacity was assessed following the SMS. Dribbling speed/accuracy and sprint speed remained unchanged throughout the SMS. Conversely, passing accuracy for both dominant (mean percentage difference [95% confidence interval, CI]: 9 [3, 15]) and nondominant (mean percentage difference [95% CI]: 13 [6, 20]) feet was better maintained during the SMS on CHO-E ($p < .05$), with passing speed better maintained in the nondominant foot (mean percentage difference [95% CI]: 5.3 [0.7, 9.9], $p = .032$). High-intensity running capacity was greater in CHO-E versus placebo (mean percentage difference [95% CI]: 13 [6, 20], $p = .010$). Capillary blood glucose concentration was higher in CHO-E than placebo at halftime (CHO-E: 5.8 ± 0.5 mM vs. placebo: 4.1 ± 0.4 mM, $p = .001$) and following the high-intensity running capacity test (CHO-E: 4.9 ± 0.4 mM vs. placebo: 4.3 ± 0.4 mM, $p = .001$). Ingesting a 12% CHO-E solution before each half of a match can aid in the maintenance of soccer-specific skill performance, particularly on the nondominant foot, and improves subsequent high-intensity running capacity.

Keywords: exercise, football, metabolism, skill

Soccer is characterized by prolonged intermittent activities involving multiple sprints, high-intensity actions, and technical motor skills. As a result, fatigue in soccer is a complex phenomenon underpinned by central and physiological mechanisms, and is most prominent during the latter stages of a match (Mohr et al., 2005; Reilly, 1997). At the metabolic level, the decline in muscle glycogen content during a soccer match (Krustrup et al., 2006) is associated with a reduced work rate (Ostojic & Mazic, 2002). As the brain is dependent on a supply of blood glucose (Duelli & Kuschinsky, 2001), enhancing exogenous carbohydrate availability could preserve central nervous system (CNS) integrity (Meeusen & Decroix, 2018) and help to attenuate the loss of motor skill performance (Russell et al., 2012). Moreover, the benefit of carbohydrate feeding on peripheral fatigue during intermittent exercise is evidenced by the previously reported better maintenance of high-intensity running capacity (McGregor et al., 1999).

Several studies have demonstrated that consuming carbohydrate beverages before and at regular intervals during sporting activities improves subsequent high-intensity exercise capacity (Davis et al., 2000; Kingsley et al., 2014; Nicholas et al., 1995; Phillips et al., 2012) and can also aid the retention of skill performance (Harper et al., 2017). This benefit of carbohydrate feeding has been demonstrated in soccer (Ali et al., 2007; Currell et al., 2009; Russell et al., 2012) and in other skill-based sports, such as tennis (McRae & Galloway, 2012) and squash (Bottoms et al., 2007), where weaker shots on the backhand side were most affected. In relation to soccer, from a practical perspective, carbohydrate beverages can only be supplied to players during the warm-up and during scheduled breaks in play (Clarke et al., 2008). Thus, opportunities available for soccer players to ingest sufficient carbohydrates (∼30–60 $g \cdot hr^{-1}$) are limited (Ali & Williams, 2009; Cermak & van Loon, 2013).

Rodriguez-Giustiniani, Witard, and Galloway are with the Faculty of Health Sciences and Sport, Physiology, Exercise and Nutrition Research Group, University of Stirling, Stirling, Scotland, United Kingdom. Rollo is with Gatorade Sports Science Institute, PepsiCo Global Nutrition R&D, Leicester, United Kingdom. Address author correspondence to Stuart D. R. Galloway at s.d.r.galloway@stir.ac.uk.

A potential strategy to overcome the limited opportunities to consume carbohydrates during soccer match-play is to administer highly concentrated carbohydrate beverages. In this context, the negative consequences of ingesting concentrated carbohydrate solutions on gastrointestinal comfort (Clarke et al., 2008) can be partially alleviated by the ingestion of maltodextrin plus fructose combinations (Jeukendrup, 2010; O'Brien & Rowlands, 2011). Accordingly, a recent study simulated a real-world soccer context by providing soccer players with a highly concentrated (12%) maltodextrin:fructose formulation at the end of the warm-up and at halftime (Harper et al., 2017). Skill performance and intermittent endurance performance were improved on the 12% carbohydrate trial compared with placebo, as evidenced by the maintenance of dribbling speed and self-paced soccer-specific exercise performance during the latter stages of the soccer match simulation (SMS; Harper et al., 2017). Crucially, players reported minimal gastrointestinal discomfort despite the provision of a 12% concentrated carbohydrate solution.

Although an excellent study, the work of Harper et al. (2017) does raise several applied questions. First, the impact of carbohydrate ingestion per se was not isolated because the players also were dehydrated by ~2% body mass during both trials. Second, participants ingested only a small standardized breakfast ~135 min before exercise, and performed the match simulation in the morning rather than in the afternoon, when professional youth team games or senior fixtures are normally scheduled in Scotland. Third, participants were University standard soccer players rather than young professionals, and no assessment of skill performance was performed on dominant and nondominant feet. To follow up the preliminary work of Harper et al. (2017), we set out to control for many of these additional factors, that is, maintain body mass loss within 1%, adopt prematch feeding guidelines prior to an afternoon kickoff, assess outcomes in professional youth players, and distinguish between potential effects in dominant and nondominant feet. Therefore, the primary aim of this study was to provide further practical insight into the influence of ingesting a 12% carbohydrate-electrolyte (CHO-E) beverage on soccer skill performance and high-intensity running capacity in professional youth academy soccer players. We hypothesized that ingesting 250 ml of a 12% CHO-E beverage before kickoff and during the halftime period, versus the ingestion of an equivalent volume of a placebo-electrolyte beverage, would improve the retention of soccer-specific skills (dribbling speed and accuracy, passing speed and accuracy), sprint speed, and anaerobic endurance running capacity during and after a 90-min SMS conducted in a cool environment.

Methods

Participants

A total of 18 male well-trained soccer players (seven midfielders, six defenders, and five strikers) were recruited from local soccer academies to participate in this investigation. A priori, we conducted a power calculation (using software G*Power version 3, Düsseldorf, Germany) of appropriate sample size based on previously published data (Harper et al., 2017). This calculation revealed that 14 participants (using a cross-over design) were required for 80% power with a mean difference in skill performance score of $0.8 \times SD$ and significance set at $p < .05$. All players had five or more years of playing experience, had been training consistently for 1 year or more, and were free from injury at the time of the recruitment and testing (age: 18 ± 2 years, body mass: 73.4 ± 6.0 kg, stature: 177.7 ± 4.8 cm, body mass index: 23.1 ± 1.0 $kg \cdot m^{-2}$, and estimated VO_2max: 55.9 ± 1.5 $ml \cdot kg^{-1}$). The experimental procedures were approved by the University of Stirling research ethics committee.

Study Design

Players attended two preliminary study visits (VO_2max estimation and full trial familiarization) before undertaking two main trials (CHO-E and placebo) with beverages administered in a double-blind randomized, cross-over manner. All visits were separated by 7–14 days. A shuttle running test protocol was used to estimate VO_2max (Yo–Yo IR1; Bangsbo et al., 2008), with the level obtained used to determine the speed corresponding to 40%, 50%, 85%, and 90% of VO_2max for use during and after the SMS protocol. Players followed 48-hr habitual diets (avoiding caffeine and alcohol) and recorded food consumed (analyzed retrospectively; Nutritics; Nutritics Ltd., Dublin, Ireland) before the familiarization visit. The prefamiliarization trial diet was replicated for both main trials. Players refrained from strenuous exercise 24 hr before the familiarization trial and main trial days. On the familiarization visit, players undertook a 10-min warm-up (incorporating light aerobic activity, dynamic stretches, and 20-m sprints), before completing the 90-min SMS protocol (Russell et al., 2011) followed by a high-intensity (90% VO_2max) running test to exhaustion. All testing sessions were performed on an indoor artificial grass pitch.

Experimental Trials

Experimental trials were scheduled to start in the afternoon to reflect times at which this cohort typically engages in soccer matches (Figure 1a). At the training ground, researchers provided players with a standardized breakfast (two eggs, two slices of bread, and one medium-sized banana, providing 423 kcal, 46-g carbohydrate, 26-g protein, and 14-g fat) and then a pretrial standardized meal 2 hr before beginning the main trials providing 459 ± 97 kcal, 2-g carbohydrate$\cdot kg^{-1}$ of body mass (pasta in a tomato sauce) plus 500 ml of water.

Soccer players performed the 90-min SMS that incorporates six blocks of activity equally split across two 45-min periods (Figure 1b). To assess dribbling performance, players dribbled a ball between six cones (3 m apart) toward a camera as fast and precisely as possible. For the sprint assessment, players ran as fast as possible through timing gates (Brower timing system, , Draper, UT) placed 15 m apart, with a 1-m run-in. At the end of each of the 12 blocks of activity, players directed alternate passes toward target zones (2.0 m × 1.0 m) placed to the left and right at a distance of 7.9 m. The passing target was divided into three equally sized areas, with the center area worth ten points and the two areas at either side worth five points. Passes that missed the areas on the target were scored as zero. The bouts of passing consisted of eight passes (four with the dominant foot followed by four with the nondominant foot).

Digitization (Kinovea version 0.8.15; www.kinovea.org) yielded dribbling speed, dribbling precision, passing accuracy, and passing speed. Dribbling, sprint, and passing performance were expressed as means for each 15-min of the protocol. After the SMS protocol was completed, players performed a fixed high-intensity running capacity test to the point of volitional fatigue. The test consisted of 20-m shuttles at a speed corresponding to 90% of their VO_2max until volitional exhaustion (the duration of the test

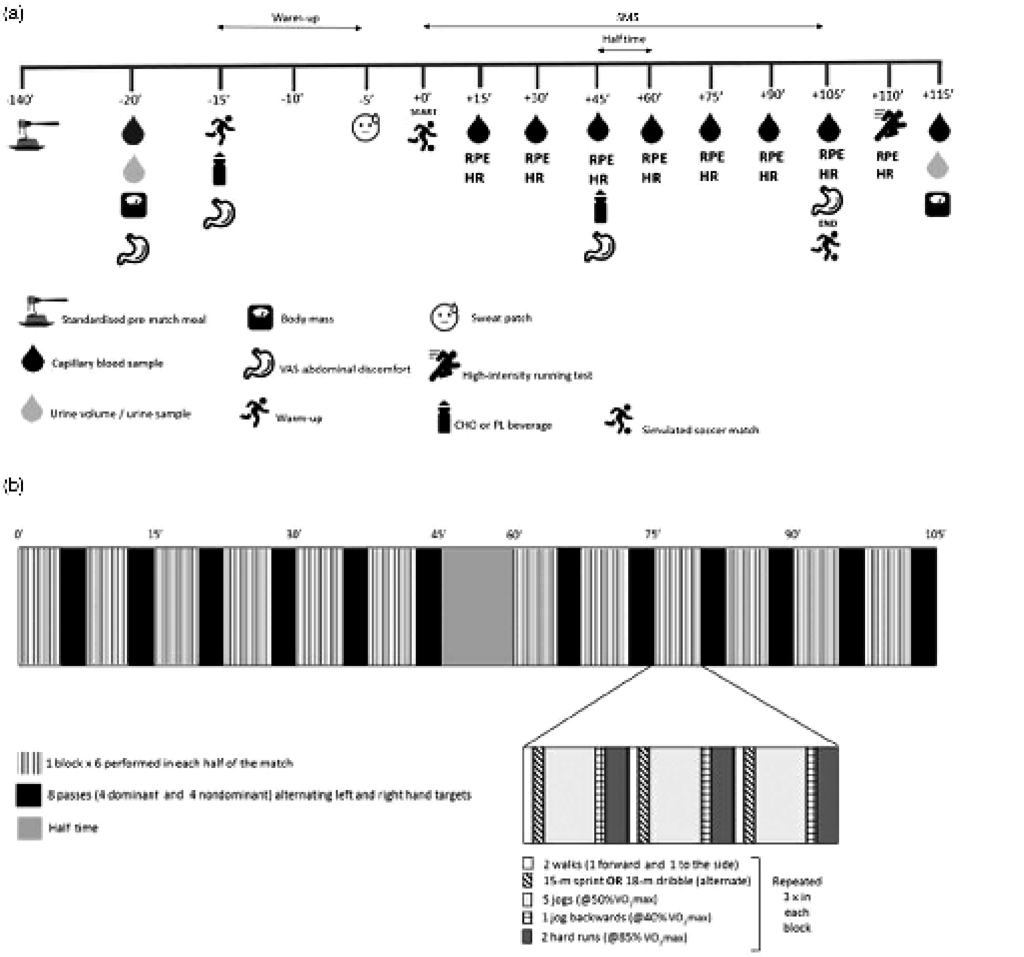

Figure 1 — Schematic diagram of study protocol (a) and outline of the SMS protocol (b). SMS = soccer match simulation; RPE = ratings of perceived exertion (Borg, 1973); HR = heart rate; VAS = visual analogue scale; CHO = carbohydrate; PL = placebo.

ranged from 1 min 21 s to 1 min 42 s). Exhaustion was defined as the inability to maintain the required pace for two consecutive shuttles. Running capacity performance was expressed as the total running distance completed during the test. Following completion of exercise, body mass loss was calculated from the difference between pre-exercise and post-exercise body mass (SECA Quadra 808, Hamburg, Germany), corrected for fluid intake and urine output. Abdominal discomfort ratings (nausea, fullness, and bloatedness) were assessed routinely by asking players to place a vertical line on a 100-mm visual analogue scale.

Beverages (250 ml) were ingested on two separate occasions. First, 15 min before beginning the SMS and, second, immediately upon completing the first half (15 min before beginning the second half). The CHO-E and placebo beverages were ready to drink formulations (PepsiCo International Ltd, Harrison, NY), matched for flavor, color, and texture, containing comparable amounts of Na^+ (41 mg·100 ml^{-1}). The CHO-E drink was a 12% solution (blend of maltodextrin and sucrose, 60 g·500 ml^{-1}, Gatorade Football Energy; PepsiCo Inc.) delivering 40 g of carbohydrate per hour during the SMS. The placebo drink was noncaloric and taste matched using artificial sweeteners. Although water was made available ad libitum, no player consumed any water during either trial.

