A comparison between UK and Cyprus based male football athletes in terms of dietary intake, markers of physiological stress and training load.

Konstantinos Rostantis

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Abstract:

Few studies have examined the relationship of nutritional intake and training load between different football teams. Additionally, there are not any comparison studies regarding training load in terms of energy cost and whether this is sufficiently balanced with energy intake. The present study aimed to examine if there was a difference in nutrition intake and training load between teams from different geographical locations across football training season, and how both influence training stress markers regarding training sessions. **Methods:** Following ethical approval, participants (n=45; age 24.47 ± 6.07 years; height 1.75 ± 0.08 m; mass 74.86 ± 9.57 kg) were male footballers from three different teams; one UK based professional team and two Cyprus based teams; professional (UKpro), semi-professional (CYsem) and recreational (CYrec). Data was collected from all teams at three time points across an annual training programme (pre, mid, end season). A food recall 4-day diary was used to record nutritional intake and metabolic equivalent (MET) values of physical activities method was used to quantify the energy cost of training. Saliva samples were collected during a standard training week on a fixed day pre- and post-training, and at rest 24hours post training. Samples were analysed in duplicate via enzyme linked immunoassay (ELISA) for secretory IgA, cortisol and testosterone. **Results:** It was found that across all three testing blocks none of the groups received inadequate nutritional energy intake. All groups showed consumption of the investigated micronutrients in higher than recommended daily amounts (RDA) but only vitamin C was significantly higher (> 20%) for UKpro compared to Cyprus groups . Furthermore, findings showed that pre-season average daily energy cost was not the highest across season and neither difference between blocks was significant. In parallel, both average daily energy intake and s-IgA did not show important changes. **Conclusions:** The collected results revealed that energy expenditure for UK based professional players was not significantly higher neither regarding the average daily or training day energy cost between all three testing blocks and with balanced nutritional energy intake. Monitoring Salivary IgA in football athletes may be an effective way to monitoring recovery.

Acknowledgements

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CHAPTER 1

INTRODUCTION

1.0 Introduction

Football game performance studies have examined the differences between players or teams with reference to ranking, national league, players playing position and various other aspects. During a typical football season, teams have to participate in their National leagues and depending on ranking they have between 9-11 months of training sessions, friendly and official games. According to the time of the season, training programs are structured in order to achieve or maintain high level performance, as such will be reflected in the finishing position in league. Naturally, individual footballers will react and adapt differently over individual times frames even when presented with identical training regimes (Naclerio, Moody and Chapman, 2013).

The ranking between teams is also applied in the European cups, with categorized groups of different level teams. Previous investigators have shown significant differences between teams in the same league regarding their physiological and anthropometric characteristics (Kalapotharakos, Strimpakos, Vithoulka, Karvounides, Diamantopoulos and Kapreli, 2006). Based on the authors' results, it is suggested that body fat (%) can play an important role for high football performance. Any additional load in training exercises delivers higher impact into the joints, the movements demand more energy and footballer is at higher risk for injuries. Earlier studies, found that players with a lower body fat percentage consistently have better performance and that body fat percentage could be considered as a direct measure of the intensity of training (Ostojic and Zivanic, 2001). Additionally, researchers who have compared various distances covered for the period of several National leagues, have stated the differences in total covered distances as well as differences in the number of high-intensity bouts (Mohr, Krustrup and Bansgbo 2003). Furthermore, important differences were indicated in physical performance during matches, with total distance covered at the end of the season being much greater compared to the values of early and mid-season (10.72 ± 0.13 vs 10.34 ± 0.21 and 10.13 ± 0.35 km, respectively). By season planning (periodization) most researchers state that teams have enhanced performances during the last part of the season. Naturally, this could be applied for teams that maintain their targets until the end of season.

On the other hand, in case of detachment from original targets, psychological stress and several other reasons could certainly influence performance. Whether this improvement is existent and furthermore if it can be planned and manipulated as to result positive changes in terms of better performance, still needs more investigation. There are boundless various psychological, financial, social and definitely physiological factors that can influence footballers' performance. Furthermore, a proper seasonal plan has to follow a common training program for footballers with respect to individual differences. Through an assessment of parallel comparison in-between teams of different rank, the corresponding influences for improved performance during the end-season in comparison to the rest of the season could be identified. In any case, the changes of the parameters which may have an impact on footballers' performance all through the season, should be investigated and eventually compared. Such parameters could primary be the weekly training load and training energy cost, nutritional intake and energy balance, physiological stress arising from training sessions and various secondary parameters.

Dietary intake as total energy intake logically should match the training demands over any typical season. However little is known about specific differences between types of team ie: professional versus recreational or from different geographical locations. There are several arguments contrary to the credence that top athletes eat an optimal diet, or that they all consume the suggested carbohydrate (CHO) intakes (Burke, Cox, Cummings and Desbrow, 2001). An inherent relationship exists between physical activity and nutrition and thus manipulation of nutrition could improve football performance, by means of special attention to macro- and micronutrients quantities, and with optimal timing consideration of ingestion. In line with previous researchers, guidelines should be given for pre-exercise eating, nutrition during exercise and post-exercise recovery. Management of nutrition prior to hard training sessions or matches with adequate carbohydrate intake, allows footballers to preserve as fully as possible repleted muscle fibres with glycogen and counter the estimated fuel costs.

Furthermore, the evidence from previous studies indicate the importance of refuelling glycogen between games, and providing adequate fuel that is necessary for training energy expenditure. Guidelines based on Burke, Loucks and Broad (2006), suggested that intervals between exercise sessions which don't exceed the 8 hours,

should be targeted with carbohydrate ingestion immediately by the end of first training session. This could support a more active recovery and allow footballers to sustain the intensity and duration impact arising from a second session. Observably in terms of football training sessions, this approach is necessary for the period of preparation season and later during main season in-between daily double training sessions. According to Jentjens and Jeukendrup (2003) positive results to the early recovery post-training and performance could be established from frequent ingestion of small carbohydrate quantities. Recommendations from this study, indicated the carbohydrate energy intake post-game and post-training (0 – 4 hours) at 1.0–1.2 $g.kg^{-1}.hr^{-1}$.

Burke (2001) similarly stated a number of factors that can interfere with the achievement of such targets, particularly with the higher intakes recommended for endurance athletes, and these include:

- Restricted energy intake
- Inadequate practical nutrition skills or food composition knowledge
- Background dietary practices and food culture of the country are inadequate in terms of CHO intake
- Poor availability of CHO-rich foods in the immediate eating environment
- Gastrointestinal limits to bulky, high fibre food intake
- High-fat diets
- Chaotic lifestyle and constant travel commitments.

Following the same principle, studies have shown that glycogen supercompensation protocols including carbohydrate-loading, can enhance performance for team games involving repeated high-intensity sprints such as football (Balsom, Wood, Olsson, and Ekblom, 1999). In the absence of severe muscle damage, glycogen stores can be normalized with 24 hours of reduced training and adequate fuel intake (Burke, Kiens and Ivy, 2004).

With an increasing scientific interest in the beneficial effects of Mediterranean, and in particular, polyphenol and antioxidant rich diets on aspects of cardiovascular health, less is known about the potential influence of cultural diets on aspects of athletic training and performance. Most athletes are aware of the potential stress caused by intense training. Based on this, antioxidant nutrient requirements have been gaining scientific interest during the last decade. So far, whether acute or chronic physical exercise induces a change in antioxidant and trace element requirements has not been sufficiently discussed while the adequacy of recommended dietary allowances (RDA) being questioned in terms of athletic sufficiency has yet to be addressed. There may also be a risk from higher than RDA antioxidants intake, a supposed impairment of adaptive effects and a still unknown long-term risk (Margaritis and Rousseau, 2008). Actual health and performance benefits from this nutrition pattern have yet to be investigated, as well as the possibility that very high intakes may suppress natural adaptations to training.

Several epidemiological reports imply that athletes engaging in heavy acute or chronic exercise are associated with an increased risk of URTI (upper respiratory tract infection). Particularly in periods of fixture congestion with national and international games, the footballers may be unusually susceptible. One of the most common symptoms of intense training is increased susceptibility to infection (Bishop and Gleeson, 2009). A parallel impact from nutritional choices influences the immune system status, as well as the immune response to pathogens.

For many years saliva has been used as a biological fluid for the detection of different biomarkers such as electrolytes, hormones, drugs and antibodies in medicine. More recently saliva diagnostics in football, concentrate on the antibodies sIgA and sIgG, the hormones cortisol and testosterone as well as the ratio between the two hormones, which may provide an indication of potential acute immunosuppression or increased stress response. Salivary IgA has often been investigated as the primary marker of overtraining / immune health. Over-reached athletes often exhibit depressed levels of immunoglobulin-A and an elevated cortisol/testosterone ratio. Not only does this relate to the susceptibility to infection but also it may relate to underperformance and insufficient recovery. Among several macronutrients and micronutrients, only the ingestion of carbohydrates appeared to be able to actually assist resistance to exercise-induced immune suppression

(Gleeson, Nieman, and Pedersen, 2004). Furthermore, while antioxidants and glutamine have been investigated, the findings until now cannot identify their influence on changes occurred from training impact on the immune system.

Although in recent years many studies on football have been published, very few have been focused on repeated measurements and monitoring individuals physiological, anthropometric and energy balance changes during the season. Limited previous research has demonstrated the physiological profile of footballers from several national leagues. Cometti, Maffiuletti, Pousson, Chatard and Maffulli (2001) have examined the differences of isokinetic strength and anaerobic power of elite, subelite and amateur French soccer players. An analogous comparison from Reilly, Bangsbo, Franks (2000), has investigated the physiological characteristics from Danish championship footballers, and compared them with footballers from other major European championships.

Therefore this study aimed to compare the habitual diets of both recreational and professional trained football athletes in contrasting geographical locations to assess whether specific dietary trends, food choices, macro- micro- and antioxidant nutrient intake may relate to management of training load and markers of physiological stress during the pre-season, mid-season and end of season.

1.1 Aims of the current study:

- To investigate dietary intake between selected Cyprus based football cohorts and UK based football cohorts, especially in terms of macro and micronutrients over selected points of a training season.
- To assess training load between above cohorts at selected points of a training season.
- To assess salivary markers of physiological stress between above cohorts at selected testing periods of a training season.

1.2 Main Experimental Questions:

Q1) is there a difference in nutrition intake between and within cohorts at the pre-, mid- and end-season monitoring points of the training season in terms of:

- Total energy intake (kcal)?
- Main macronutrients (CHO, Protein, Fat and Fibre)?
- Micronutrients (vitamins C, E, zinc and magnesium)?

Q2) is there a difference in training load between and within cohorts at the pre-, mid- and end-season monitoring points of the training season in terms of:

- Average energy cost for the training days (TD)?
- Average energy cost for all days of the week (AD)?

Q3) is there a difference in markers of physiological stress between and within cohorts at the pre-, mid- and end-season monitoring points of the training season in terms of:

- (i) IgA at rest? (ii) IgA after hard training? (iii) IgA after 24h recovery from hard training?
- (i) Testosterone/ Cortisol (T/C) ratio at rest? (ii) T/C ratio after hard training? (iii) T/C ratio after 24h recovery from hard training?

Q4) is there a difference in body fat percentage (%) between and within cohorts at the pre-, mid- and end-season monitoring points of the training season.

1.4 Hypotheses

Main Experimental hypotheses:

H1) All nutritional intake variables will be significantly greater for the UK professional cohort at all testing blocks. During pre-season all groups will present higher values compared to the following two testing blocks, in regards to:

(i) Total calorie intake
(ii) Carbohydrate intake percentage of total energy
(iii) Fat intake percentage of total energy
(iv) Protein intake percentage of total energy

(v) Selected micronutrients

H0a) There will be no significant difference in any of the nutritional parameters between groups over time.

H2) There will be a significantly greater energy cost for the all groups during pre-season compared to mid- and end-season separately, in regards to

(i) Training Day energy cost (TD).
(ii) Average week Day energy cost (AD).

H0b) There will be no significant difference in energy cost between mid- and end-season within all groups.

H3) There will be a significant difference in all physiological stress markers between and within groups at all three testing blocks of the training season in regards to:

- (i) IgA after hard training (ii) IgA after 24h recovery from hard training
- (i) T/C ratio after hard training (ii) T/C ratio after 24h recovery from hard training

H0c) There will not be a significant difference in IgA at rest or Testosterone/ Cortisol (T/C) ratio at rest, between all testing blocks within groups.

H4) There will be significantly higher body fat percentage for CYsem and CYrec groups, compared to UKpro overall and at each testing block.

H0d) There will be no significant difference within groups during time (testing-blocks).

CHAPTER 2

REVIEW OF LITERATURE

2.0 Review of Literature.

2.1 The physiological and metabolic demands of football.

The physiological demands arising for footballers during official games and training sessions have been well investigated in the literature. Performance in football due to the duration of play is largely aerobic with interval and high intensity efforts. According to Iaia, Rampinini, Bangsbo (2009) improvement of high-intensity intermittent exercise such as football performance can be achieved during season by using both aerobic and speed-endurance training. Aerobic energy production is extremely important in football, with mean and peak heart rates of around 85 and 98% of maximal values, respectively (Krustrup, Mohr, Ellingsgaard et al., 2005; Bangsbo, Graham, Johansen and Saltin, 1994). Unfortunately it is possible that the heart rates measured during a match lead to an overestimation of the oxygen uptake, since various factors such as mental stress, environmental conditions and dehydration may influence the heart rate elevation.

Previous research indicated that the rate of rise in oxygen uptake due to several high-intensity actions needed in football games and training sessions could be essential for performance. Generally footballers' performance during official matches retain rather high heart rates (> 65% HR), which suggests that the main recruited muscles are probably retaining high oxygen needs. However, this low to high intensity intervals exercise would need to retain adequate blood oxygen to the recruited muscles, but various limitations arise including the oxidative capacity (Krustrup, Hellsten, & Bangsbo, (2004a); Bangsbo, Gibala, Krustrup, Gonza´lez-Alonso and Saltin, 2002). Anaerobic periods of energy production in football were indicated to be an essential component of performance, as they comprise the most crucial events during the game (Mohr et al., 2003). The ability to level the anaerobic system to higher requirements can be increased laterally with the level of competition.

Athletic performance during football game according to Williams (1987) are divided by the parameters influencing the physiological markers, into the 'multiple sprints' sports and the 'endurance' sports. The fatigue as a result of high number of sprints is linked to the increase of the end products of metabolism while for largely aerobic

exercises the fatigue is most probably related to the depletion of glycogen stores. Football however, is a mixture of these mechanisms and therefore any attempt to explain the metabolic demands as well as the fatigue process should be undertaken with caution. A number of interrelated elements influence athletic performance. Football is generally classified as intermittent exercise and according to Strudwick and Reilly (2001), during a game players perform approximately 1,525 discrete activities, amounting to a change in activity every 3.5 seconds. Elite players have been observed to perform approximately 19 sprints (>20km/h) during a game, the mean duration of each sprint being ~2.0 seconds (Bangsbo, Norregaard and Thorsoe, 1991).

More recently, Strudwick (2001) has reported that during a game 22 sprints were observed, which equates to a maximal effort approximately every 4 minutes, with an average duration of 3.2+-1.6 s for English Premier League players. The total distance covered in a match (TD) ranges from 10 to 13 kilometres with differences related to rank and tactical role. Comparative total distances covered between the two halves in a game, show a greater distance of 5-10% covered in the first half. On average, players spend 70% of the total match duration performing low-intensity activities such as fast walking and jogging, while the remaining 30% incudes actions of high-intensity for running small distances usually under 15m. Sprinting by means of running speed ranging from 19 to 25 $km.hr^{-1}$, amounts to 5%–10% of the total distance covered during a match, thus corresponding to 1%–3% of the match time; average sprint duration is 2–4 s, and average sprint occurrence is 1 in 90s (Di Salvo, Baron, Tschan, Calderon, Bachl and Pigozzi, 2006). In less trained professional athletes at moderate-level (3^{rd} division), estimations of running and sprinting distances appear to be 28% and 58% lower than professional athletes (Mohr et al., 2003).

Overall, studies so far indicate important performance differences between high-level professional level and moderate or recreational level footballers. Total distance covered and high-intensity running are two major differences indicated by Mohr (2002). Also important findings correlated to this study, suggest the improvement in players performance towards the end-season by increasing high-intensity running distances. In one of the few studies that have compared different season points, (Clark, Edwards, Morton and Butterly, 2008), investigated similarities between three

football seasons in terms of physiological footballers profiles within a professional team, for the positive purposes of identifying normative responses before pre-season training, mid-season, and end-of-season. Footballers were tested with a specific battery of tests through a 3-year period: 1) basic anthropometry, 2) countermovement jump (CMJ) tests, 3) a combined aerobic threshold (AT) and maximum oxygen uptake (VO_{2max}) test. Interestingly, anthropometrics values did not significantly change. Generally, VO_{2max} values did not vary significantly through the research, in agreement to previous studies that estimated threshold values for professional footballers at $60ml \cdot kg^{-1} \cdot min^{-1}$. Also, the AT was suggested to provide a better indication of critical training sessions between break from previous football season and the preparation period of the new season.

On the contrary, several earlier studies found that rank among teams from the same national leagues was in agreement with their maximum oxygen uptake (VO^{2max}) results (Kalapotharakos, 2006; Wisloff, Helgerud and Hoff, 1998; Apor, Reilly and Murphy, 1988). Also, Kalapotharakos (2006) reported important physiological differences between three teams of different grading place from the national Greek championship. In that study, multiple comparison tests revealed that the best team among various superior physiological results showed significantly ($p < 0.05$) lower body fat (%) values in comparison to the middle and last team of the league. Regarding the body fat percentage results are stated to be comparable with the body fat in Spanish soccer players, in Icelandic elite soccer players and in Saudi elite soccer players. Overall, the sum of previous studies indicates the relationship between different national or international ranking of football teams and their physiological differences.

A comparison of physical and technical performance in professional soccer players of FA Premier League and La Liga, indicated significant differences in physical aspects including covered distances. Analysis of physical and technical aspects evidence different behaviours when their teams were or were not in ball possession, especially regarding covered distances in sprinting (Dellal, Chamari, Wong, Ahmaidi, Keller, Barros, Bisciotti and Carling 2011). Important role for these differences seem to play the different tactics, pending also on players' position. In line with the game performance differences, researchers have found significant physiological differences between teams of different ranking. Although someone may suggest that

physiological limits can compromise with teams tactics. Rampinini, Impellizzeri, Castagna, Coutts, Wisloff (2009) investigated the match performance parameters of Serie A, 2008 games. Totally 416 individual matches from 186 footballers (27 ± 4 years, 76 ± 5 kg, and 181 ± 5 cm) were monitored via a video match-analysis system. The investigated game parameters included among several others, total distance covered (TD), high-intensity running distance (HIR), very high-intensity running distance (VHIR), total distance with the ball (TDB), high-intensity running distance with the ball (HIRB), and very high-intensity running distance with the ball (VHIRB). Additionally, counting some skill contributions was also assessed. The higher ranking teams managed to show higher distances covered and had better higher successful involvement with ball to football specific actions like passing and dribbling compared to the lower ranking teams. Despite the fact that the total duration of active play in football all over the world is obviously the same, the total distances covered in official games, analysed by motion analysis systems, appear to differ between countries and ranking positions. In line with the total distances, a comparison of the distance covered in the first and second halves of football match-play shows greater distances covered in the first half +-4% (Alghamnam, 2011), evidence which is in agreement with Rampinini's findings of performance in Serie A during 2008.