Analytical Procedures

Capillary blood samples (30 µl aliquots) were dispensed into 300 µl of ice-cold 0.3-N perchloric acid, and shaken vigorously, before being placed in an ice bath. On completion of the trial, samples were centrifuged and stored at −70°C until analysis. Analyses of blood glucose and lactate concentrations were completed using the method of Maughan (1982). Urine samples were collected into a 0.5-L plastic container, and total mass (to the nearest 0.1 g) was assessed to determine urine volume. A 5 ml aliquot was then dispensed into a plain screw-cap tube and stored at 4 °C until analysis of osmolality on the evening of the trial days (freezing point depression method; Löser Micro-Digital Osmometer M15, Schwerte, Germany).

Statistical Analysis

Data are presented as mean (*SD*) with 95% confidence intervals (CIs) for mean differences shown in the text. Statistical significance was set at $p < .05$. Effect sizes based on using Cohen's *d* with threshold values for trivial, small, moderate, large, very large, and extremely large effects set at <0.2, 0.2. 0.6, 1.2, 2.0, and 4.0 (Hopkins et al., 2009) were reported, along with a written description. Performance variables were analyzed using two-way repeated measures analysis of variance whenever data contained multiple time points. When a significant Time × Trial interaction was observed, post hoc pairwise comparisons with Bonferroni confidence interval adjustment were performed to identify at what time point(s) differences existed between conditions. If no Time × Trial interaction was observed, main time or trial effects only were reported. Paired samples *t* tests were used to examine mean differences between trials when data were collected at one time point only (e.g., high-intensity anaerobic endurance running capacity).

Results

No significant trial order effect was observed for any performance variable, therefore, any differences between conditions were considered to be due to treatment effects. Two out of the 18 participants correctly identified the order of the treatments. Dietary analysis revealed no difference in nutritional intake between CHO-E (total energy: 2120 ± 532 kcal·d^{-1}; carbohydrates: 290 ± 63 g·d^{-1}; proteins: 101 ± 47 g·d^{-1}; fats: 70 ± 17 g·d^{-1}) and placebo (total energy: 2345 ± 437 kcal·d^{-1}; carbohydrates: 293 ± 62 g·d^{-1}; proteins: 89 ± 27 g·d^{-1}; fats: 87 ± 27 g·d^{-1}; all *p*s > .05) trials in the 48-hr period before the main experimental trial days. Ambient temperature (CHO-E: 6 ± 1 °C and placebo: 6 ± 1 °C) and humidity (CHO-E: 59 ± 10% and placebo: 61 ± 13%) were similar between trials ($p > .05$).

Physiological Responses to Soccer Match Simulation

There was no significant Time × Trial interaction for heart rate, ratings of perceived exertion, and hydration status responses to the SMS ($p > .05$). Heart rate increased from the 15-min time point (144 ± 9 bpm, CHO-E and placebo trials combined) throughout the SMS protocol (time effect: $p = .01$), rising to 158 ± 6 bpm (CHO-E and placebo combined) by the end (90 min) of the SMS protocol, with no difference between trials ($p = .42$). Likewise, ratings of perceived exertion scores increased from the 15-min time point (10 ± 2, CHO-E and placebo trials combined) throughout the SMS protocol (time effect: $p = .01$), rising to 15 ± 3 (CHO-E and placebo combined) by the end (90 min) of the SMS protocol, with no difference between trials ($p = .17$). No differences were observed for any of the fluid balance and hydration status variables recorded before and after the SMS protocol (pretrial urine osmolality: 513 ±159 mOsm·kg^{-1}; post-trial urine osmolality: 464 ± 137 mOsm·kg^{-1}; body mass loss: 0.7 ± 0.4%, all *p*s > .05 for CHO-E and placebo trials combined).

Dribbling Speed and Accuracy

Dribbling speed did not decline throughout the SMS on both CHO-E and placebo trials (Figure 2a), with no Time × Trial interaction ($p = .42$), time ($p = .49$), or trial ($p = .38$) effects. The mean difference (95% CI) in dribbling speed between CHO-E and placebo trials was 0.03 [−0.24, 0.09] m·s^{-1}, with an effect size (Cohen's *d*) of 0.2 (small effect). Likewise, dribbling accuracy did not change over time in either CHO-E or placebo trials (Figure 2b) and was no time ($p = .54$), trial ($p = .41$), or Time × Trial interactions ($p = .38$). The mean difference (95% CI) in dribbling accuracy between trials was −0.03 [−0.23, 0.16] m, with an effect size (Cohen's *d*) of 0.1 (trivial effect).

Passing Accuracy and Speed

There was a significant Time × Trial interaction ($p = .02$) on passing score (accuracy) for both feet. Passing accuracy was greater in CHO-E than placebo at 15 min ($p = .005$) and 90-min ($p = .02$) time points for the dominant foot (Figure 3a) and at 60-min ($p = .012$) and 75-min time points ($p = .001$) for the nondominant foot (Figure 3c). Passing accuracy, calculated as mean scores measured during the SMS, also was greater in CHO-E than placebo when passes were completed with either the dominant (Figure 3b) or nondominant (Figure 3d) foot. The mean difference (95% CI) in passing score for the dominant foot between CHO-E and placebo trials was 5 [1, 9] points, with an effect size (Cohen's *d*) of 0.7 (moderate effect). The mean difference (95% CI) in passing score for the nondominant foot between trials was 6 [3, 10] points, with an effect size (Cohen's *d*) of 0.8 (moderate effect).

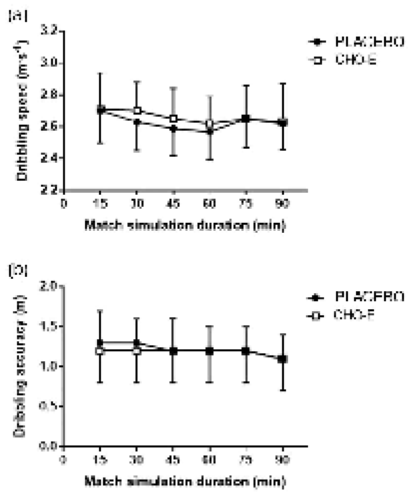

Figure 2 — Dribbling speed (a) and dribbling accuracy (b) during the SMS on placebo and CHO-E trials. No main effects of trial, time, or Trial × Time interaction were observed. CHO-E = carbohydrate-electrolyte; SMS = soccer match simulation.

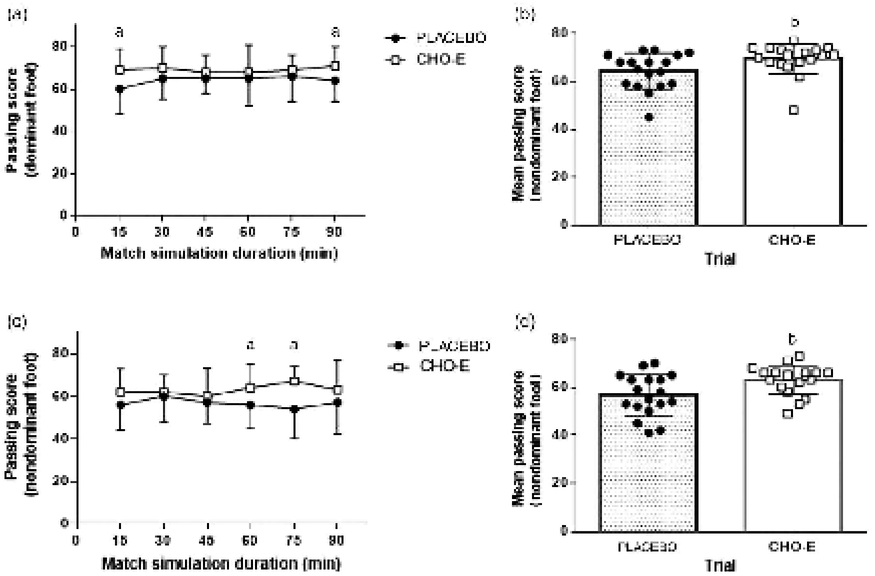

Figure 3 — Passing score (a) during the SMS and mean passing score (b) for the dominant foot. Passing score (c) during the SMS and mean passing score (d) for the nondominant foot. Significant main effects of trial ($p < .05$) and Trial × Time ($p < .05$) interaction were observed. [a]Time points at which significant differences were evident between placebo and CHO-E trials. [b]Significant difference in mean passing score. CHO-E = carbohydrate-electrolyte; SMS = soccer match simulation.

Passing speed was similar on both trials for the dominant foot with no time, trial, or Time × Trial interaction effects observed (all $ps > .05$) (Figure 4a). The mean difference (95% CI) in passing speed between trials for the dominant foot was 0.4 [−0.1, 0.9] $km{\cdot}hr^{-1}$, with an effect size (Cohen's *d*) of 0.2 (trivial effect). By contrast, for the nondominant foot, a significant Time × Trial interaction was observed ($p = .032$). Post hoc analyses revealed that passing speed was better maintained from 75 min onwards in CHO-E trial compared with placebo ($p = .001$; Figure 4c). Mean passing speed was greater in CHO-E than placebo for the nondominant foot only ($p = .04$; Figures 4b and 4d). The mean difference (95% CI) in passing speed for the nondominant foot between trials was 0.6 [0.1, 1.2] $km{\cdot}hr^{-1}$. The effect size (Cohen's *d*) was 0.4 (small effect).

Sprint Speed

Sprint speeds did not decline throughout the SMS with no time ($p = .38$), trial ($p = .47$), or Time × Trial inactions ($p = .31$), detected. In addition, average sprint speed during the 90-min SMS was similar between trials. The mean difference (95% CI) in sprint speed between trials was 5.9 [5.8, 6.1] $km{\cdot}hr^{-1}$. The effect size (Cohen's *d*) was 0.2 (small effect).

High-Intensity Anaerobic Endurance Running Capacity

Anaerobic endurance capacity, expressed as running distance completed at 90% of the VO_2max, was 11.8% better on the CHO-E trial than placebo ($p = .01$; Figure 5). The mean difference (95% CI) in running capacity between trials was 54 [15, 94] m, with an effect size (Cohen's *d*) of 0.4 (small to moderate effect).

Gastrointestinal Comfort

Visual analogue scale scores recorded for nausea were low and remained constant (15-min time point: 12 ± 15 and 90-min time point: 21 ± 16) throughout the SMS with no time ($p = .536$), trial ($p = .11$), or Time × Trial ($p = .37$) effects. There was a significant main effect of trial ($p = .014$) and a Time × Trial interaction for fullness ($p = .007$), with participants reporting greater fullness scores in the CHO-E condition pretrial (39 ± 22) and at the end (90 min) of the SMS protocol (37 ± 25). Perceived feelings of bloatedness increased during the SMS, with participants feeling more bloated after ingesting the second drink at half time (45 ± 23), compared with pretrial feelings (30 ± 22), on both trials. However, there was no Time × Trial ($p = .78$) interaction for bloatedness scores.

Blood Analytes

There was a significant Time × Trial interaction ($p = .001$), main time effect ($p = .001$), and main trial effect ($p = .002$) for capillary blood glucose concentration. Whereas a 26% decline in glucose concentration from halftime to the first 15 min of the second half in the CHO-E condition was observed, glucose concentrations remained constant in placebo. Glucose concentrations were greater in CHO-E than placebo at halftime and after completing the high-intensity running capacity test (Figure 6a). No significant

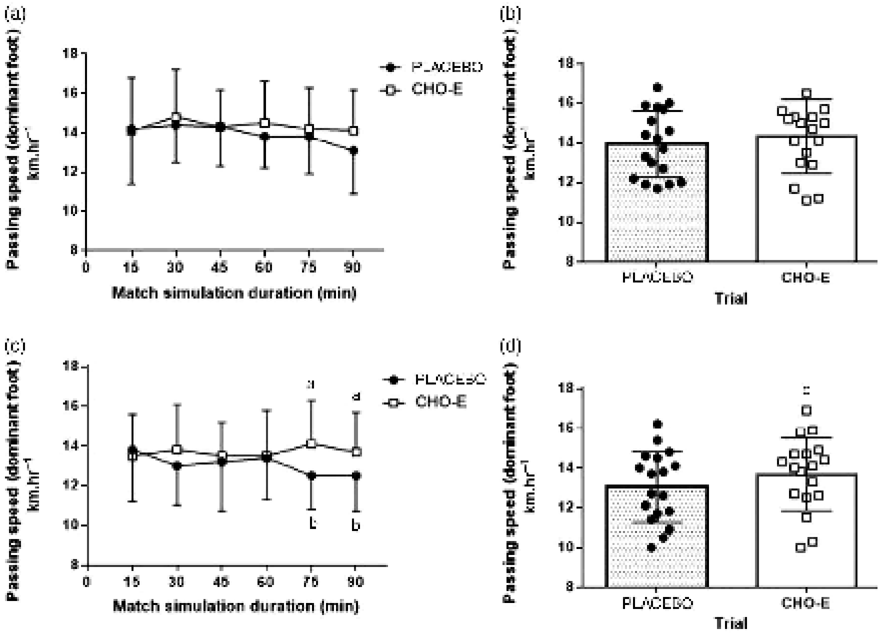

Figure 4 — Passing speed (a) during the SMS and mean passing speed (b) for the dominant foot. Passing speed (c) during the SMS and mean passing speed (d) for the nondominant foot. Significant main effects of trial ($p < .05$) and Trial × Time ($p < .05$) interaction were observed on nondominant foot only. [a]Time points at which significant differences in passing speed were evident between placebo and CHO-E trials. [b]A significant decrease in passing speed compared with 15-min value on PLACEBO trial only. [c]Significant difference in mean passing speed. CHO-E = carbohydrate-electrolyte; SMS = soccer match simulation.

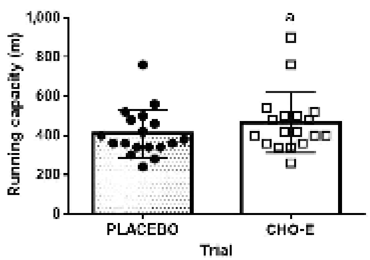

Figure 5 — High-intensity anaerobic endurance running capacity on completion of the SMS on placebo and CHO-E drink ingestion trials. [a]Significant effect ($p < .05$) with greater running capacity on CHO-E than placebo. CHO-E = carbohydrate-electrolyte; SMS = soccer match simulation.

Time × Trial interaction effect ($p = .12$) or main trial effect ($p = .27$) was observed for blood lactate concentrations over the course of the SMS (Figure 6b). However, lactate values increased above baseline at 15 min and, with the exception of halftime, values remained constant during the 90-min SMS. Lactate concentrations increased after the high-intensity anaerobic endurance running capacity test.

Discussion

The primary aim of this study was to investigate the influence of ingesting 60 g of carbohydrate as a 12% CHO-E solution, prior to and at halftime during a 90-min SMS, on soccer-specific skill performance, speed, and high-intensity running capacity in academy soccer players. The SMS protocol was performed on an indoor artificial grass surface 2 hr following intake of a prematch meal, compliant with the recommended carbohydrate guidelines. We demonstrated that ingesting the 12% CHO-E solution versus an electrolyte-matched placebo better maintained passing accuracy in both the early and latter stages of the SMS protocol and passing speed during the latter stages of the SMS protocol with minimal impact on gut comfort. Although there was no benefit of ingesting the 12% CHO-E solution on dribbling speed and accuracy, or sprint speed, compared with placebo, postmatch high-intensity running capacity was improved on the CHO-E trial. In terms of practical application, these data suggest that ingesting a 12% CHO-E solution before and during a soccer match may benefit soccer-specific skill performance and anaerobic endurance capacity.

A recent study reported the better maintenance of ball dribbling speed during the final 30 min of an SMS when ingesting a 12% CHO-E solution prematch and during halftime versus an electrolyte or water placebo condition (Harper et al., 2017). By contrast, we demonstrated no influence of ingesting a 12% CHO-E

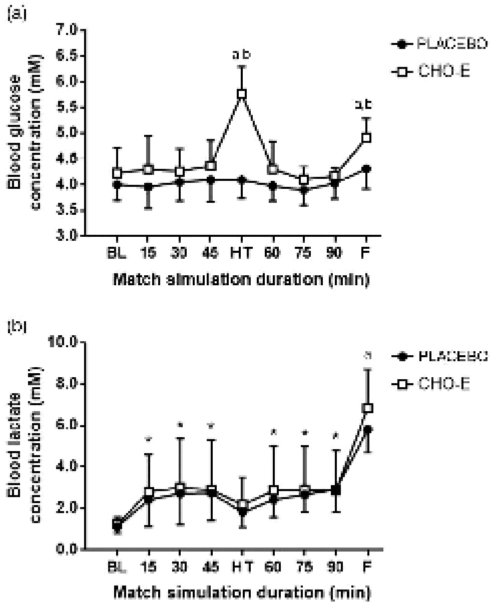

Figure 6 — Blood glucose (a), lactate (b) concentration during the SMS on placebo and CHO-E drink ingestion trials. [a]Significant difference ($p < .05$) from zero time point. [b]Significant difference between trials at halftime and final time point. CHO-E = carbohydrate-electrolyte; SMS = soccer match simulation; BL = baseline; HT, halftime; F, final trial sample taken following running capacity test.

solution on dribbling speed versus an electrolyte-matched placebo. We speculate that this discrepant finding may be attributed to several methodological factors. Unlike previous studies, our experimental trials were performed on an artificial grass surface and players wore their own soccer boots, which may have facilitated the better execution of skills. Moreover, we chose to perform the trials in the early afternoon instead of the morning with the aim to better reflect competitive practices of this group of players in Scotland. In accordance with published carbohydrate recommendations for exercise (Thomas et al., 2016; Williams & Rollo, 2015), we also provided a standardized breakfast containing 46 g of carbohydrate in addition to a prematch meal consisting of 2 g carbohydrate per kg of body mass ingested 2 hr prior to starting the SMS protocol. By contrast, Harper et al. (2017) provided soccer players with a breakfast containing 10% of daily energy requirement (~35 g of carbohydrate) ingested ~135 min before exercise. Interestingly, whereas Harper et al. (2017) reported a statistically significant decline in dribbling speed over time, in this study, dribbling speed remained constant throughout the 90-min SMS protocol. Although muscle glycogen concentration was not measured in this study, we speculate that including a carbohydrate-rich prematch meal prevented the decline in muscle glycogen content that has previously been associated with impaired performance (Mohr et al., 2005). Hence, the benefit of ingesting a 12% CHO-E solution on ball dribbling performance appears to be context specific, for example, when it is not possible to comply with prematch carbohydrate intake guidelines, and/or only when fatigue has a markedly detrimental impact on dribbling performance.

In this study, whereas ingesting the 12% CHO-E solution resulted in the better maintenance of passing accuracy in both dominant and nondominant feet, passing speed was better maintained with carbohydrate ingestion during the latter stages of the SMS protocol in the nondominant foot only. To our knowledge, this is the first study to differentiate between dominant and nondominant feet when measuring soccer passing performance. Previous work demonstrates that if a task is familiar to an individual, then there will be an element of automaticity, and fewer CNS resources are required for optimal performance (McMorris & Graydon, 1997). Conversely, when a task becomes more complex, the task outcome is more likely to be influenced by arousal (McMorris & Graydon, 1997). Thus, it is intuitive that performing the passing test with the nondominant foot required a greater allocation of CNS resources than when performing the passing test with the dominant foot. It also has been proposed that fatigue is associated with a decrement in central control (Welsh et al., 2002), thus it can be inferred that the nondominant side would demand more activity from the CNS, and therefore, be more susceptible to fatigue. Previous work has demonstrated that carbohydrate ingestion enhances CNS activity and motor control (Liu et al., 2000; Welsh et al., 2002). In this regard, Bottoms et al. (2007) showed that when compared with placebo the ingestion of a carbohydrate solution resulted in skill retention, specifically in the backhand drive (weaker shot) in squash players. Therefore, it is intuitive that ingesting the 12% CHO-E solution had a more profound effect on passing performance with the nondominant foot than with the dominant foot. Consistent with this proposed mechanism, it appears from our data that the impact of carbohydrate feeding is observed primarily with the nondominant foot, particularly toward the end of the SMS.

Studies showing a deterioration in soccer-specific skills (Ali & Williams, 2009; Ali et al., 2007; Ostojic & Mazic, 2002) when players did not consume carbohydrates have argued that lowered glucose concentrations are associated with declines in skill performance. In this study, blood glucose concentrations at halftime and at the end of the full protocol were significantly higher when ingesting the 12% CHO-E solution versus placebo. However, ingesting the 12% CHO-E solution failed to prevent the decline in blood glucose concentration 15 min into the second half of the SMS, likely reflecting increased glucose disposal at the onset of the second half of the SMS. Other studies (Russell et al., 2012) have reported skill performance decline in placebo condition versus carbohydrate consumption despite mean blood glucose concentration remaining euglycemic. Altogether, it appears that blood glucose concentration per se is not a key driver for the changes in skill performance detected between trials.

Blood lactate concentration during the SMS protocol was similar on both trials and reflects typical match intensity responses (Krustrup et al., 2006). Although marginal, the higher lactate concentrations in the CHO-E trial following the high-intensity anaerobic running test likely reflects greater capacity for flux through glycolysis in the face of additional substrate availability on that trial. Although sprint speed over 15 m did not decline throughout the SMS, mean values were comparable with those reported during actual match play (Krustrup et al., 2006). Interestingly, Balsom et al. (1992) previously demonstrated that 15-m sprints could be performed at 30 s intervals without impaired performance in the absence of carbohydrate supplementation. In this study, 18 × 15-m sprints were performed over the 90-min SMS protocol separated by a minimum period of 5 min, during which lower intensity exercise intervals were performed. This protocol would suggest that there was sufficient time for phosphocreatine resynthesis between sprints, thus preventing a decline in sprint speed over the SMS on both trials. We reported that high-intensity running capacity following the SMS was significantly better in the 12% CHO-E versus placebo trial. Similar results were reported by Alghannam (2011) and Nicholas et al. (1995), supporting the argument that carbohydrate ingestion exhibits an ergogenic benefit on anaerobic endurance capacity after prolonged intermittent exercise.

In conclusion, ingesting a 12% CHO-E solution before an SMS protocol and at halftime aided the retention of soccer-specific skill performance, particularly passing performance toward the end of the SMS protocol and enhanced high-intensity running capacity after simulated match play. Soccer players typically experience fatigue toward the end of the match (Bradley et al., 2009; Mohr et al., 2005), and the number of goals conceded increases during the latter stages of the game (Reilly, 1997). This study adds to the evidence base that optimization of carbohydrate ingestion strategies appears to have a practically relevant benefit on key skill (passing) and physiological-related (high-intensity running capacity) factors that likely influence performance toward the end of a soccer match.

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APPENDIX 2

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A preliminary study of the reliability of soccer skill tests within a modified soccer match simulation protocol

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A preliminary study of the reliability of soccer skill tests within a modified soccer match simulation protocol

Paola Rodriguez-Giustiniani[a], Ian Rollo[b] and Stuart D.R. Galloway[a]

[a]Physiology, Exercise, and Nutrition Research Group, Faculty of Health Sciences and Sport University of Stirling, Stirling, UK; [b]Gatorade Sports Science Institute, PepsiCo Life Sciences, Global R&D, UK

ABSTRACT

Aim: This study examined test-retest reliability of soccer-specific skills within a modified version of the soccer match simulation (SMS) protocol.

Methods: Ten professional youth academy soccer players (18 ± 1 years) from the United Kingdom completed 30 minutes of the modified SMS on two occasions under standardised conditions. During each trial, participants performed 20-m dribbling, short passing (4.2-m), long passing (7.9-m), shooting skills, and 15-m sprints within four blocks of soccer specific activity.

Results: Collapsed normative data (mean (SD)) for trial 1 and trial 2 for dribbling speed was 2.7 (0.2) m/s, for sprint speed 5.9 (0.4) m/s, for short pass speed 11.1 (0.5) km/h, for long pass speed was 12.2 (0.5) km/h, and for shooting speed was 13.3 (0.4) km/h. Mean results from trial 1 and trial 2 were not different for all measures evaluated ($P > 0.05$). Good to excellent reliability (ICC 0.76-0.99) was observed for long and short passing speed, shooting speed, sprint speed, and long pass accuracy, with CVs typically < 5-10%. Moderate reliability (ICC 0.50-0.75) was observed for dribbling speed. Poor reliability (ICC <0.50) was observed for dribbling accuracy and shooting accuracy.

Conclusions: The reliability of the modified version of the SMS protocol is promising for most of the skills assessed, with the exception of dribbling and shooting accuracy in this group of professional youth soccer players. The modified protocol is easy to implement within professional clubs without specialist equipment, but due to the limited sample size the reliability requires further confirmation in a larger sample.

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KEYWORDS
Football; reproducibility; accuracy; speed

Introduction

Soccer simulation protocols aim to replicate movement patterns and physiological demands of match-play (Drust et al. 2000; Nicholas et al. 2000; Thatcher and Batterham 2004; Russell et al. 2011). Free running intermittent exercise simulation protocols are designed to simulate the activity pattern characteristics of soccer, however, several factors, such as the omission of game-specific skills (Russell et al. 2011), and the use of a non-grass surface might reduce the ecological validity of these protocols (Russell et al. 2011). As such, modified versions of protocols have been implemented to investigate soccer-specific skills (Ali et al. 2008; Rostgaard et al. 2008; Foskett et al. 2009).

Russell et al. (2011) developed the Soccer Match Simulation (SMS) protocol. The SMS is a modified version of the Loughborough Intermittent Soccer Test (LIST) which was the first intermittent exercise simulation protocol designed to simulate the activity pattern characteristics of soccer. The LIST includes 75-min of intermittent activity followed by a run to exhaustion (Nicholas et al. 2000), however, it does not include game-specific skills. The SMS includes additional movement components, and soccer-specific skills are embedded to enhance the ecological validity of the protocol (Russell et al. 2011). This means the SMS has application to studies investigating interventions on both the physical and skill components of soccer players performance (Russell et al. 2011; Harper et al. 2017). The SMS protocol has been successfully used to evaluate performance, physiological responses, and the efficacy of nutritional interventions (Kingsley et al. 2014; Harper et al. 2017; Rodriguez-Giustiniani et al. 2019). However, it is important to know the reliability of sport-specific tests like the SMS, as day to day variation needs to be understood in order to determine differences between trials in intervention studies. Reliability refers to the reproducibility/precision of values from a test, assay, or other measurement in repeated trials on the same individuals (Hopkins 2010). Russell et al. (2010) assessed the reliability of the skills contained in the SMS and reported moderate, to moderately strong, relative reliability for passing (speed, precision, and success), shooting (accuracy), and dribbling (speed and accuracy). When assessed over 120-min (two 45-min halves plus 2 additional 15-min periods) Harper et al. (2016) reported the physiological and performance responses were reliable but did not include skill outcome measures of passing or shooting.

In our recent study, we utilised a modified version of the SMS protocol to assess soccer skill performance (Rodriguez-Giustiniani et al. 2019). The protocol was modified for ease of use in a professional soccer academy setting, as well as to provide greater insight into potential differences between dominant and non-dominant foot. In order to assess the

CONTACT Stuart D.R. Galloway s.d.r.galloway@stir.ac.uk Physiology Exercise and Nutrition Research Group, Faculty of Health Sciences and Sport, University of Stirling, Stirling, FK9 4LA, UK

passing variables, we utilised readily available passing targets (i.e., soccer mannequins) instead of banners with target boxes and an illumination system (Russell et al. 2011). Furthermore, for the first time, the assessment of passing and shooting was discriminated between the dominant and non-dominant foot. This involved increasing the number of passes and shots taken during the match simulation protocol. Discriminating between feet could be of importance as fatigue is associated with a decrement in central control (Welsh et al. 2002) and may be more likely to affect skill performance with the non-dominant foot (Rodriguez-Giustiniani et al. 2019).