Comprehension of the energy cost during a football match is decisive in order to plan the nutritional strategy for footballers. Based on both laboratory studies and match analysis, it was shown that carbohydrates (CHO) stores are the most crucial for football performance. Post-blood glucose levels In case sufficient pre-exercise liver glycogen content, the intense nature of football will result in close or slightly above resting levels (Baldwin, Snow and Gibala, 2003). For the period of sustained high-intensity sports lasting approximately 1 hour, small amounts of carbohydrate, including even mouth-rinsing, enhance performance via central nervous system effects. While 30–60 $g.h^{-1}$ is an appropriate target for sports of longer duration, events > 2.5 hours may benefit from higher intakes up until $90g.hr^{-1}$ (Burke, Hawley, Wong and Jeukendrup, 2011). The utilization of glycogen stores during a football match was suggested to be 155-160 $mmol.kg.dw^{-1}$ from muscle glycogen stores, with an estimated 600 kcal of energy provided, while blood glucose derived from the liver may account for approximately 210 kcal of energy during the game. In accordance, endogenous CHO stores are suggested to supply ~ 55% of the energy requirements of match-play, and a substantial utilization of lipids and proteins must also be taken

into account. In agreement, Krustrup (2005) found that blood glucose was maintained at elevated levels throughout friendly matches, whereas there was a progressive increase in the FFA concentration. More interestingly, muscle biopsies were collected for investigating relative glycogen content in ST, FTa, and FTx fibers as well as all fibers before and immediately after a soccer match. Data revealed that the muscle glycogen concentration at the end of the game was reduced to 150 – 350 mmoL kg d.w^{-1}. Thus, there was still glycogen available, but the histochemical analysis revealed that about half of the individual muscle fibers of both types were depleted or almost depleted for glycogen. Therefore, it is possible that such a depletion of glycogen in some fibers does not allow for a maximal effort in single and repeated sprints.

Furthermore, evidence exists that glycogen depletion is prominent, particularly in type 2 muscle fibres not only after 45 minutes of high intensity match play, but also at the end of a game. Data analysis though, showed that athletes were not all fully repleted. Whilst this may not be the only mechanism to explain player fatigue, evidence exists that if players start training sessions or matches in a pre-depleted state (ie: 50% glycogen depleted) then part of muscle fibres may be near depleted at the end of the game (Mohr et al., 2003). More recently, Krustrup, Mohr, Steensberg, Bencke, Kjær & Bangsbo (2006) examined muscle and blood metabolites for possible changes in sprint performance. Muscle glycogen was decreased ($p < 0.05$) from 449 =/- 23 to 255 T 22 mmoL kg d.w^{-1}. While blood glucose remained elevated during the game, muscle glycogen reduction was 42+-6% lower after game. Whereas pre game all fibres were fully repleted (73 ± 6%), post-game all fibres reserved in average only 19+- 4%. The 10 subjects that were analyzed for fibre type –specific glycogen depletion during the game had 58.5 ± 3.5% ST fibers, 26.9 ± 2.6% FTa fibers, and 14.6 ± 3.0% FTx fibers. Before the game, 73 ± 6% of all fibers were rated as full with glycogen, whereas this value was lower ($p < 0.05$) after the game (19 +/- 4%). After the game, a total of 36 ± 6% of the individual muscle fibers were almost empty, and another 11 ± 3% were completely empty of glycogen (Refer to Figure 2.1).

The current popular explanation is that when the concentration of glycogen content during prolonged exercise reduces, the capacity for ATP generation depends more on glycogenolysis. Initially, the required rate of ATP production is appropriate for that

exercise intensity and can be provided by ATP derived from the oxidation of intramuscular and circulating fat. Once the maximum rate of fat oxidation has been reached, a deficit in the rate of ATP generation is expected. Hence the work rate falls, with the rates of ATP production and ATP use returning again to balance. However, a more recent study by Krustrup (2006) comparing both halves of three friendly matches, did not find any relationship between the decrease in sprint performance during the game and either muscle lactate, muscle pH, total glycogen content or muscle ATP (r2 = 0.05 and 0.07, $p > 0.05$). Additionally, in line with the lack of evidence that muscle fibers can be completely empty from glycogen, Noakes and Gibson (2004) suggested that ATP concentrations are clearly homeostatically regulated at levels that are appropriate for the exercise intensity (Refer to Figure 2.2).

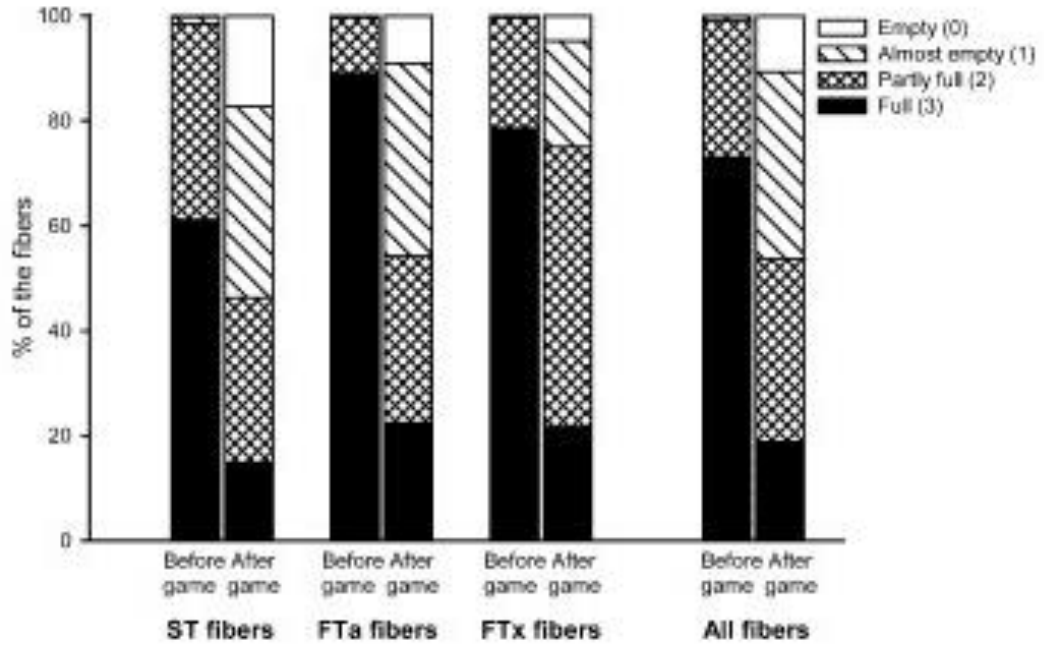

Figure 2.1 Before and immediately after a soccer match relative glycogen content in all fibers. Taken from, Krustrup et al., (2006).

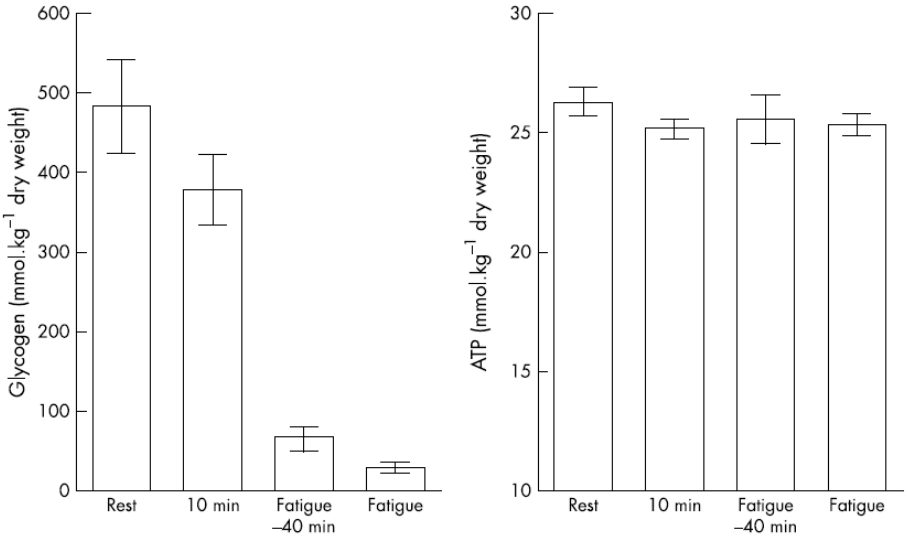

Figure 2.2 Muscle ATP concentration is not reduced at fatigue during prolonged exercise even though muscle glycogen concentrations fall substantially. Cited by, Febbraio and Dancey (1999).

As in most sports, performance can be limited from the premature onset of fatigue. Fatigue is the result of a number of events and reactions during any activity. In this state the working muscles are insufficiently provided with energy, descent their capability to generate force and therefore force output reduces over the 90 minutes of a football game duration. Most obviously, this working load deficit is mirrored in the decline of work-rate towards the last 15 minutes of the game. Additionally, the important role of each factor involved in exercise performance is subject to inter-individual variability. Evidence from the literature indicates that fatigue, notably the ability to perform high intensity sprints whilst executing technical skills, declines in the latter half of match play, particularly in the last 15 minutes of a typical 90 minute game (Stolen, Chamari, Castagna and Wisloff, 2005). During the second half of a match, total distance and high-intensity running decline noticeably with the amount of high-intensity being running 20% to 40% lower in the last 15 minute of the game, compared with the initial 15-minutes period. When more activity is performed in the first half a greater decrement in running is observed. Additionally, in the 5 minutes following the most demanding 5-minute period of the game, the distance covered at high intensity is reduced by 6% to 12% in comparison to the match typical distance. Conclusively, the findings of that research suggested that footballers experience fatigue mainly at the end of second half, but also in small various periods through a

typical football game duration. In line, a reduction in sprints numbers follows these periods of fatigue, while also it may influence the accuracy of executed ball actions as a result of limited technical performance, especially for footballers of low fitness level. Apparently, footballers must retain suitable fitness level to sustain the high energy expenditure and fatiguing parameters from official matches and training (Iaia et al., 2009). Furthermore, technical, tactical and psychological elements, as well as nutritional and hydration demands also impact on player/ team performance (Currell and Jeukendrup, 2008).