Previous studies on skill-based sports like squash (Bottoms et al. 2007) and tennis (McRae and Galloway 2012) have shown positive effects of nutritional interventions on the maintenance of skill performance, particularly on weaker/non-dominant shots such as backhand drive-in squash. Indeed, passing speed was better maintained in the non-dominant foot when evaluating the effects of a carbohydrate-electrolyte beverage on soccer skill performance using a simulated soccer match protocol (Rodriguez-Giustiniani et al. 2019). Taken together, it seems reasonable to distinguish between the dominant and the non-dominant foot when investigating the reliability of soccer-skill assessments. Due to the changes that we have made in the delivery of the SMS, and the skills within, it is relevant to assess the reliability of this modified version of the protocol. We theorised that this modified protocol would demonstrate similar reliability to previous studies using the SMS. Specifically, we aimed to quantify the absolute and relative test–retest reliability and compare the magnitude of these statistics to previous investigations on the SMS.

Methods

Participants

Ten male well-trained professional outfield soccer players (5 midfielders, 3 defenders, 2 strikers) from the United Kingdom, who were accustomed to skill assessments as part of their regular training, were recruited from a local Professional Football Club development squad in order to participate in this investigation. All players had five or more years of playing experience, had been training consistently for 1 year or more, were regularly participating in match-play with their squad, and were free from injury at the time of the recruitment and testing (age: 18 ± 1 years, body mass: 75.0 ± 6.5 kg, stature: 179.2 ± 5.6, body mass index: 23.4 ± 1.0 kg/m^2). The experimental procedures were approved by a local Ethics of Research Committee and the study was conducted in accordance with the declaration of Helsinki.

*Study Design*Players attended one preliminary visit for a familiarisation session before undertaking two main trials in which test re-test reliability was assessed. All visits were expected to be separated by a minimum of 2 days and a maximum of 7 days. From the ten players, three completed their first main trial 2 days after the familiarisation visit, four after 4 days of the familiarisation, two 6 days after the familiarisation, and one player completed the first trial 7 days after the familiarisation. All the participants completed the two main trials 7 days apart. Players followed 48-hr habitual diets (avoiding caffeine and alcohol) and recorded food consumed before the familiarisation visit. The pre-familiarisation diet was replicated for both main trials. Players refrained from strenuous exercise 48-hr before the familiarisation and main trial days. All testing sessions were performed on an indoor artificial grass pitch (length: 37-m; width: 19-m; ceiling height: 6.5-m). Soccer balls were inflated to a pressure of 14 psi before each trial.

Familiarisation and main trials procedures

All trials started in the afternoon to reflect the time at which this group typically engages in soccer match play. At the training ground, researchers provided players with a standardised breakfast (2 eggs, 2 slices of bread, 1 medium-sized banana providing 423 kcal, 46 g carbohydrate, 26 g protein, 14 g fat). A pre-trial standardised meal also was provided 2-h before beginning the main trials, with the meal containing 2 g carbohydrate □ kg^{-1} of body mass (pasta in a tomato sauce) plus 500 ml of water. Upon attendance for testing (familiarisation and main trials), body mass (SECA Quadra 808, Hamburg, Germany) was assessed immediately after voiding of bladder and bowels.

Familiarisation and main trials commenced after a 15-min standardised warm-up (consisting of running, dynamic stretching, and 20-m sprints) that preceded each trial. During each trial, participants were required to perform soccer dribbling, passing, and shooting skills, and 15-m sprints throughout four blocks of a modified soccer match simulation (SMS) protocol (Russell et al. 2011) lasting a total of 30 minutes (Figure 1(a)). Each block of the SMS protocol consisted of 3 repeated cycles of three 20-m walks, one walk to the side, five 20-m jogs, one 20-m backwards jog, two 20-m strides and an alternating timed 15-m sprint or a 20-m dribble (Figure 1(b)), followed by passing and shooting assessments (Russell et al. 2011).

Simulated soccer match protocol, skills testing and analysis

The SMS includes exercise blocks consisting of 3 repeated cycles of three 20-m walks, one walk to the side, an alternated timed 15-m sprint or a 20 m dribble, a 4-s passive recovery period, five 20-m jogs at a speed corresponding to 40% VO_2 max, one 20-m backwards jogs at 40% VO_2max and two 20-m strides at 85% VO_2max followed by passing and shooting assessments. So as to assess the reliability of our modified version of the protocol, the participants completed four blocks of the abovementioned cycles. In order to assess dribbling speed and accuracy, players dribbled a ball between 7 cones (cones 2–7 were placed 3-m away from the preceding cone, and cones 1 and 7 were 1 m away from each end of the course; Figure 2(a)). Participants were required to dribble the ball as quickly and accurately as possible from one end to the other over the 20-m total distance. Participants dribbled towards a video camera that was placed directly in line with the cones. For the sprint assessment, players ran as fast as possible through timing gates (Brower®, USA) placed 15-m apart, with a 1-m run-in. At the end of each block of activity, players

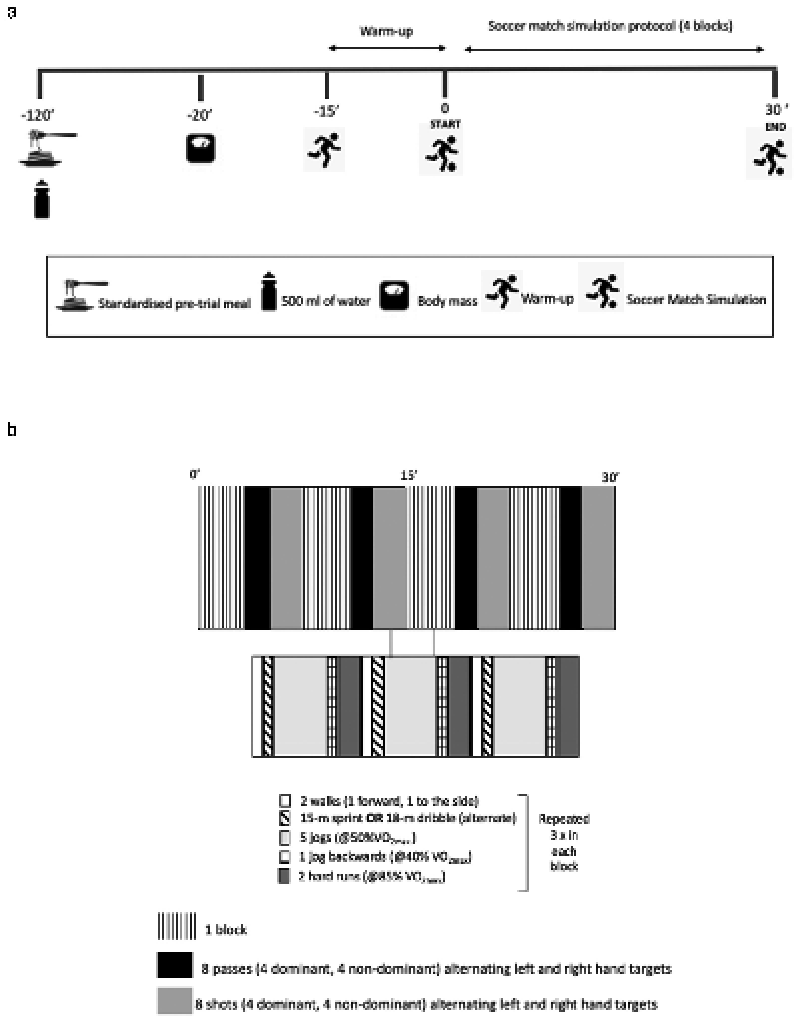

Figure 1. Protocol schematic (a) and simulated soccer match outline (b).

performed a bout of passing where they directed alternate passes towards target zones placed to the left and right at distances of 4.2-m (short pass) and 7.9-m (long pass).

Soccer mannequins (Diamond Football®, Senior Pro Free Kick) with their bases were used as passing targets. The base (0.5-m wide) was the central zone of the target, with cones at a distance of 0.5-m of each side as the lateral zones of the target. A pass to the centre area was worth 10 points and the two lateral areas were worth 5 points. Passes that missed the target areas were scored as 0. Passing bouts consisted of 8 passes (2 with the dominant and 2 with the non-dominant foot to the short pass target, and 2 with the dominant and 2 with the non-dominant foot to the long pass target; Figure 2(b)). Then, a shooting skill assessment was performed, for this, participants were instructed to kick the ball as firmly and accurately as possible to a shooting target. Shooting target zones were at a distance of 15-m in the four corners of the goal. These target areas have been identified as optimal ball placement to beat a goalkeeper when shooting (Ali et al. 2007). Each shooting target was divided into two areas, a centre area (75 cm x 60 cm) and an extended area (100 cm x 90 cm) with the centre area worth 10 points and the extended area worth 5 points. Shots that missed the areas on the target were scored as 0. The bouts of shooting consisted of 8 shots (4 with the dominant foot and 4 with the non-dominant foot; Figure 2(b)). To enhance ecological validity, no prior touches were allowed to control the ball before a pass or a shot (Dooan et al. 2001).

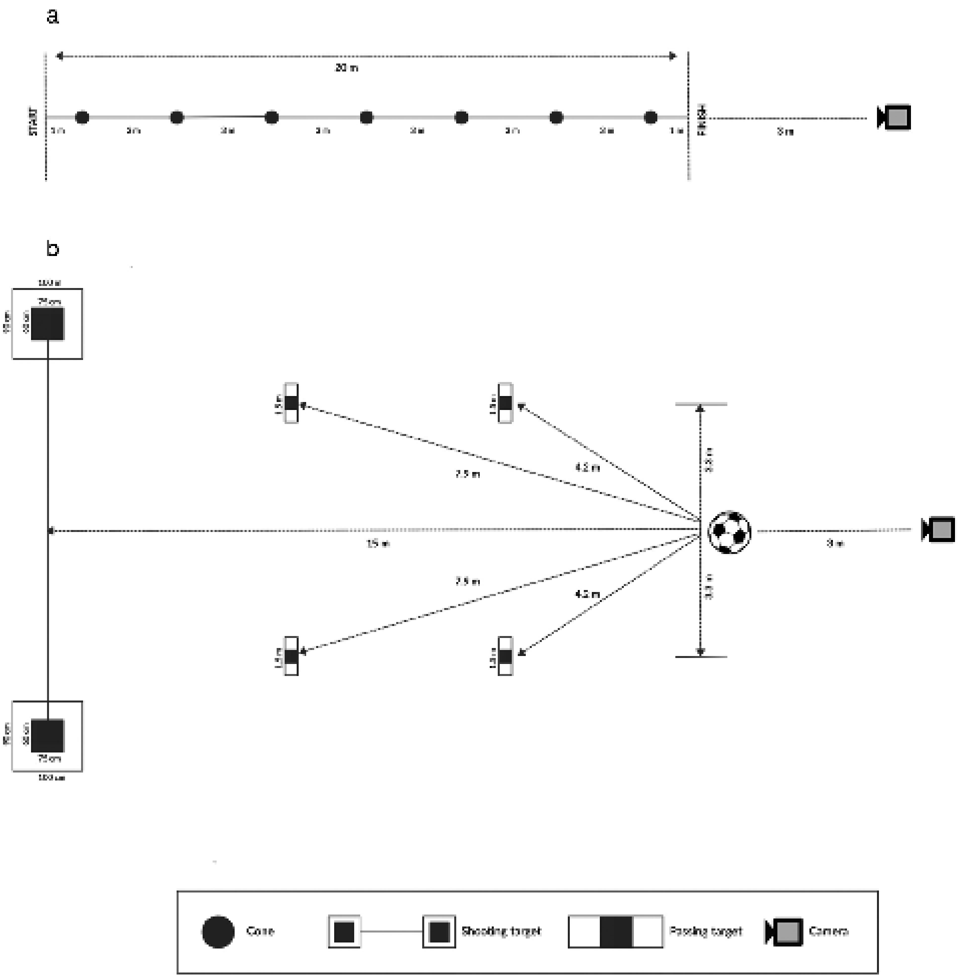

Figure 2. Schematic representation of the (a) dribbling assessment and the (b) passing and shooting assessments.

Video footage of the skill tests was captured using GoPro cameras (GoPro®, Hero 5), one was placed 1-metre apart from the last cone of the dribbling course and the other 1-metre behind the passing and shooting zone. Manual digitisation (Kinovea® version 0.8.15; Kinovea Org., France) yielded dribbling speed, dribbling precision, passing accuracy, and passing speed, as well as shooting accuracy and speed. Passing and shooting speed was calculated from the time interval between ball contact with the foot and subsequent ball contact with the target area.

Inter and intra rater reliability

In order to determine inter-rater and intra-rater reliability, we selected data from three participants across the two trials for each one of the variables evaluated through digitisation. For the assessment of inter-rater reliability two experienced video analysts within a professional football club analysed the data which was compared with the data gathered by the investigators of this study. To assess intra-rater reliability we compared repeated measures from one of the authors of the study and two video analysts for each one of the variables assessed.

Statistical analysis

Statistical analysis was carried out using SPSS software (Version 16.0; SPSS Inc., USA) and a custom-made spreadsheet (Hopkins 2015). Systematic bias was evaluated as the mean change between trial 2 and trial 1, determined using paired t-tests and statistical significance set at $P < 0.05$. Soccer skills relative and absolute reliability was assessed using the intra-class correlation coefficient (ICC) and the coefficient of variation (CV), respectively. The ICC was derived from the methods of a two-way mixed effects model for consistency in a single measure (i.e., ICC3,1). Interpretation of the ICC values was based on guidelines provided by Koo and Li (Koo and Li 2016): ICC's less than 0.50 were indicative of poor reliability, values between 0.50 and 0.75 indicated moderate reliability, values between 0.76 and 0.90 indicated good reliability, and values greater than 0.90 indicated excellent reliability. The coefficient of variation was calculated from the standard error of measurement. All the aforementioned statistics were presented with 95% confidence intervals (CI).

Results

Descriptive and reliability statistics along with mean differences (95% CI) for each of the repeated skill assessments are presented in Table 1 and 2. Mean results from trial 1 and trial 2 were not significantly different for all measures evaluated ($P > 0.05$). Reliability statistics for the assessed skills are also presented in Table 1 and 2. Long passing speed with both feet, with the dominant foot, and with the non-dominant foot were the most reliable outcomes that were identified since the ICC showed excellent reliability.

Short pass speed with both feet and shooting speed with both feet showed good reliability along with a CV <5%, whereas sprint speed also presented good reliability with a CV <10%. Good reliability and a CV in the 10–20% range was reported for both short and long pass accuracy with both feet. Dribbling speed was moderately reliable with a CV <10% whereas shooting accuracy and dribbling precision both showed poor reliability. When assessing passing and shooting performance with the dominant and the non-dominant feet separately (Table 2), we observed that long pass accuracy with the dominant foot showed excellent reliability. We reported good reliability with a CV <5% for short pass and shooting speed with the non-dominant foot, whereas short pass speed with the dominant foot had good reliability and a CV <10%. Shooting speed with the dominant foot, short pass accuracy with the dominant foot, long pass accuracy with the non-dominant foot, and shooting accuracy with the non-dominant foot were all moderately reliable while short pass accuracy with the non-dominant foot and shooting accuracy with the dominant foot showed poor reliability.

Inter-rater and intra-rater error reliability was good to excellent for almost all variables studied, except for long pass with non-dominant foot which only achieved moderate intra-rater reliability in analyst 2 for trial 1 (Table 3). The coefficient of variation was below 10%for most of the variables assessed except in four cases (dribbling precision, intra-rater reliability, analyst 2; shooting accuracy with both feet, intra-rater reliability, researcher; shooting accuracy non-dominant foot, intra-rater reliability, analyst 1; shooting speed dominant foot, analyst 1) in which the CV values were between 10–20%. We did not observe any of the CV values to be > 20%.

Discussion

The aim of this study was to examine the preliminary reliability of soccer skill tests within a modified version of the SMS protocol (Russell et al. 2011). In particular, we have observed that when assessing long passing speed (both feet, dominant foot, and non-dominant foot), sprint speed, short pass accuracy with both feet, long pass accuracy (both feet and with the non-dominant foot) short pass speed (both feet, with the dominant foot, and with the non-dominant foot), and shooting speed (both with and with the non-dominant foot) this version of the SMS protocol demonstrated good to excellent reliability. However, the modified protocol revealed poor reliability for dribbling precision, short pass accuracy (with the non-dominant foot) and shooting accuracy. To our knowledge this study is the first protocol of this kind that assesses the reliability of soccer specific skills performed with the dominant and non-dominant foot in professional youth soccer players, on an artificial grass surface.

Reliability of dribbling speed and precision

The number of successful dribbling tasks has been identified as a key contributor to match success (Zago et al. 2016). The present study found moderate reliability for dribbling speed but poor reliability for dribbling precision. This finding is in contrast to good reliability for both dribbling speed and dribbling precision reported previously (Russell et al. 2010). The reason for the difference may, in part, be due to how the skills were assessed. Specifically, integrating the dribbling skill assessment within the SMS in the present study may have reduced the reliability, in comparison to when the dribbling assessment was performed in isolation (Russell et al. 2010). Under circumstances where the player performs the dribbling test between efforts, they are likely to take time to focus exclusively on the required skill.

Harper et al. (2016) investigated the reliability of physiological and performance responses to the SMS across 120 minutes of soccer-specific exercise. In order to do this, these authors used an extended version of the SMS in which they included two additional 15-min periods of intermittent exercise and skill testing, on top of the two 45-min halves of the original SMS protocol. All performance variables assessed were expressed as an average per 15-min of exercise. Harper et al. (2016) demonstrated moderate reliability for dribbling speed in both the 0–15 min ($r = 0.71$) and the 16–30 min ($r = 0.52$) time-points within the SMS. Hence dribbling speed over a 20-m seems to be a moderately reliable soccer skill to assess when embedded within the SMS protocol. On the contrary, dribbling precision seems to be poorly reliable within the SMS protocol. Harper et al. (2016) demonstrated moderate reliability during the first 15-min of activity only ($r = 0.64$). However, correlation values (using Pearson's correlation) thereafter (30–120 mins) corresponded with poor reliability. As both mental and physical fatigue influence skill performance (Reilly and Holmes 1983; Ramsbottom et al. 1988; Rampinini et al. 2009; Smith et al. 2015), Harper et al. (2016) attributed the respective lower reliability values to the fact that skill performance was measured in a fatigued state (Foskett et al. 2009). Thus, with respect to

Table 1. Descriptive and reliability statistics obtained from two trials assessing soccer-skills performance within a modified SMS protocol.

Skill	Trial 1	Trial 2	Mean change (95% CI) Raw values %	t-test (P-value)		ICC (95% CI)	CV (%) (95% CI)
Dribbling Speed (m/s)	2.7 ± 0.2	2.7 ± 0.2	0.0 (−0.1, 0.1)	−0.1 (−5.3, 4.9)	0.99	0.68 (0.14, 0.91)	7.6 (7.5, 7.7)
Dribbling Precision (cm)	1.2 ± 0.4	1.1 ± 0.3	−0.1 (−0.4, 0.3)	0.1 (−0.4, 0.3)	0.61	0.15 (−0.49, 0.69)	23.1 (22.9, 23.3)
Sprint Speed (m/s)	5.9 ± 0.4	5.9 ± 0.4	0.0 (−0.1, 0.2)	0.0 (−0.2, 0.1)	0.69	0.87 (0.58, 0.97)	6.9 (6.7, 7.2)
Short Pass Accuracy Both Feet (points)	56 ± 10	54 ± 6	−2 (−6, −2)	2.2 (−1.9, 6.1)	0.25	0.85 (0.43, 0.95)	13.9 (8.9, 18.9)
Long Pass Accuracy Both Feet (points)	44 ± 7	43 ± 5	−1 (−3, 2)	0.1 (−0.2, 0.3)	0.65	0.76 (0.22, 0.92)	15.5 (11.8, 19.2)
Short Pass Speed Both Feet (km/h)	11.1 ± 0.4	11.0 ± 0.5	−0.1 (−0.3, 0.2)	0.6 (2.3, −3.4)	0.58	0.84 (0.44, 0.96)	3.4 (3.2, 3.7)
Long Pass Speed Both Feet (km/h)	12.1 ± 0.6	12.2 ± 0.4	−0.1 (−0.1, 0.2)	0.1 (−0.2, 0.1)	0.33	0.95 (0.81, 0.99)	4.3 (3.9, 4.6)
Shooting Accuracy Both Feet (points)	69 ± 6	71 ± 5	2 (−2, 6)	−1.9 (−0.1, 0.2)	0.33	0.40 (−0.27, 0.81)	6.3 (2.9, 9.7)
Shooting Speed Both Feet (km/h)	13.6 ± 0.3	13.6 ± 0.4	0.1 (−0.2, 0.1)	−0.1 (−0.2, 0.1)	0.27	0.89 (0.62, 0.97)	2.5 (2.3, 2.7)

Data presented as mean ± standard deviation, CI: confidence interval. P-value determined from test re-test data using paired sample t-test for all measurements' outcomes (n = 10).
SMS: soccer match simulation, CI: confidence interval, ICC: intra-class correlation coefficient, CV: Coefficient of variation.

previous observations our data would suggest that dribbling precision over 20-m when assessed within an SMS protocol has poor reliability even when not fatigued, particularly beyond the initial 15 minutes of activity (Harper et al. 2016).

Reliability of passing speed and accuracy

To retain possession of the ball, accurate passing is a frequent and essential skill throughout soccer match-play (Hughes and Franks 2004; Rampinini et al. 2009). Longer passing sequences and a greater number of successful passes are associated with more goals scored (Hughes and Franks 2004). Russell et al. (2010) reported moderate reliability (ICC = 0.51) when assessing passing accuracy. As previously highlighted by Russell et al. (2010), it is possible to make comparisons between studies that report reliability in different ways. Using the intraclass correlation coefficient, our data revealed good reliability for passing accuracy using both feet, for both short and long passes, however, the CV revealed that there was only a moderate rating on variability. Regarding passing speed, we observed good reliability for the short pass and excellent reliability for the long pass with both feet. Russell et al. (2010) observed similar reliability for passing speed with an ICC of 0.76. Ali et al. (2007) aimed to assess the reliability of the Loughborough Soccer Passing Test (LSPT) in elite and non-elite soccer players. While the outcomes from the LSPT (time taken, time penalties for incorrect actions and total time) are not aligned to the outcome measures of the SMS passing assessment (i.e., accuracy, speed), the reliability of these outcomes are comparable with those shown by Russell et al. (2010). When comparing reliability for passing accuracy using the ICC, in the current study to the total time for the LSPT (as global marker of precision) we demonstrated greater reliability. Thus, passing performance (accuracy, speed) with both feet appears to be a reliable skill to assess within a SMS. We suggest the improvement observed in the present study may be due to players being able to use their routine soccer footwear (boots), instead of trainers (indoor surface). In addition, replacing the passing markers with mannequins may have also helped the players passing performance by providing a more realistic target.

Skill performance declines with fatigue (Mohr et al. 2005), and it appears that the non-dominant side may be more susceptible to this decline (Bottoms et al. 2007; McRae and Galloway 2012; Rodriguez-Giustiniani et al. 2019). Therefore, it is important to discriminate between dominant and non-dominant foot when assessing passing performances. We previously reported that long passing speed was better maintained with carbohydrate ingestion versus placebo during the latter stages of a SMS protocol in the non-dominant foot only (Rodriguez-Giustiniani et al. 2019). To our knowledge, that was the first study to differentiate between dominant and non-dominant foot when measuring soccer passing performance. The present data reveal that reliability of passing accuracy for the long pass is good for the dominant foot, and moderate for the non-dominant foot. However, this level of reliability was not evident for the short pass, which was moderate for the dominant foot and poor for the non-dominant foot.

By reducing the passing distance between the player and target, a reasonable assumption would have been improved, or at least similar, reliability to that of the long pass. We observed that reliability for passing speed was excellent (ICC: > 0.90) for both the dominant and the non-dominant foot for the long pass, whereas it was classified as good for both the dominant and the non-dominant foot for the short pass. Thus, since short pass targets were positioned at a 30° angle from the ball contact point whereas long pass targets were located at a 60°angle from the ball contact point, we speculate that the more lateral positioning of the short pass targets may have negatively influenced the reliability. The effect of increasing the passing distance further, to >8 m (typical of goal keepers and common for all outfield players), on reliability of passing remains to be established. Nevertheless, dominant, and non-dominant foot passing skills (accuracy, speed) within the SMS seem to be reliable assessments within this group of players, on an artificial grass surface when football mannequin targets are used.

Reliability of shooting speed and accuracy

The aim of soccer is to score more goals than the opposition, thus, shooting is a crucial skill (Stone and Oliver 2009). When assessing the reliability of soccer-skills, Russell et al. (2010) revealed poor reliability (ICC = 0.38) when assessing shooting accuracy. Our data also show poor reliability for shooting accuracy when considering data for both feet combined. As previously stated, the skill assessments in Russell's study (Russell et al. 2010) were evaluated in isolation and not integrated within the blocks of running *per se* as in the present study. As

Table 2. Descriptive and reliability statistics obtained from two trials assessing soccer passing and shooting performance with the dominant and non-dominant foot within a modified SMS protocol.

Skill	Trial 1	Trial 2	Mean change (95% CI) Raw values	%	t-test (P-value)	ICC (95% CI)	CV (%) (95% CI)
Short Pass Accuracy Dominant Foot (points)	31 ± 5	30 ± 3	−1.0 (−2.0, 4.0)	1.0 (−2.0, 3.9)	0.47	0.53 (−0.10, 0.86)	11.7 (9.2, 14.2)
Short Pass Accuracy Non-Dominant Foot (points)	25 ± 5	23 ± 5	−2.2 (−2.5, 6.9)	2.2 (−2.6, 6.7)	0.32	0.22 (−0.34, 0.66)	15.5 (12.4, 18.6)
Long Pass Accuracy Dominant Foot (points)	24 ± 6	24 ± 3	−0.1 (−1.2, 1.4)	0.1 (−1.2, 1.4)	0.86	0.92 (0.72, 0.98)	18.6 (15.8, 21.4)
Long Pass Accuracy Non-Dominant Foot (points)	20 ± 4	19 ± 3	−0.7 (−1.6, 3.0)	0.7 (−1.6, 3.0)	0.51	0.56 (0.05, 0.84)	15.3 (13.1, 17.5)
Short Pass Speed Dominant Foot (km/h)	11.6 ± 0.6	11.4 ± 0.8	−0.2 (−0.2, 0.5)	0.2 (−0.2, 0.5)	0.36	0.76 (0.39, 0.92)	5.6 (5.2, 6.0)
Short Pass Speed Non-Dominant Foot (km/h)	10.7 ± 0.3	10.7 ± 0.3	0.0 (−0.2, 0.14)	0.0 (−0.2, 0.1)	0.63	0.78 (0.33, 0.94)	2.9 (2.2, 3.1)
Long Pass Speed Dominant Foot (km/h)	10.7 ± 0.3	10.8 ± 0.5	0.1 (−0.3, −0.4)	0.2 (−0.3, 0.0)	0.09	0.95 (0.81, 0.99)	3.7 (3.5, 4.0)
Long Pass Speed Non-Dominant Foot (km/h)	11.4 ± 0.5	11.3 ± 0.5	−0.1 (0.0, 0.2)	0.1 (0.2, 0.2)	0.02	0.96 (0.85, 0.99)	3.7 (3.4, 4.0)
Shooting Accuracy Dominant Foot (points)	39 ± 5	38 ± 4	−1.0 (−2.1, 4.1)	1.1 (−1.9, 4.0)	0.43	0.43 (−0.23, 0.82)	8.5 (5.7, 11.3)
Shooting Accuracy Non-Dominant Foot (points)	29 ± 7	32 ± 6	3.5 (−6.7, −0.3)	−3.6 (−7.0, −0.2)	0.04	0.75 (0.26, 0.93)	18.3 (14.3, 22.3)
Shooting Speed Dominant Foot (km/h)	14.1 ± 0.3	14.0 ± 0.3	−0.1 (−0.2, 0.4)	0.1 (−0.2, 0.4)	0.49	0.56 (−0.07, 0.87)	2.4 (2.2, 2.6)
Shooting Speed Non-Dominant Foot (km/h)	13.0 ± 0.5	13.1 ± 0.5	0.1 (−0.1, 0.2)	0.0 (−0.1, 0.2)	0.65	0.87 (0.55, 0.97)	4.1 (3.8, 4.4)

Data presented as mean ± standard deviation, CI: confidence interval. P-value determined from test re-test data using paired sample t-test for all measurements' outcomes (n = 10).
SMS: soccer match simulation, CI: confidence interval, ICC: intra-class correlation coefficient, CV: Coefficient of variation.

comparisons are possible where reliability is reported in dimensionless units, both the present data and that presented by Russell et al. (2010) appear to be more reliable than the Loughborough Soccer Shooting Test (Ali et al. 2007). Ali et al. (2007) evaluated the reliability of the Loughborough Soccer Shooting Test (LSST) in elite and non-elite soccer players, they reported poor reliability (ICC = 0.26) for shooting success and precision in both groups. Moreover, when discriminating between the dominant and the non-dominant foot the present data also demonstrate poor or moderate reliability for shooting accuracy with the dominant and the non-dominant foot, respectively.