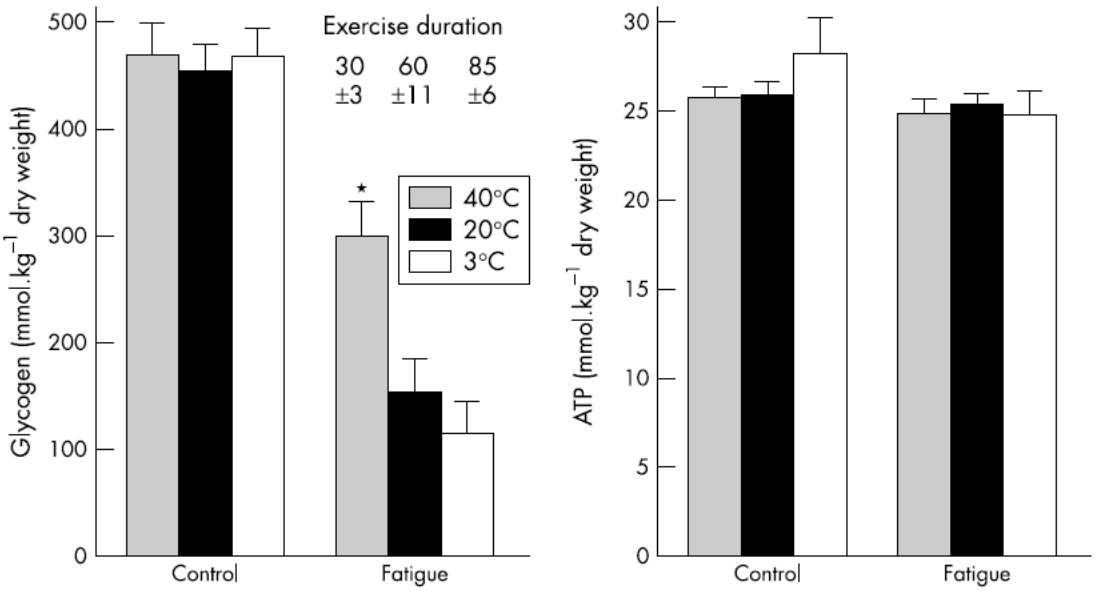

Figure 2.3 The effects of prolonged submaximal exercise on muscle glycogen during different temperatures and in parallel how much the muscle ATP concentrations are influenced. Both glycogen and ATP are not responsible for exercise termination in high temperatures. Adapted from Parkin, Carey, Zhao et al., (1999)

However, Baldwin (2003) found that when prolonged submaximal exercise is undertaken in different environmental conditions (3, 20, 40^{0}C), exercise terminates after different durations (85, 60, and 30 minutes respectively) and at different muscle glycogen concentrations, but at the same time ATP concentrations that are not different from values measured at rest. This indicates that prolonged exercise in the heat does not terminate as a result of depletion of either muscle glycogen or ATP stores. Also muscle ATP concentrations are regulated such that fatigue always

occurs without a substantive change in their concentration. The most probable explanation is that the central nervous system regulates the exercise level to ensure that a dangerous elevation in the core body temperature does not occur. Thermoregulatory strain may also be encountered, resulting in a fall in physical performance, or there may be a reduced central drive from the nervous system.

2.2 Training load in sports.

Measuring training load by a valid and reliable method is necessary while investigating various aspects of training, especially the relationships between injury, training load and overtraining, efficacies of various training methods. While high-intensity running in football competition performance seems to be the major difference between teams of different level (Iaia, 2009; Mohr, 2003; Bansbo, 1991), it is unclear whether training sessions differ considerably in terms of duration, exercise intensity or other variables. Bansgbo and Mohr (2005) suggested that weekly training schedules must include both aerobic and anaerobic sessions. In line with most studies, footballers training should focus on high-intensity sessions according to game demands, but aerobic training can improve recovery during intervals. Thus, a carefully short- or long-term training plan must define teams' and individuals' programs always with respect to the principles of periodization.

In theory, physiological measurements associated with exercise intensity can be considered as valid markers of the training load. A methodology which can show the activity's intensity could be the oxygen consumption (metabolic rate) and/ or blood lactate values. Unfortunately, while accurate measurement of oxygen consumption can be done in the field as well, laboratory's stable and repeatable conditions are considered most accurate. Practically the capability to receive accurate values for metabolic rate and internal training load through measurements from training sessions or competition wouldn't be possible. The absence of an absolute accurate and objective source of comparison, combined with the football specific requirements of various intense actions, assessment of training load and energy cost becomes

really challenging (Iaia et al., 2009). Additionally the lactate levels concentration in relationship to training load can be quite inaccurate due to various factors including the collection method using the earlobe or finger blood exceeds the values that can be found in muscles (Krustrup et al., 2001). Secondly, portable equipment could present greatly different results than the laboratory equipment due to numerous conditions of collection and analysis such as uncontrolled environmental circumstances.

The quantification and comparison of training loads has traditionally been evaluated by monitoring heart rate using telemetry systems, estimating physiological intensity of training sessions in relation to each individual's maximum heart rate. However, while this method can give an accurate picture of aerobic training load, quantification of anaerobic sessions load by HR cannot be considered as accurate (Bangsbo and Mohr, 2005). The rating of perceived exertion scale has also been used as an evaluation tool after each training session using a modified Borg scale (Jeong, Reilly, Morton, Bae and Drust, 2011). A very interesting self-report estimation and classification of the energy cost of human physical activity was developed through metabolic equivalents (MET) that were published from laboratory or field experiments. Similarly to PRE method, there is no need for using further equipment e.g. heart rate monitors, but instead of asking participators to value sessions' intensity, researchers can make this estimation according to MET values (kilocalories= MET x weight in kilograms x duration of training in hours). This simplicity offers a valid indirect methodology to estimate energy cost during training sessions. The compendium of physical activities provides linking categories and types of physical activities with their respective MET intensity values. Standard MET levels in the 2011 Compendium are a direct estimation of the weight-specific energy costs, computed by taking the energy costs (VO_2, $mL^{-1 \cdot}min^{-1}$) and dividing by 3.5 $mL^{-1 \cdot}kg^{-1 \cdot}min^{-1}$. As such, they are an estimation of expressing the weight-specific energy cost of activities. As recommended in 2011 Compendium of physical activities by Ainsworth et al., 2011 estimation of the caloric cost of PA, can be achieved with the equation, kilocalories= MET x weight (kg) x duration (hours).

Bangsbo (2005) indicated that there are major individual differences in the physical demands of players during a game related to physical capacity and tactical role in the team. These differences should be taken into account when planning the training and

nutritional strategies of top-class players, who require a significant energy intake during a typical week. Organizing training programs with the progression of increased knowledge among sports scientist, has become a challenge due to the high demands of a high level football season. With the elevated demands of frequent games in parallel to longer competitive periods, the concept of achieving maximum peaking and tapering is not possible. The effect of any training session is determined by the training load orientation (Oca and Navaro, 2011). Load orientation will depend on qualitative and quantitative factors. In terms of football training, qualitative factors are the aerobic and speed endurance training, documented as the predominant physical capacity that should be trained and is identified by the intensity of exercise. On the other hand, by quantitative factor the amount of work performed is related to the volume and the given intensity. The principle of individualisation suggests that athletes will react and adapt differently over individual times frames even when presented with identical training regimes (Naclerio et al., 2013). An optimal programme would prevent undertraining, overtraining and injury (Meeusen et al. 2006), and produce favourable physiological adaptations towards desired outcomes at specific times (Lambert and Borresen, 2006). It is well established that adequate regeneration periods in training programmes are crucial, so that adaptation can be achieved. In a typical week for a professional football team with one match to play, the players may have six training sessions in 5 days (i.e. one day with two sessions), with the day after the match free. If there is a second match in midweek teams will often train once a day on the other days. However, there are marked variations depending on the experience of the coach (Bangsbo and Mohr 2005). From the same study, an estimation of daily energy expenditure among Danish professional footballers (National team) has been thru via two weeks of training sessions' analysis by using heart rate zones methodology. With special reference to positional and tactical role of players, daily energy expenditure has been quantified between 5.6 and 7.6MJ and duration of training sessions was 79-85.9min.

Periodization may be the most important parameter in any sport, including football. Stone (1999) defined it as "a logical phasic method of manipulating training variables in order to increase the potential for achieving specific performance goals". Programming the season training non-linearity is a main principle of periodization. A proper periodization aims are anticipated as follows: (a) diminish the chances for overtraining and (b) selection of peak phase of training load in macro- and meso-

cycles with respect to the importance of competition, transition and active rest periods within season. Manipulation of the intensity and volume while respecting the tactical and technical parameters fraction can lead to the anticipated results (Refer to Figure 2.4).

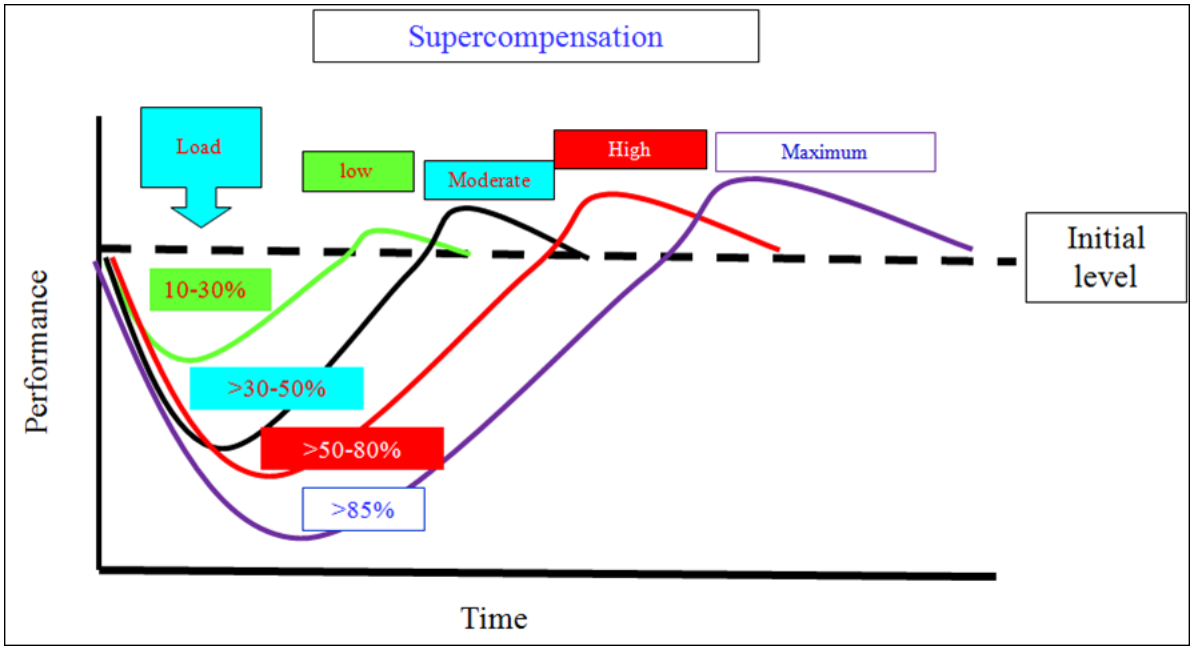

Figure 2.4 The theory of supercompensation adaptations following different performance volume (Adapted from, Naclerio et al., 2013).

During a comparison study of professional futsal players during pre-season, end of pre-season and in-season with regards to their physical performance changes, yo-yo intermittent recovery test total distance was significantly improved at the end of pre-season while during in-season performance was maintained (Oliveira, Leicht, Bishop, Barbero-Álvarez, Nakamura, 2013). For the period of monitoring the training load of the same three weeks by rating og perceived exertion (PRE) method, there was no difference between the two first weeks (P=0.13), while the training load of the third week was reduced approximately by 38% and 33% respectively in comparison to the two previous weeks. The data indicate how most seasonal training programs are designed, with heavier energy cost during pre-season in terms of weekly training sessions' number and duration (Bangsbo and Mohr, 2005).

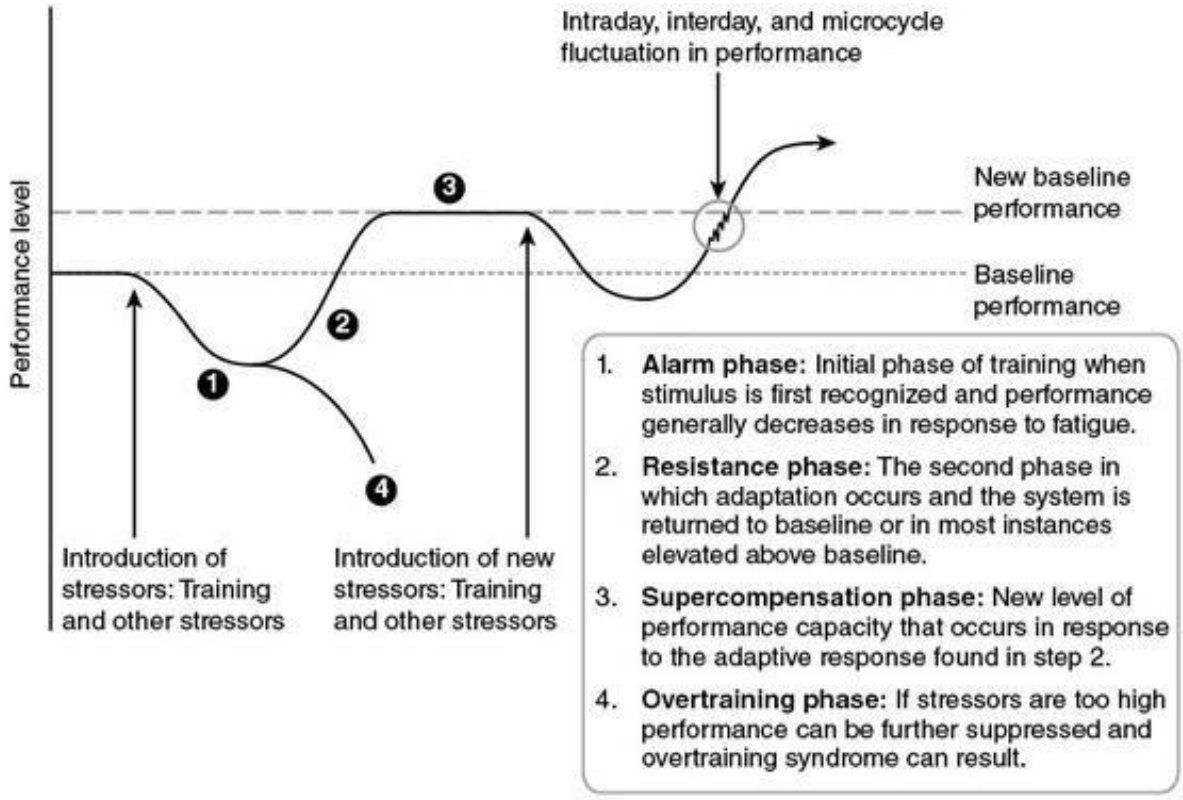

Figure 2.5 The general adaptive syndrome and its application to periodization. Adapted from, Fry et al. (1992) and Stone et al. (1996).

Overall, the training load between weekly training programmes during the season differ greatly in terms of number of training sessions and the average percentages of technical, tactical and physical elements, especially with regards player position as well. Pre-season is often targeted with having a higher training load, although this may differ between clubs with regards to player standards (Jeong et al., 2011). Modern use of methods to assess training load vary within the literature ranging from mathematical models of variable intensity within a session to advanced individual player tracking devices and real-time video-based assessment to optimise squad and player development. Such techniques can provide useful information to quantify training load based on exercise intensity, specific exercise duration, percentage of performed at specific high intensity match play and total distance covered.

Any attempt for the quantification of exercise in physiological or metabolic terms should first identify the high intensity and submaximal exercise. The reference point for aerobic exercise is the maximal oxygen uptake for any athlete. Difficulties for a similar reference point to help estimations of anaerobic capacity of athletes, have lead science to describe anaerobic power of an athlete only in terms of the absolute values achieved during tests. Some other approaches are based on the concept of maximal oxygen deficit and oxygen dept. In an attempt to quantify training energy cost based on actual match metabolites averages, Osgnach, Poser, Bernardini, Rinaldo and di Prampero (2010) have developed a theoretical model which estimates energy cost and metabolic power. Any estimation of energy cost is taking into account the fact that running on a football field is approximately 30% more costly than running on compact homogenous terrain. Similarly, any training load energy cost in order to be as accurate as possible should also be taking in account the running surface. In advanced championship teams all over the world, software and game reports seem to concentrate mostly on total distance covered, maximum speed and average pace, energy expenditure etc. Based on game performance parameters, a more accurate estimation of the energy expenditure during training could be also calculated. As such are the equivalent distance; signifies the distance that the athlete would have run at a steady pace on grass using the total energy spent over the match, the anaerobic index; is the ratio between the energy expenditure above a certain metabolic power threshold (TP) selected by the investigator (e.g. power output corresponding to VO_{2max} or to anaerobic threshold) and the total energy expenditure ($J \cdot kg^{-1}$) over the whole match or in the period considered. More importantly, the energy cost estimation of accelerated and decelerated running can be estimated, leading to a new assessment of metabolic demands. This approach allows the calculation of metabolic power by multiplying the EC by running speed, but more importantly all field-training variables are calculated according to actual performance average values.

2.3 Football nutritional demands.

2.3.1 Physical activity and dietary intake.

Increased training load can evidently raise the requirement for macronutrients and probably micronutrients. Principally at a top level football, with frequent periods of playing two games per week, this additional nutritional intake might be important. According to Burke (2006), establishing good eating practices and a well-balanced diet which would not allow significant energy deficit should be the primary nutritional strategy to support optimum performance in football. Primarily, daily energy intake shouldn't be less than 30 kcal per kilogram of fat-free mass daily, plus the required energy expenditure needed for training. Hawley, Tipton & Millard-Stafford (2006) stated that despite different cellular adaptations as result of homeostasis and glycogen stores alterations during exercise, significant chronic training adaptations arise occur mainly during the recovery phase, both in the early-post exercise and long-post exercise until return to baseline levels. Naturally, a theory parallelising the chronic training adaptations with short-term nutritional strategies, a longer seasonal nutritional periodization could probably be manipulated based on the general sport training requirements.

Heavy exertion increases the generation of free radicals and reactive oxygen species (ROS) through several pathways (Urso and Clarkson, 2003). Neutrophils and macrophages migrate to the site of contraction-induced muscle damage, infiltrate the muscle tissue, activate the release of cytokines, and produce additional ROS. Most ROS are neutralized by a sophisticated antioxidant defence system consisting of a variety of enzymes and non-enzymatic antioxidants including vitamin A, E, and C, glutathione, ubiquinone, and flavonoids. Intensive and sustained exercise, however, can create an imbalance between ROS and antioxidants, leading to oxidative stress that not only causes lipid peroxidation and protein oxidation, but may also impact immune function (Nieman and Bishop, 2006).

Manipulation of foods to meet fluctuating energy needs should be the primary strategy for every footballer, especially for young players instead of the use of dietary supplements to compensate for potential dietary insufficiencies. Physical exercise may increase the body's requirements for some micronutrients, but it is generally

agreed that these can be met by a well-balanced and energy-adequate diet, with no need for further supplementation unless the athlete is restricting food intake to maintain or reduce body weight. It is crucial to understand each macro-micronutrient's role in energy metabolism and furthermore to optimize the interaction between food intake and storage, and training or performance.

2.3.2 Macronutrients.

2.3.2a Physical activity and carbohydrates.

Muscle glycogen and blood glucose serve as the primary fuels during intense anaerobic exercise beyond 10-seconds duration. Trained muscular system demonstrates a higher ability to oxidize carbohydrate. Deficiency in carbohydrates ingestion depletes muscle and liver glycogen which directly affects the performance.

Research has shown that athletes undertaking endurance type activities should include a high percentage of total calories from carbohydrates, approximately around 65% of total energy intake. Nevertheless, studies agree that the possibilities of development higher endurance capability should include sufficient refuelling via carbohydrate energy intake, especially for high-intensity and intermittent training (Burke et al., 2006). Originated from the basal metabolic rate, each athlete's total energy expenditure and requirements occur upon the principle of individuality (Manore, Meyer and Thompson, 2009). A guideline for carbohydrate energy intake for individuals and footballers (based on Burke et al., 2004), recommends that generally footballers must try to reach their game and training energy costs to enhance restoration of muscle and liver glycogen stores. In line, sufficient energy requirements will be succeeded among double training sessions and continuous matches. Clearly, if a person regularly performs excessively heavy training sessions or games, daily allowances must be adjusted to permit optimal glycogen resynthesis to sustain high level performance. Since muscle and liver glycogen stores need at least 24 hours to be restored after heavy training, rest or light activity is suggested, and diets high in carbohydrates (±79% of energy intake) in order to facilitate carbohydrate replenishment.