There may be several reasons for poor shooting reliability, such as the increased distance versus the long pass (15-m v 8-m). The increased shooting speed generated when performing a shot (Table 2) may also add variation to the skill, as well as technique performed in the execution of shooting (front of foot/laces) versus passing (instep) per se. Our results are consistent with data presented by both Russell et al. (2010) and Ali et al. (2007) who reported poor shooting reliability, over distances of 15 m and 16.5 m respectively. It should be noted that only two of the 10 participants in the present study were classified as strikers. Players adopt playing positions due to their suitability and skill. Therefore, position-specific participants may be required to increase the reliability and assessment of specific skills such as shooting.

*Intra- and inter-rater reliability*We observed good to excellent intra-rater reliability for almost all of the variables when assessed by the study researcher and two video analysts within a professional football team. This indicates that the analysts' assessments were on the whole consistent on repeated analysis. When multiple raters assessed the studied variables, excellent inter-rater reliability was observed. This excellent inter-rater reliability indicates that different raters can consistently assess the experimental trials. Therefore, it is recommended that experienced video analysts/researchers are in charge of the digitisation when assessing skill outcomes using this modified version of the SMS protocol. For large-scale projects, the ability to use multiple experienced researchers/analysts offers important methodological considerations on data assessment and input

Limitations

It has been stated that proficient skill performance is affected by cognitive factors such as decision-making and game intelligence (Williams and Reilly 2000). In the current study, we did not use a randomised lighting system for target identification when assessing passing and shooting as used in the original version of the protocol (Russell et al. 2011). This modification was made to increase ease of implementing the protocol in professional club settings. Therefore, it is unknown how inclusion of decision-making and visual searching, would have influenced the skill reliability. We acknowledge that including such perceptual demands would increase the ecological validity. However, we believe that the modified version of the SMS used in the present study could be more practically applicable in professional club settings as the equipment used (mannequins) are readily available.

Due to the fact that more than 40 participants are recommended for reliability studies (Atkinson and Nevill 1998; Hopkins 2010) we recognise that our results are only preliminary and further reliability assessment of this version of the SMS is warranted. However, we believe that our results are applicable to professional youth football players. Russell et al. (2010) also reported construct validity when they assessed the reliability of the skills contained within the SMS and observed that professional football players performed better than recreational players. There are no data regarding this aspect for our modified version of the protocol, but it would be of interest to test for construct validity in a further larger scale study.

Table 3. Inter-rater and intra-rater reliability.

	ICC (95% CI)							
	Inter-rater reliability		Intra-rater reliability: researcher		Intra-rater reliability: analyst 1		Intra-rater reliability: analyst 2	
Skill	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Dribbling Speed (m/s)	0.99 (0.96, 1.00)	0.98 (0.84, 0.99)	0.99 (0.54, 1.00)	0.99 (0.89, 1.00)	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)	0.97 (0.21, 0.99)	0.89 (0.72, 0.92)
Dribbling Precision (cm)	0.99 (0.97, 1.00)	0.98 (0.82, 1.00)	0.98 (0.40, 1.00)	0.98 (0.33, 0.99)	0.99 (0.97, 1.00)	0.99 (0.96, 1.00)	1.00	0.94 (0.38, 0.99)
Short Pass Accuracy Both Feet (points)	0.99 (0.96, 1.00)	0.99 (0.90, 1.00)	1.00	0.98 (0.56, 1.00)	0.99 (0.80, 1.00)	0.98 (0.65, 1.00)	0.83 (0.21, 0.98)	0.91 (0.44, 0.99)
Short Pass Accuracy Dominant Foot (points)	0.95 (0.63, 0.99)	0.96 (0.05, 0.99)	1.00	0.99 (−0.01, 0.99)	0.95 (0.30, 0.99)	0.87 (0.31, 0.99)	0.97 (0.22, 0.99)	0.96 (0.41, 0.99)
Short Pass Accuracy Non-Dominant Foot (points)	0.99 (0.96, 1.00)	0.98 (0.83, 0.99)	0.98 (0.62, 0.99)	0.98 (−0.46, 0.99)	0.99 (0.91, 1.00)	0.97 (0.34, 0.99)	0.99 (0.43, 0.99)	0.78 (0.13, 0.90)
Long Pass Accuracy Both Feet (points)	0.99 (0.95, 1.00)	0.97 (0.68, 0.99)	1.00	0.99 (0.93, 1.00)	0.99 (0.79, 1.00)	0.97 (0.03, 0.99)	0.85 (0.23, 0.97)	0.99 (0.82, 1.00)
Long Pass Accuracy Dominant Foot (points)	0.99 (0.86, 0.99)	0.99 (0.92, 1.00)	0.98 (0.77, 1.00)	0.96 (0.41, 0.99)	0.99 (0.81, 1.00)	0.96 (0.20, 0.99)	0.98 (0.89, 0.99)	0.97 (0.74, 1.00)
Long Pass Accuracy Non-Dominant Foot (points)	0.91 (0.14, 0.99)	0.99 (0.36, 1.00)	1.00	1.00	0.98 (0.59, 1.00)	0.98 (0.50, 0.99)	0.91 (0.30, 0.99)	0.95 (0.30, 0.99)
Short Pass Speed Both Feet (km/h)	0.97 (0.36, 0.99)	0.98 (0.83, 0.99)	1.00	1.00	0.99 (0.61, 1.00)	0.96 (0.10, 0.99)	0.99 (0.91, 1.00)	0.99 (0.91, 1.00)
Short Pass Speed Dominant Foot (km/h)	0.99 (0.94, 1.00)	0.98 (0.85, 0.99)	1.00	1.00	0.98 (0.03, 0.99)	0.96 (0.03, 0.99)	0.95 (0.53, 1.00)	0.99 (0.79, 1.00)
Short Pass Speed Non-Dominant Foot (km/h)	0.98 (0.84, 1.00)	0.96 (0.66, 0.99)	1.00	1.00	0.91 (0.22, 0.99)	0.93 (0.37, 0.99)	0.99 (0.81, 1.00)	0.99 (0.81, 1.00)
Long Pass Speed Both Feet (km/h)	0.98 (0.85, 0.99)	0.91 (0.18, 0.99)	0.96 (0.31, 0.99)	0.94 (0.41, 0.99)	0.98 (0.47, 0.99)	0.86 (0.61, 0.99)	0.98 (0.59, 1.00)	0.85 (0.32, 0.97)
Long Pass Speed Dominant Foot (km/h)	0.99 (0.96, 1.00)	0.97 (0.69, 0.99)	0.94 (0.94, 0.99)	0.97 (0.48, 0.99)	0.98 (0.58, 0.99)	0.98 (0.14, 1.00)	1.00	0.89 (0.74, 0.93)
Long Pass Speed Non-Dominant Foot (km/h)	0.97 (0.80, 0.99)	0.95 (0.66, 0.99)	0.96 (0.67, 0.99)	0.88 (0.43, 0.99)	0.93 (0.07, 0.99)	0.92 (0.67, 0.99)	0.73 (0.38, 0.84)	0.97 (0.73, 0.99)
Shooting Accuracy Both Feet (points)	0.95 (0.61, 0.99)	0.97 (0.75, 0.99)	0.96 (0.25, 0.99)	0.97 (0.56, 0.99)	0.93 (0.11, 0.99)	0.87 (0.42, 0.99)	0.91 (0.77, 0.99)	0.98 (0.33, 0.99)
Shooting Accuracy Dominant Foot (points)	0.94 (0.59, 0.99)	0.91 (0.59, 0.99)	1.00	1.00	0.95 (0.12, 0.99)	0.93 (0.10, 0.99)	0.94 (0.59, 0.99)	0.98 (−0.03, 1.00)
Shooting Accuracy Non-Dominant Foot (points)	0.99 (0.90, 1.00)	0.95 (0.61, 0.99)	0.91 (0.58, 0.99)	0.90 (0.67, 0.99)	0.88 (0.30, 0.99)	0.92 (0.48, 0.99)	0.89 (0.81, 0.98)	1.00
Shooting Speed Both Feet (km/h)	0.98 (0.88, 1.00)	0.98 (0.89, 0.99)	0.96 (0.56, 0.99)	0.99 (0.74, 1.00)	0.95 (0.31, 0.99)	0.96 (0.01, 0.99)	0.93 (0.78, 1.00)	0.98 (0.46, 0.99)
Shooting Speed Dominant Foot (km/h)	0.99 (0.88, 1.00)	0.96 (0.64, 0.99)	0.97 (0.73, 0.99)	0.96 (0.79, 0.99)	0.84 (0.12, 0.99)	0.97 (0.46, 0.99)	0.95 (0.64, 0.99)	0.99 (0.93, 1.00)
Shooting Speed Non-Dominant Foot (km/h)	0.97 (0.56, 0.99)	0.98 (0.85, 0.99)	0.96 (0.26, 0.99)	0.90 (0.61, 0.99)	0.97 (0.48, 0.99)	0.98 (0.43, 0.99)	0.98 (0.85, 0.99)	0.96 (0.41, 0.99)

In conclusion, this modified version of the SMS protocol showed encouraging reliability, especially for dribbling speed, sprint speed, short and long pass speed, shooting speed, and long pass accuracy. How skill reliability may change as the academy players transition to senior teams, and the reliability of other soccer-specific skills such as heading, and ball control remain to be established. This testing protocol has potential application for research settings out of the laboratory when investigating strategies that aim to improve skill performance in professional soccer players.

Disclosure statement

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APPENDIX 3

Quantification of chlorogenic acid and related metabolites in drink samples

Contents

1. Quantification of chlorogenic acid, caffeic acid, and ferulic acid by LC/PDA

Materials and Reagents. Methanol, acetonitrile, and formic acid were of chromatography grade. Water was prepared with a Milli-Q EQ 7000 water system. The standard for caffeic acid was obtained from Sigma-Aldrich, ferulic acid was obtained from Selleckchem.

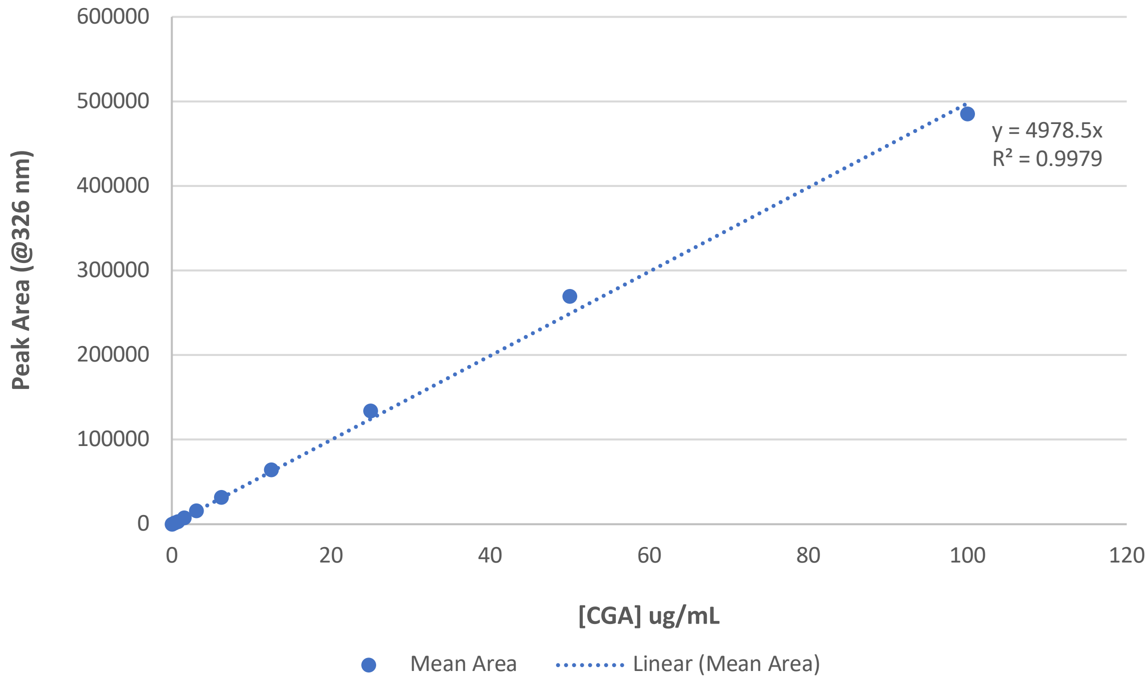
LC-PDA Analysis. The analysis was carried out on a Shimadzu Nexera-i UPLC fitted with a diode array detector. Linear gradient conditions were used for the separation of analytes using a Shimadzu Shim-pak LC column. Mobile phase conditions included: Solvent A 0.1% acetic acid and Solvent B chromatography grade acetonitrile. The following protocol was used during the run: 0 min 93% Solvent A: 7% Solvent B; 0-5 min 80% Solvent A: 20% Solvent B at a flow rate of 0.5 mL/min. Conditions were re-equilibrated for 5 min before subsequent injections The column temperature was kept at 45°C and detection wavelength set at 326 nm for optimal detection of chlorogenic acid, caffeic acid, and ferulic acid. Sample injection was 1 μL.