In general, the importance of the different carbohydrate forms concerns the rates of digestion and absorption. Thus, the starches with a relatively large amount of

amylopectin digest and absorb much faster than starches with high amylose content. Consequently, the importance of these rates is much higher when feeding time is relative to exercise or performance

2.3.2b Physical activity and lipids.

Standards for optimal lipid intake have not been firmly established. Consumption of dietary lipid intake varies according to personal taste, money spent on food, and the availability of lipid-rich foods. In conditions of regular aerobic exercise profoundly improves ability to oxidize long-chain fatty acids, particularly from triglycerides stored within active muscle during mild to moderate intensity exercise (Nicklas, 1997). Training enhances catabolism of fat. During constant-load prolonged exercise, energy from fat oxidation significantly increases following aerobic training, while corresponding decreases occur in carbohydrate breakdown. Due to lipolysis and these training adaptations, endurance athletes can train at a higher absolute level of submaximal exercise before experiencing the fatiguing effects of glycogen depletion than an untrained person. However, even the top level endurance athletes when training in near-maximal, continual aerobic levels, can rely almost entirely on oxidation of stored glycogen.

In a comparison study between usual high-carbohydrate loading diet and a 5-day of high fat diet followed by one day of carbohydrates restoration, the levels of pyruvate dehydrogenase (PDH) activity at rest were reduced and predicted rates of glycogenolysis were decreased post the assessment of basic 1 min sprint once the high-fat diet period was completed. While the researchers could not identify the metabolic signals that changed in muscle substrate use during cycling at 70% VO_{2peak} cycling, that is close to a football game aerobic average metabolism. According to the researchers, results emerge that the dominate suggestion of previous studies about availability of muscle glycogen due to fat-adaptation may possibly be due to diminished glycogenolysis rhythm. Therefore, in anaerobic sports similar to football this modification wouldn’t be of assistance (Stellingwerff, Trent, Spriet, Watt, Kimber, Hargreaves, Hawley and Burke, 2006). Hence, with low fat diets during training, it becomes difficult to increase carbohydrate and protein intake to furnish sufficient energy to maintain body weight and muscle mass.

2.3.2c Physical activity and protein.

Protein for most sportsmen is considered to participate in muscle build and recovery, more than energy itself during performance. Indeed muscle protein synthesis is stimulated after resistance exercise (Borsheim, Tipton, Wolf, and Wolfe, 2002). Protein mass and thus gains in muscle size and strength can be gained through resistance training and ingestion of carbohydrates-protein mixture during and after resistance training sessions (Beelen, Koopman, Gijsen, Vandereyt, Kies, Kuipers, Saris and van Loon, 2008).Bovine colostrum was suggested to be used for improving recovery between intervals during intermittent sports, although this hasn't been assessed via specific sport protocol (Buckley, Abbott, Brinkworth, and Whyte, 2002).

The main determinants of an athlete's protein needs are training regimen and habitual nutrient intake, but individuals' needs may be increased after resistance training or intermittent exercise (Tipton, Arny, Ferrando, Phillips and Wolfe, 1999). Protein serves more as an energy fuel depending on nutritional status (low CHO intake) and the intensity of training and performance. As energy source is marked in circumstances of low carbohydrates intake, when the process of gluconeogenesis can assist in restoring part of glycogen (Williams, 2007). Amino acids particularly branched chain amino acids (BCAA) concentrate researchers attention regarding their importance for recovery after training or performance.

Primary general recommendations for elite athletes were suggesting protein intakes between 0.94 g/kg/day and 1.37 g/kg/day wet and 3.5-6.9 g/kg body wet for carbohydrates (Wootten and Williams, 1989; Meredith, Zackin, Frontera and Evans, 1989), but more recently evidence agree that any protein ingestion strategy should be applied by a nutrition professional, depending on the sport, their training load, habitual nutrient intake and individual's needs (Tipton and Wolfe, 2004). Since resistance training is part of modern football training, recommendations for a football player's beneficial protein consumed quantities are between 1.4-1.7 g/kg/day (Lemon, 1994). Generally studies agree that protein availability is critical for optimizing many of the adaptations that take place in muscle in response to both endurance and resistance training. Even though resistance exercise inhibits protein synthesis, muscle protein breakdown is also increased, which produces a balanced situation in blood protein. More recently, Alghannam (2011) suggested that a mixture

of carbohydrates and protein (CHO+Pro) could be advantageous compared to carbohydrates alone for sports like football. Endurance running capacity of players ingested the mixture supplement was increased by 43% at the end of a controlled game compared to players who ingested only CHO supplement with equal energy content.

2.4 Nutritional intake and performance in football.

A variety of factors ultimately impact athletic performance, including tactics, technical skills and nutrition. An inherent relationship exists between physical activity and nutrition. Both training and performance result in different energy expenditure among individual footballers. In detail, Shephard (1999) recommended that daily energy intake for footballers should be, 14–15MJ.day^{-1} (3346–3585kcal.day^{-1}); carbohydrate, 8g.kg body mass^{-1}.day^{-1}; protein, 1.5g.kg body mass^{-1}.day^{-1}. Earlier, researchers determined the effects of a ‘‘moderate’’ (6.5 g.kg BM^{-1}.day^{-1}) or high (12 g.kg BM^{-1}.day^{-1}) carbohydrate intake for swimmers when combined with intensive anaerobic training for a period of 9 days. Due to the lack of any additional benefits to the high intensity of training, there was an indication suggesting the possibility of highest daily carbohydrate intake (500–600g.day^{-1}) and additional amounts in daily intake would improve neither performance nor energy restoration (Lamb, 1990). Athletes generally seem to periodize the quantities of carbohydrates ingested, according to the training and the period of the season. Burke (2011) suggests that researches so far indicate that athletes should concentrate more on sufficient CHO availability for high-intensity training sessions or high levels of technique, instead of trying to adapt a more general seasonal pattern.

Nutritional targets can also be categorized into three sub-targets, related to time; pre-exercise, during exercise and post-exercise. Through a combination of a high energy intake prior to match with a sports drink ingested during the match, resulted in a greater exercise capacity than a high-carbohydrate meal alone. Wu and Williams (2006) found that pre-exercise low-GI meals superior high-GI meals (breakfast), when consumed before running test to exhaustion at 70% VO_{2max} by running times 109min and 101min respectively. Greatest obvious advantage of low-GI carbohydrate pre-exercise meal is the stability of plasma glucose concentrations. Also low- GI pre-match meal results in feelings of satiety for longer and produces a

more stable blood glucose concentration than after a high-GI meal. There are also some reports of improved endurance capacity after low-GI carbohydrate pre-exercise meals. A study with long distance runners has investigated the influence of pre-exercise meals on endurance capacity (Chryssanthopoulos, Williams, Novitz, Kotsipoulou, & Vleck, 2002). When runners ate a high-carbohydrate breakfast (2.5 g carbohydrate·kg BM^{-1}) 3 hours before exercise and drank a carbohydrate-electrolyte solution during a subsequent run to exhaustion, their endurance capacity was greater than when they ran after a high-carbohydrate breakfast alone. The pre-exercise carbohydrate meal and the carbohydrate-electrolyte solution increased running time by 9% (125 min) more than when only the meal was consumed (115 min) but 21% more (103 min) than when the runners completed the test without breakfast and had fasted overnight. Same lead researcher has examined the influence on skeletal muscle glycogen concentration after 3hours of a carbohydrate rich meal (2.5g $CHO.kg^{-1}$ body mass) ingested by recreational endurance runners. Biopsies results showed an increase of 10.6 ± 2.5% in vastus lateralis muscle glycogen concentration. However, glycogen store could probably be higher considering that part of the consumed carbohydrates was still in digestion system after 3hours.

Nutritional educational program must be able to recognise that many coaches request that footballers retain lean bodies and most of the times diets with low energy intake and low carbohydrate fraction are suggested to the players are struggling to reduce body fat percentage. Nevertheless, the energy balance is disrupted with higher energy expenditure than energy intake with negative consequences for both performance and health status. In literature, earlier studies established that low carbohydrate ingestion cannot resynthesize muscle glycogen stores post-game and post-training (Bangsbo, Mohr & Krustrup, 2006). Additionally, inadequate glycogen ate diets that do not allow players to cover their daily energy expenditures appear to suppress the immune system and so make them more susceptible to viral infections (Nieman & Bishop, 2006). In summary, the more players and coaches understand about training load and nutritional intake, the better they comprehend the needs for sufficient energy to balance daily energy expenditures.

Most published studies, found significant relationship between exercise time to fatigue and muscle glycogen stores levels. Therefore, a probable positive performance adaptation may occur thru deficiency of energy availability. This method

of ‘‘train-low, compete-high’’ is investigated by Hawley (2005) and indicated the importance of applying such method with care in real training circumstances. Researchers recommend these positive adaptations for endurance training under conditions of deficit glycogen stores in comparison to training cost requirements, expecting an increase of the transcription rate of specific genes which are considered to be intricate in the body systems responses (Febbraio, 2003; Pilegaard, Keller, Steensberg, Helge, Pedersen, Saltin and Neufer, 2002; Keller, Steensberg, Pilegaard, Osada, Saltin, Pedersen et al., 2001).

Burke (2011) suggests, that estimations of the amount of carbohydrate required replenishing glycogen stores including the small quantities of CHO ingestion during exercise should be provided in absolute amounts rather than scaled to body mass, with a sliding scale according to the training or competition energy cost. The athlete’s needs are not static, but rather move between categories according to changes in the daily, weekly or seasonal goals and exercise commitments in a periodized training programme. A number of studies regarding peak oxidation rates of exogenous fuel (CHO) during exercise showed no correlation to body size. Products containing special blends of different carbohydrates may maximize absorption of carbohydrate at such high rates. In general, most researchers agree to the benefits of carbohydrates ingestion before training. In contrast, studies involving branched chain amino acids (BCAA) infusion before (70minutes) and during performance to fatigue, found that it didn’t affect times to fatigue, perceived exertion or various measures of cardiovascular and metabolic function (Davis et al., 2000).

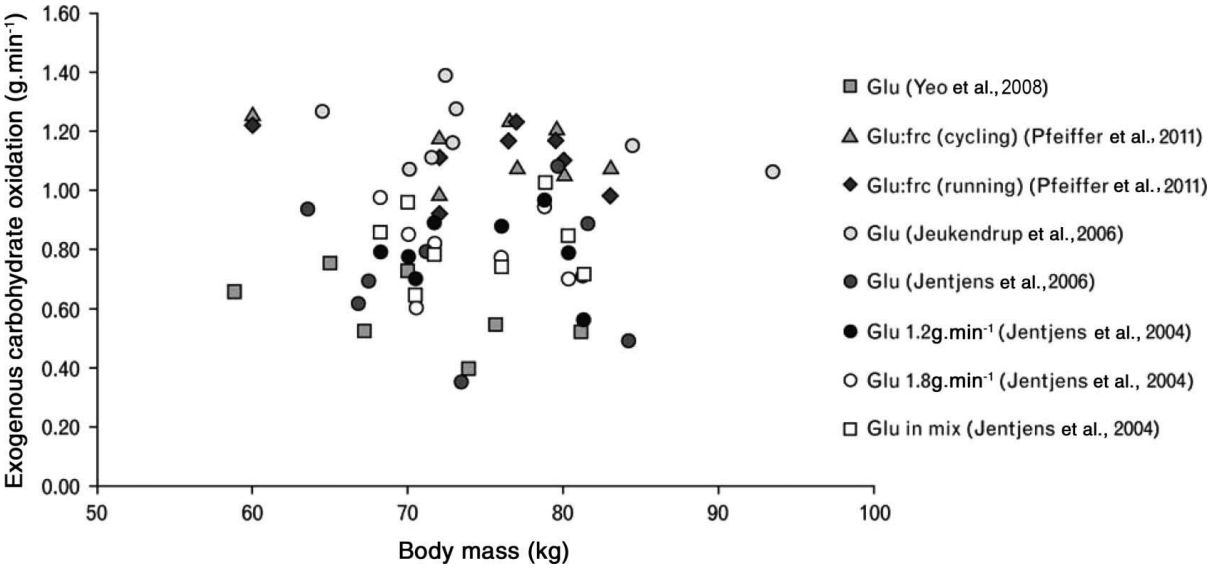

Figure 2.6 Results of peak oxidation rates of exogenous carbohydrate consumed during exercise versus body mass for individuals involved in a number of studies. Taken from Burke et al., 2011 (adapted from Jeukendrup et al., 2006). These data show that there is no correlation between exogenous fuel use and body size.

Prado (2006) hypothesized that there may be a genetic basis for food choices. Research investigated the functional variant of a serotonin receptor subtype, 5-HT_{2A} T102C, which has been reported to be associated with several eating disorders. Accordingly, while all subjects had the same amount of weekly energy intake (1627 ± 524, 1474 ± 551, and 1586 ± 529 kg.Cal/week) for the three genotypes respectively, $P < 0.02$), subjects with TT genotype have higher protein intake than either CC or TC genotype. Additionally, subjects with TT genotype had significant lower BMI then the other two groups, probably as a consequence of their increased protein intake.

According to researches, despite the fact that long-term muscle adaptations are considered to be the result of the growing effect of repeated bouts of exercise, the primary responses that lead to these long-lasting changes occur during and after each training session (Pilegaard et al., 2000). In line with training load progression method, manipulation of nutritional intake may assist the improvement of footballers' performance. Training and performance energy expenditure require specific choices of food, targeting the energy needs from the consequent metabolic reactions similar

to a long-term nutritional periodization. With respect to the daily total energy balance, additional requirements arise for the timing of ingesting foods prior, during and post-training or post-game. Parallel to the shorter training adaptations and consistent with supercompensation, similarly time-adapted food intake can be estimated.

The energy balance equation is the relationship between food intake, energy expenditure and the fuel stores of the body. Methods using the oxygen consumption or heart rate are usually assessed to calculate the energy expenditure, although it should be always considered a compromised approach as the estimation of energy expenditure (Montoye et al., 1996). Energy expenditure quantification is not only the energy demanded when the individual participates in any activity, but it also includes the energy expenditure needed to fulfil the domestic requirements of the body. Basal metabolic rate (BMR) or kilocalories/ 24h, can be estimated from equations based on the age, weight and sex of the individual. It is clear out that a matching of energy intake and energy expenditure does not appear to occur on a day by day basis. Hassapidou (2000) found that despite the mean energy intake of Greek professional footballers during the competitive season was above their calculated mean energy expenditure, six athletes were not in energy balance. Half of the athletes consumed carbohydrates in less than 50% of their total energy intake, meaning that half of the players had inadequate carbohydrates intakes, with a possible consequence of reduced performance.

Restoration of water and salt losses is an important part of the post-exercise recovery process. Along with replacement of muscle glycogen stores and the provision of a source of amino acids to support protein synthesis, the recovery meal should contain sufficient water and salt ensuring a return to euhydration (Maughan Shirreffs and Leiper 2007). Excessive sweating from training and competition equals to significant losses of water and minerals. Sweat loss during exercise usually does not increase the minerals requirement. During football season, teams and individuals particularly at elite level may be required to perform in various diverse geographical areas with significant climate and environmental alternations. McGregor (1999) found that football specific drills accomplishments were worsen when drinking was not allowed during activity by 5% compared to drills assessment with drinking allowance. Despite the fact that thereis not a specific agreement for the quantities of fluids that should be consumed before exercise in warm environments, a general consent of the benefits

of drinking water than nothing exists. Moreover, researches agree that a mixture of CHO and electrolytes can enhance performance instead of simply water drinks (Sawka, Burke, Eichner, Maughan and Montain, 2007).

Some researchers state, that the average requirements of vitamins and minerals for athletes should be higher than basic healthy individuals RDA (Magkos et al., 2003; Clark et al., 2003). Based on several studies however, it seems that if energy intake is sufficient, balanced and varied, supplementation for vitamins and minerals may not be necessary. Exceptional cases would be sportsmen with weight limitations (e.g. judo, wrestling), or specifically in relation to this study for footballers, it would apply for individuals with increased body fat percentage level or special eating habits (e.g. vegetarian). Hassapidou and Mastrantoni (2001) when compared the dietary intake of Greek elite female athletes from different sports with a non-athletic group, reported a lower than recommended intake of iron in both athletic and non-athletic groups, but evidence showed higher than recommended intake of vitamin C in all participants. Interestingly, the researchers stated that these results were characteristic of the population of the Mediterranean countries. Generally, athletes' needs are higher than other healthy untrained individuals, although a well-balanced diet that manages to balance the higher energy demands most probably manages to deliver the necessary vitamin C supply (>200mg/d). Some athletes who are involved in heavy exercise while facing problems with upper respiratory tract infections may benefit from supplementing of vitamin C. On the other hand, supplementation of vitamin C has generally failed to show consistent results in improving oxidative burst activity. High-training-stress periods are key periods for vitamin C deficit awareness. Naturally though, higher energy demands from training are met with higher nutritional intakes, consequence a parallel greater ingestion of micronutrients in those quantities. In general, the background literature agrees that athletes' individual nutritional goals should be established by a qualified professional.

Similarly the correct relationship between training load, nutritional intake and the micronutrients quantity effect has not been evidently proven. Margaritis (2008) interpreted the established relationships between antioxidant micronutrient intake and the adaptive response of antioxidant systems. Also it disclosures, that real needs of antioxidant micronutrients in athletes' diets are still to be addressed. With analysing if physical exercise modifies the status of antioxidant vitamins, carotenoids

and flavonoids, some notable conclusions are revealed. The evidence of high antioxidants intake needs for footballers are still to be established. Having as a fact that oxidative stress cannot be avoided; the attention should be concentrated in minimising the oxidative damage by balancing the oxidants and antioxidants. There has been the assumption that training induces a biochemical adaptive response which might require an increase in the ingestion of foods rich in antioxidants, followed or parallel to the absorption of trace elements and vitamins. More importantly, antioxidant-rich foods have bioactive properties which may support training adaptations and individuals in Mediterranean countries including athletes may naturally consume higher quantities of polyphenol rich compounds.

Dietary assessments to investigate the effect of food consumed that contain antioxidants require accurate reports. Antioxidants and trace element intakes are necessary to allow endogenous adaptation and to avoid excessive stress as a result of heavy exertion (Urso and Clarkson, 2003). Increased generation of free radicals and reactive oxygen species (ROS) are deactivated by a defence system based on antioxidants including vitamin A, E, and C, glutathione, ubiquinone, and flavonoids. Unfortunately, while this may be adequate for athletes when assessing moderate exercise sessions, ROS might defeat antioxidants countermeasures with effect on immune system when high-intensity exercise sessions are not combined with sufficient recovery intervals and nutritional intake countermeasures. The long-term risks of deficiency, as well as excessive intakes of antioxidant micronutrients, are largely unknown especially in football because they are still poorly investigated in athletes (Nieman et al., 2006).

There may be a variety of reasons (social, religious, psychological, travelling, digestive, medical, geographical location) that influences a players habitual eating pattern, which in turn may result in transient nutrient insufficiency (both macro and micro-nutrients) which could results in inadequate refuelling or recovery during regular training periods (Leiper, Junge, Maughan, Zerguini and Dvorak 2008; Bangsbo et al., 2006). Probably eating the “Mediterranean way” is less of a diet and more about a healthy approach to eating. It is based on foods that are traditionally found in countries surrounding the Mediterranean. Basic daily diet includes vegetables, fruits, nuts, fish, olive oil and whole foods with high antioxidant potential (e.g. whole grain).