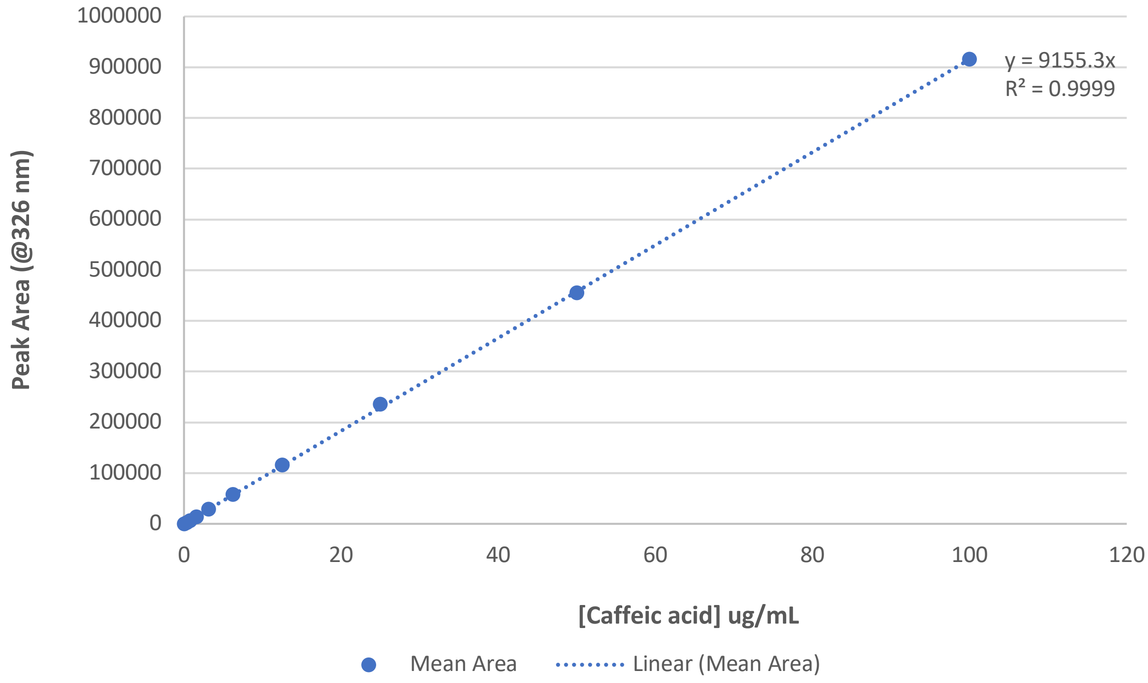
Standard Curve Preparation. A stock solution of chlorogenic acid, caffeic acid, and ferulic acid was prepared at 1 mg/mL in methanol. For each compound an eight step, 1 in 2, serial dilution was prepared in methanol. The concentration for these dilutions ranged from 100 μg/mL to 0.39 μg/mL. These serial diluted standards were used to determine identification information about each compound, as well as the linear quantification ranges for each compound.

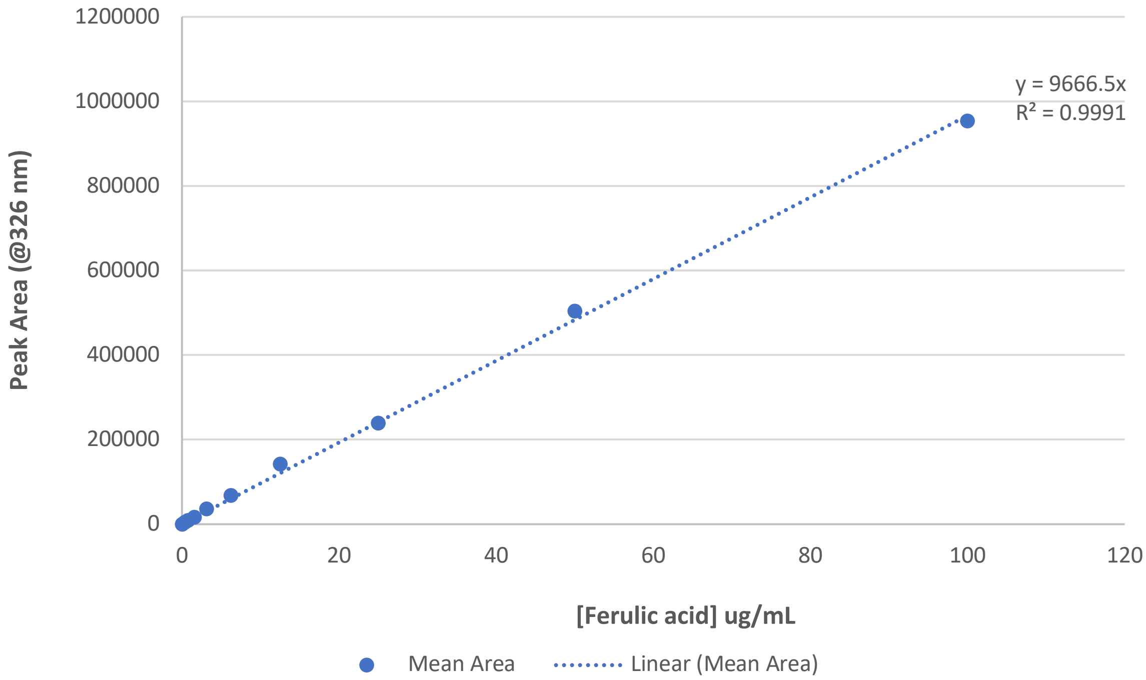
Table 1. Diagnostic information for chlorogenic acid, caffeic acid, and ferulic acid

Compounds	Retention time (min)	Detection Wavelength	
Chlorogenic acid	2.82 ± 0.01	326 nm	
Caffeic acid	4.01 ± 0.01	326 nm	`
Ferulic acid	2.389 ± 0.004	326 nm	

Linearity. The calibration curves for chlorogenic acid, caffeic acid, and ferulic acid are presented in the figures below.

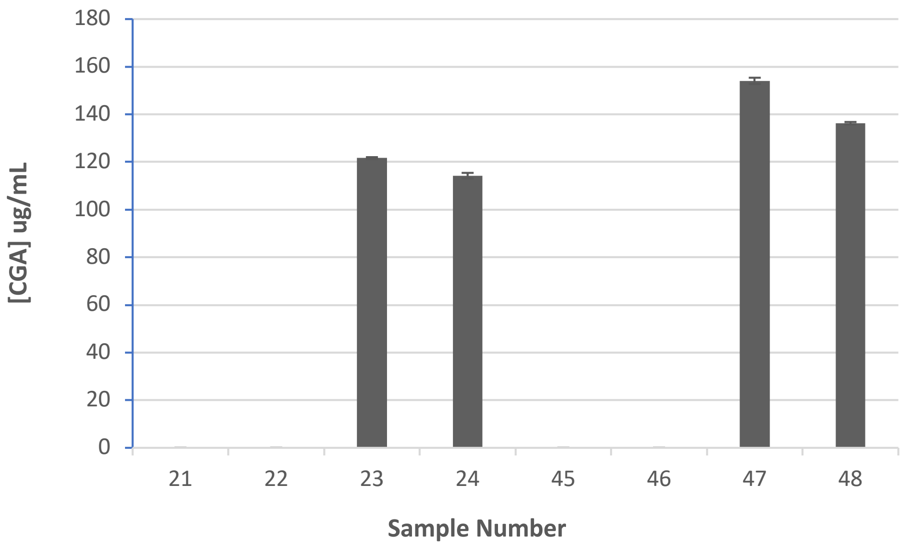

600000
500000
400000
300000
200000
100000
0
0
20
40
60
80
100
120
Peak Area (@326 nm)
[CGA] ug/mL
y = 4978.5x
R² = 0.9979
Mean Area
Linear (Mean Area)


1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
20
40
60
80
100
120
Peak Area (@326 nm)
[Caffeic acid] ug/mL
y = 9155.3x
R² = 0.9999
Mean Area
Linear (Mean Area)



Quantification of chlorogenic acid, caffeic acid, and ferulic acid in drink samples.

Drink samples were diluted with an equal volume of acetonitrile, centifuged (12,000 *x g*; 30 min), and filtered (0.4 µm) prior to injecetion. The results for chlorogenic acid detection shown in the figure and table below.



Sample	Mean [CGA] ug/mL	Std Dev	Mean Rt (min)	Std dev	%RSD
21	0	0			
22	0	0			
23	121.6608304	0.377253219	2.822	0.003	0.106308
24	114.2678516	1.166143599	2.832	0.005	0.176554
45	0	0			
46	0	0			
47	154.0590165	1.324450454	2.839	0.006083	0.214257
48	136.2984561	0.509731852	2.838	0.003	0.105708

Both ferulic acid and caffeic acid were detected in the drinks samples 23, 24, 47, and 48 but at very low concentrations. A number of unkwond compounds with similar UV spectral profiles were detected in these drink samples at concentrations similar in strength to chlorogenic acid. These could be effecitvely be identified by MS/MS analysis.

No chlorogenic acid, ferulic acid, or cafeic acid was detected in drink samples 21, 22, 45, and 46.

2. Quantification of caffeine by LC/PDA

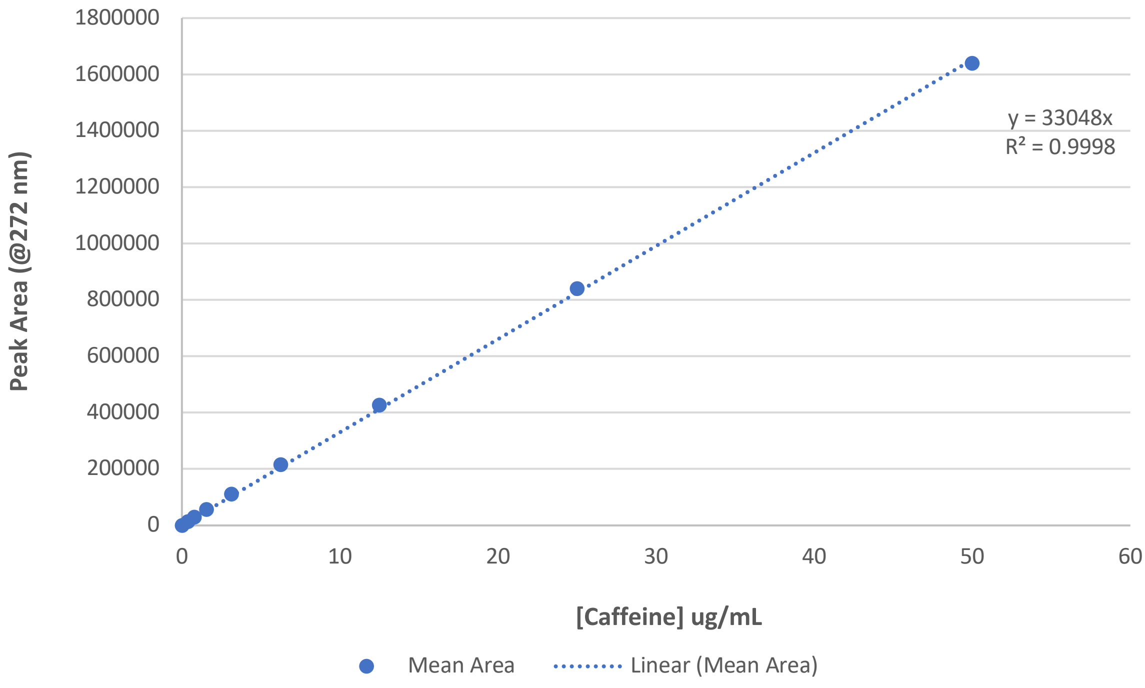
Materials and Reagents. Methanol, acetonitrile, and formic acid were of chromatography grade. Water was prepared with a Milli-Q EQ 7000 water system. The standard for caffeine was obtained from Sigma-Aldrich.

LC-PDA Analysis. The analysis was carried out on a Shimadzu Prominence UPLC fitted with LC-20AB binary pumps, SIL-20 HT Autosampler and SPD-M20A diode array detector. Isocratic conditions were used for the separation of analytes using a Grace 250 mm x 4.6 mm, 5 um reverse phase column fitted with matched guard column. Mobile phase conditions included: Solvent A was 0.05M NaH_2PO_4 (adjusted to pH 5.66 using triethylamine) and Solvent B chromatography grade methanol. The following protocol was used during the run: 0–20 min 70% solvent A: 30% solvent B at a flow rate of 1 mL/min. The column temperature was kept at 40°C and detection wavelength set at 272 nm for optimal caffeine detection. Sample injection was 10 µL.

Standard Curve Preparation. A stock solution of caffeine was prepared at 1 mg/mL in methanol. For each compound an eight step, 1 in 2, serial dilution was prepared in methanol. The concentration for these dilutions ranged from 100 µg/mL to 0.39 µg/mL. These serial diluted standards were used to determine identification information about each compound, as well as the linear quantification ranges for each compound.

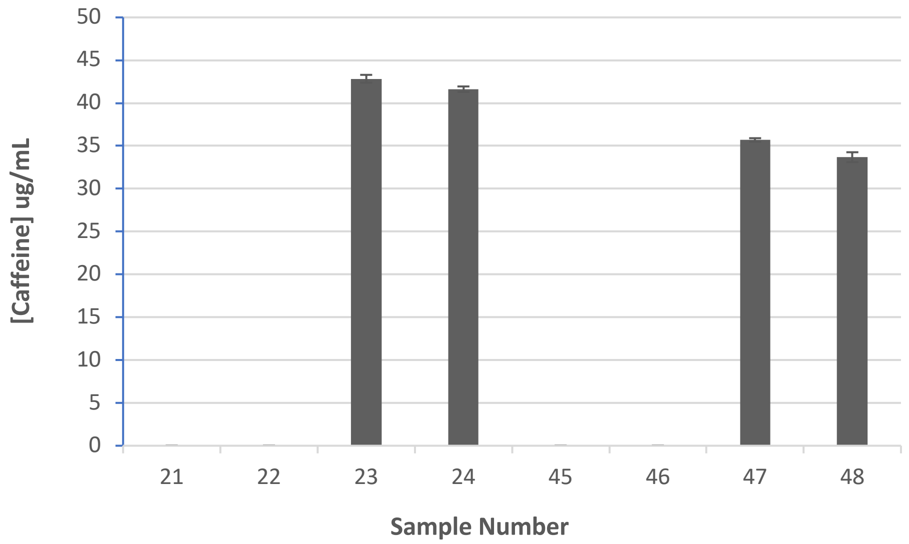
Compounds	Retention time (min)	Detection Wavelength
Caffeine	8.16 ± 0.02	272 nm

Linearity. The calibration curve for caffeine is shown in the figure below.



Quantification of caffine in drink samples.

Drink samples were diluted with an equal volume of methanol, centifuged (12,000 *x g*; 30 min), and filtered (0.4 µm) prior to injecetion. The results for caffeine detection are shown in the figure and table below.



Sample	Mean [Caffeine] ug/mL	Std Dev	Mean Rt (min)	Std dev	%RSD
21	0	0			
22	0	0			
23	42.83244272	0.45951404	8.179666667	0.000577	0.007058
24	41.61973774	0.320373595	8.175666667	0.001528	0.018684
45	0	0			
46	0	0			
47	35.69374725	0.205000111	8.177	0.004359	0.053307
48	33.68444965	0.576092795	8.182	0.003464	0.042338

Caffine was detected and quantified in the drinks samples 23, 24, 47, and 48. No caffeine was detected in drink samples 21, 22, 45, and 46.

3. Quantification of chlorogenic acid and nine related compounds by LC/MS

A total of ten standards were obtained including quinic acid, caffeic acid, ferulic acid, dihydrocaffeic acid, 5-feruloylquinic acid, *p*-coumaric acid, chlorogenic acid, 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid (**figure 1**). Except for 5-feruloylquinic acid calibration curves were successfully created for the remaining nine standards.

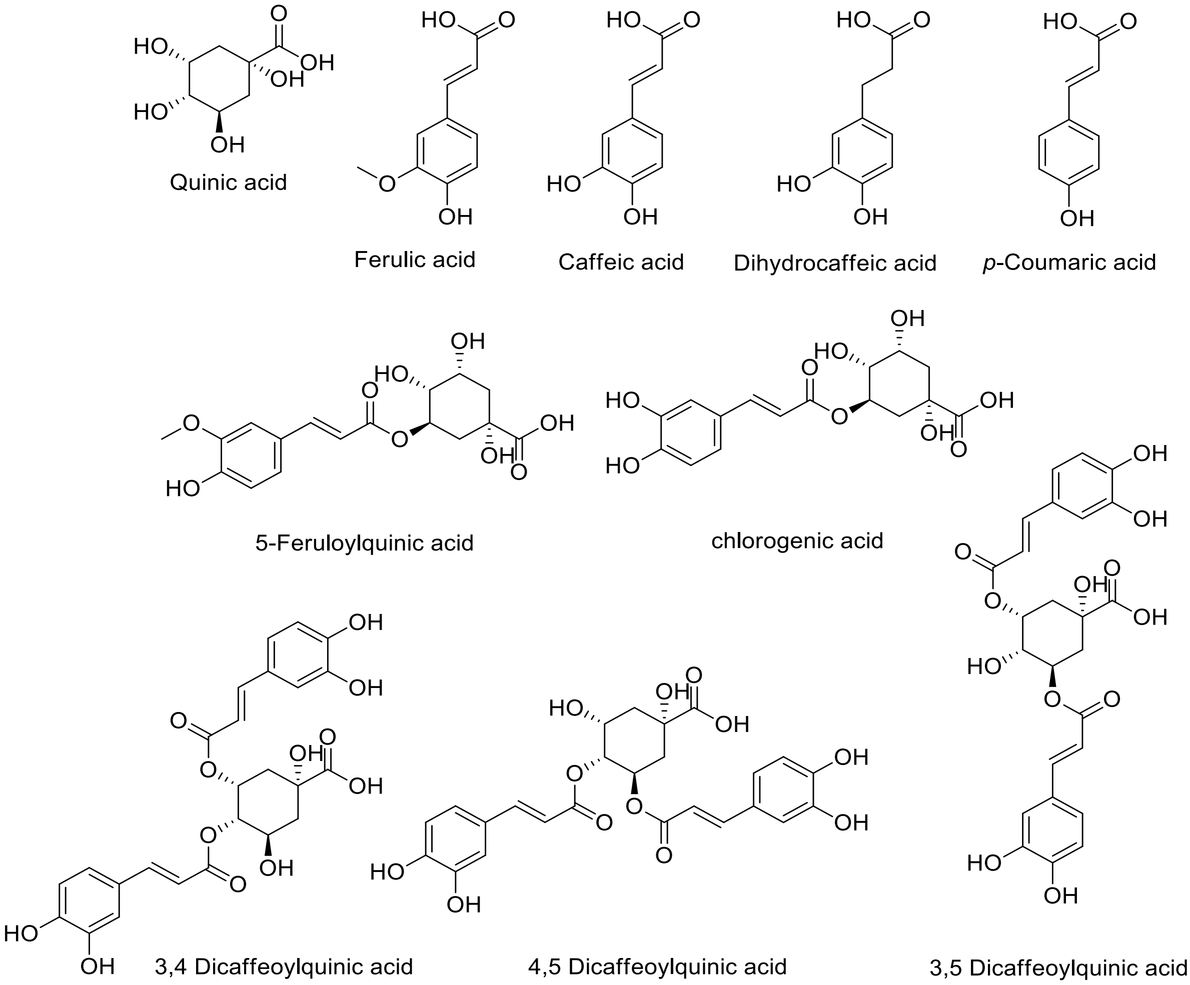


Figure 1. Structures of the ten compounds.

Materials and Reagents. Methanol, acetonitrile, and formic acid were of LCMS grade. Water was prepared with a Milli-Q EQ 7000 water system. The standards, caffeic acid, quinic, and *p*-coumaric acid were obtained from Sigma-Aldrich, 5-feruloylquinic acid, chlorogenic acid were obtained from Cayman chemicals, ferulic acid was obtained from Selleckchem and dihydrocaffeic acid, 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid were obtained from sapphire bioscience.

Standard Curve Preparation. A mixed stock of all compounds was prepared at 1 mg/mL in acetonitrile. This stock was used for optimization of the chromatographic gradient.

For each compound a ten step, 1 in 2, serial dilution was prepared in acetonitrile spiked with 0.1% formic acid. The concentration for these dilutions ranged from 250 ng/mL to 0.49 ng/mL. These serial diluted standards were used to determine identification information about each compound, as well as the linear quantification ranges for each compound.

LC-MS Analysis. The LC-MS analysis was carried out on an XEVO G3 QTof equipped with Zspray LockMass II ESI source, a Waters Acquity premier sample manger (ACQ-FTN) and a Waters Acquity premier quaternary solvent manager (ACQ-QSM). Chromatographic separation was performed on a Waters Acquity premier HSS T3 column (2.1 × 100 mm, 1.8 μm). The injection volume for all samples was 2 µL. The mobile phases consisted of water (A) and methanol (B), both of which were spiked with 0.1% formic acid. The gradient was 5% B-13% B (0–3min); 13% B–25% B (3–3.2 min); 25% B–55% B (3.2–10 min); 55% B-80% B (10–10.1 min); 80% B-5% B (10.1–11 min). The column was then re-equilibrated for 9 min in 95%-A. The column temperature was maintained at 40°C and the flow rate was set at 0.300 mL/min. Data was acquired in negative MSe mode and the source setting included the capillary set to 40 kV, and the source and desolvation temperatures were set to 100°C and 500°C, respectively.

Linearity and LLOQ. A calibration curve was determined individually for each standard plotting the peak area ratio of each compound (y) versus the concentration (x) of each compound using Waters UNIFI software. The lower limit of quantification (LLOQ) was defined as the signal-to-noise ratio (S/N) was higher than 10 and the relative standard deviation (RSD) was less than 25%.

During method development several chromatographic gradients were attempted using a mixed standard of all compounds at 1 mg/mL. The gradient documented in the LC-MS analysis section was chosen as it provided good separation of the ten compounds. Both negative and positive modes with solvents spiked with and without 0.1 % formic acid were used during method development. Negative mode spiked with 0.1 % formic acid was found to provide the sharpest peaks.[1,2]

After method development was completed, a ten step, 1:2, serial dilution was prepared for each compound. The concentration for each compound ranged from 250 ng/mL to 0.48 ng/mL. Each concentration was injected onto the MS in triplicate. While all ten compounds could be detected by MS, only nine compounds could be quantified. The compound that could not be quantified was 5-feruloylquinic acid.

MSe data (**table 1**) was acquired for each compound and for each concentration. From the low energy MSe data the retention times and experimental accurate mass data was determined. The retention times for each compound were highly reproducible between replicates and at different concentrations. The accurate mass data was also highly reproducible with observed masses [m/z] differing by less the 3 ppm from the calculated. High energy MSe is used to generate fragmentation information like traditional MS/MS. High energy MSe was acquired for each of the ten compounds. Plausible daughter ions were generated for seven compounds including ferulic acid, *p*-coumaric acid, 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, caffeic acid and chlorogenic acid, these daughter ions were also consistent with the literature.[3] The daughter ions generated for quinic acid and dihydrocaffeic acid were implausible and 5-feruloylquinic acid ionized poorly. An example chromatogram and a low and a high energy spectrum for each compound can be found in the supplemental information (**Figures 2-12**).

The standard calibration curves for nine of the compounds were created, the tenth compound, 5-feruloylquinic acid, ionized poorly and therefore could not be quantified. The linear ranges (**table 2**) of three compounds including 4,5-dicaffeoylquinic acid and one of the peaks associated with both caffeic acid and chlorogenic acid were found to be 250-7.5 ng/mL. The low limit of quantification (LLOQ) of these samples was found to be 7.5 ng/mL. The linear ranges and LLOQ of the remaining compounds were found to be 250-15.6 ng/mL and 15.6 ng/mL, respectively. Regression equations were determined for each compound and the linear correlation coefficients (R^2) exceed 0.9939. The standard curves created for each compound are in the supplemental information (**Figures 13-16**).

For caffeic acid and chlorogenic acid two peaks were generated in their respective chromatograms. The experimental masses and daughter ions for the peaks in the caffeic acid chromatogram were consistent with what was expected for caffeic acid. This trend was also observed for chlorogenic acid. Both peaks for both compounds produced similar linear ranges and LLOQs. It is unclear why these two compounds generated split peaks and the other compounds did not. As all available evident suggests that these peaks are real, these peaks will be monitored in subsequent experiments and if these peaks are detected both will be quantified.

Diagnostic information for each of the ten compounds.

Compounds	Retention time (min)	Calculated mass of parent ion [m/z]	Observed masses of daughter ions [m/z]
Quinic acid	0.89	191.0561	-
Ferulic acid	8.27	193.0506	134
Dihydrocaffeic acid	6.08	181.0506	-
5-feruloylquinic acid	7.30	367.1035	-
p-coumaric acid	7.81	163.0401	119, 93
3,4-dicaffeoylquinic acid		515.1195	
4,5-dicaffeoylquinic acid	9.67	515.1195	353, 191, 179, 135
3,5-dicaffeoylquinic acid	8.78	515.1195	353, 191, 179, 135
Caffeic acid,			
peak 1	6.43	179.0350	135
peak 2	6.54		
Chlorogenic acid,			
peak 1	5.89	353.0878	191, 135
peak 2	6.06		

Linear regressions, correlation coefficients (R^2), linear ranges and the low limit of quantifications (LLOQ) for the ten compounds.

Compounds	Regression equation	R^2	Linearity range (ng/mL)	LLOQ (ng/mL)
Quinic acid	y = 193x- 707	0.9995	250-15.6	15.6
Ferulic acid	y = 252x- 2e3	0.9939	250-15.6	15.6
Dihydrocaffeic acid	y = 132x- 1.86e3	0.9939	250-15.6	15.6
5-feruloylquinic acid	-	-	-	-
p-coumaric acid	y = 213x- 1.08e3	0.9970	250-15.6	15.6
3,4-dicaffeoylquinic acid	y = ?x- ?			
4,5-dicaffeoylquinic acid	y = 644x- 2.46e3	0.9979	250-7.5	7.5
3,5-dicaffeoylquinic acid	y = 710x- 7.87e3	0.9979	250-15.6	15.6
Caffeic acid,				

peak 1	y = 169x- 1.53e3	0.9977	250-7.5	7.5
peak 2	y = 193x- 424	0.9990	250-15.6	15.6
Chlorogenic acid,				
peak 1	y = 222x- 1.63e3	0.9971	250-15.6	15.6
peak 2	y = 153x- 561	0.9991	250-7.5	7.5

In summary, the LLOQ and linear range of nine of the ten compounds were successfully determined. The regression equations for the nine compounds were also determined and the high correlation coefficients obtained for each equation indicated a good linear relationship for each equation. Diagnostic information was also obtained, including RTs and experimental accurate masses that was a near match (< 3.0 ppm) to the calculated accurate masses for all nine compounds. Plausible fragmented ion(s) were detected for seven of the compounds. The RT, accurate mass and daughter ions will be used to identify each of the compounds in subsequent experiments.

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