Meat, saturated fats and high-fat dairy are consumed in much smaller quantities. Multiple studies suggest that eating in a Mediterranean style diet contributes to fat control and reduces cardiovascular disease. Probably, a life style that helps controlling body fat% can be beneficial for footballers, since fat% has been associated with teams ranking. Therefore, the higher ranking on the table demonstrates lower fat percentage (Kalapotharakos et al., 2006). Furthermore, fat percentages significant differences seem to occur between players from national leagues with different international ranking levels. Comparable results between studies present average ±10% fat in Spanish footballers (Rico-Sanz et al., 1998), ±10.5 in Greek footballers (Kalapotharakos et al., 2006) and ±12.6% in Scottish players (Maughan, 1997).

Also all the above studies stated that the energy intake of their participants had similar fractional contribution of the macronutrients to total energy intake of the general population. Mediterranean culture submits in the local countries cuisine because is associated with the rich in antioxidants sources of food along with the traditional cooking methods. Unfortunately, there haven't been any studies investigating whether these diet benefits can influence stress markers of athletes at any professional level, like footballers trying to maintain a better immune system defence through the season. Despite the high fat intake, the population of the biggest Greek island had very low rates of coronary heart disease and certain types of cancer and had a long life expectancy (Hu, Rimm, Smith-Warner, Feskanich, Stampfer, Ascherio et al., 2003). An interpretation of these results should have also described the lifestyle of the participants, since a significant percentage of Greek and Italian population actually live on islands, which create different living circumstances. Part of these differences, could be the more active lifestyle due to the "country" available jobs. In general, Mediterranean diet refers to a high-content of olive oil and its overall group of characteristics.

However, it does not appear to be any evidence of additional benefits for athletes following dietary practices similar to Mediterranean diet. There are evidence that athletes diets in Greece also consist from high fat percentages (Hassapidou et al., 2000), but it can't be concluded whether this cultural food habit improves either training performance or immune markers values. Although an important health

observation occurred via blood analysis that showed no elevated cholesterol level among athletes despite the high-percentage of fat intakes.

Most teams in top level clubs of each country, especially in Europe, contain players from different ethnic, cultural and socio-economic backgrounds. When athletes in team squads train and compete together, they share team targets and ambitions. Possibly, many players, when back in their home country, are likely to have very different eating (and possibly training) habits. The authors of a dietary survey of Italian national athletes found that the apparent contributions of CHO and fat in their diets, was different to the intakes reported in other dietary surveys of athletes from other countries. They suggested that the high proportion of CHO energy was due to the 'Mediterranean' dietary practices. Clearly, it is difficult for athletes to achieve significant dietary changes that conflict with the eating practices of the general community (Schena, Pattini and Mantovanelli, 1995).

In agreement, a study by Ono (2012) has indicated that whilst football athletes share broadly similar nutritional targets (i.e. energy intake, macronutrient intake), there would appear to be larger variance between athletes in terms of food choices, food combinations and nutrient timing as a means to achieve such targets. For example, English players despite eating in average 2.2 pasta dishes per day, they stated that they would prefer eating traditional food at home, probably because they were not comfortable to eating so many high-carbohydrate meals. Burke (2006) grouped various studies regarding reported dietary intakes of male soccer players during training (mean daily intake ± *s*) from players of different national leagues. Using similar survey methods (3-7 day food diaries) and similar professional status, total energy intakes and macronutrients contribution seem to vary greatly between studies and groups. As an example, Scottish professionals had total energy intake 11.0 ± 2.6 MJ (CHO 354 ± 95g, protein 103 ± 26g, fat 93 ± 33g) and English players had total energy intake 12.8 ± 0.8 (CHO 437 ± 40g, protein 115 ± 2g, fat 94 ± 1g). However, they can only be used for references individually, since basic principles and procedures should have been followed for all studies' assessments in order to have accurate comparisons (e.g. time of season, food record diaries design). Nevertheless these reports suggest the alterations among individuals or groups, regarding nutritional intake and macronutrient percentages.

Notably the various different environment in which footballers must train and perform during season, generate several stressful environmental conditions. Nutritional strategies e.g. high carbohydrates intake, fluid-electrolyte and creatine may help athletes to offset environmentally induced performance decrements (Armstrong et al., 2006). Eating habits within football appear to be more International based than previously observed, and players (especially at professional level) are often given choices that include multiple ethnic cuisine. However, less is known about comparisons between trained and recreational football athletes, let alone differences that may exist between ethnic groups

2.5 Parameters that may influence performance and energy expenditure.

A significant influence on performance during a typical season is the body composition of each athlete, as physiological markers improve while muscle increases and fat mass decreases. The effects of positional group (goalkeepers, defenders, midfielders, and forwards) and exposure time to play (participation time in training and matches) in relation to in-season variations have been examined (Carling et al., 2010). As expected, the research demonstrates that pre-season fat percentages and muscle mass of players, respectively improved during mid-season and end-season. Significant variation of fat percentage and fat-free mass percentage was recorded between different positional groups during the seasonal testing points. However, more investigations may be needed in relation to the energy balance succeeded between the different season monitoring points. Bloomfield (2007) has investigated the variations in height and body mass between players in different leagues. This study suggests that these differences are probably simply the result of influences due to the culture and style of each country, as they might prefer certain body types for certain positions. According to the same study, Germany's top league employs players who have greater height, BMI and body mass than their colleagues in English, Italian and Spanish leagues.

It is crucial to remember that a significant energy amount is needed in order to complete the domestic requirements of the body. The basal metabolic rate (BMI) indicates how much exactly this need is. The resting or metabolic rate accounts for about 2/3 of the daily energy expenditure and can be estimated by using equations that use participants' weight and age. However, it should be acknowledged that body

biochemically usable energy, which is ATP, can only use 40% of the individual's total nutritional intake. Transformation of the foods energy content spares about 60% of the absolute energy content for heat, which maintains resting body temperature at 37^{o}C. Training in different environments affects sweat loss and drinking behaviour. During competitions it has been observed that individuals lose 2-3 litres of sweat, while during competition in humidity and hot environment this loss increases to 4-5 litres (Grantham et al., 2010).

While trained subjects may attain core temperatures as high as 41˚C, most subjects in laboratory become unwilling to continue exercise tests when their core temperature exceeds 40˚C. It has to be acknowledged that the first protective mechanism against exertional heat illness is a reduction in the intensity and duration of the activity (Nybo & Nielsen, 2001). While background literature investigates the mechanisms influenced when training in extreme environment conditions, there are not enough evidences of the impact on training sessions and training plan. Particularly if extreme conditions like high temperature and humidity, are solid elements that influence training sessions and game performance. Undoubtedly the effect of high body temperature, cardiovascular strain and fatigue result a significant reduction in the amount of sprinting, high-intensity running, and distance covered. Numerous factors such as exercise intensity, air or water, fat percentage and physical characteristics, lead to different ways of handling cold environment's impact on performance. Heat loss may be dangerous for injuries or in extreme cases even myocardial infarction. Nutritional dietary habits of athletes performing at approximately 70% VO_{2max} in a 10˚C environment include higher quantities of carbohydrates and fats in their food. According to Nimmo (2004), evidence of a cold-induced increase in appetite is poor, with the spontaneous adaptation to extreme environments being one of an adaptation of body mass to meet the new energy balance. There is no background literature investigating these adaptations in body composition between different geographical and environmental based groups.

A very important role on individual performance can play the metabolic cost of running on different surfaces and that is substantially independent of speed. Because of the majority of training methods, several kinds of surfaces are used for training sessions. Therefore the determination of the running cost on each type of surface may be very useful to estimate training load. Sassi (2011) compared previous studies

that had tried to evaluate running energy cost on different surfaces, but also via this research they attempt to fill in the gaps. In conclusion, they found the cost of running at 4.2J.kg-1.m-1 that was similar on both natural and artificial grass football pitches that possess similar percentage shock absorption characteristics. Impressively most individuals reported higher physical effort during matches played on artificial turf than natural grass despite similar distances covered at the concerned intensities (Andersson et al., 2008). The energy cost was only 5% higher than the valued observed in the same subjects running on an asphalted track (Sassi et al., 2011). Notably the above researchers in their conclusions are clearly pointing the future steps that need to be done by exploring the effect of energy expenditure e.g. when watering the surfaces.

2.6 Relationship between training load and the immune system.

Infections can be a very bad implication that affects an athlete's performance. Depending on the seriousness an infection can even be a reason for not competing or training at all. Additionally, most upper respiratory infections (URTI) can become radically spread in the whole team. Evidence shows that the increased susceptibility to URTI in athletes actually arises from a depression in immune system, usually caused from limited functioning of the immune system, influenced by stress and heavy training overloading.

The common mucosal immune system is a network of organised structures protecting the mucosal surfaces of the body, such as those in the gut, nasal passages, respiratory passages and urinary tract. The major effector function of the mucosal immune system is the production of IgA which is considered to provide the first line of defence against pathogens and antigens presented at mucosal surfaces and it does this with support from innate mucosal defences such as alpha-amylase (ptyalin), lactoferrin and lysozyme. However, this antibody alone cannot be used to predict URTI at the individual athlete level. There was another study, where salivary IgA secretion rate decreased by nearly half (pre 4.35±0.52, post 2.13±0.30) in a group of 155 ultra-marathon runners following a 160-km race (Henson et al., 2007). An important finding of the same study was about pre and post plasma cortisol as post-race values were almost doubled in comparison to pre-race values (412 ± 29, 806 ± 97). Nearly one in four runners reported an URTI episode during the two week

period following the race, and the decrease in s-IgA secretion rate was significantly greater in these runners (54%) compared to those not reporting URTI (31%).

Should short term nutrient insufficiency manifest, this may have implications on short-term recovery, the ability to perform at repeated training sessions or matches, and specific immune function. Deficiencies in protein intake, as well as key minerals such as iron, zinc, and antioxidant vitamins have been linked to sub-optimal immune status (Gleeson et al., 2004). Furthermore, immune system suppression has been associated with both excess dietary fat, and glycogen depletion. Training in nutritionally sub-optimal states has been further associated with elevations in stress hormones e.g. cortisol, with a longer term corresponding reduction in circulating testosterone levels. As a result, many professional clubs now utilise daily or weekly testing of salivary markers (including salivary immunoglobulin A, cortisol and testosterone) as an adjunct assessment of the effects of current training load and hence player status.

Health status can be monitored by assessing biomarkers of the immune system which is compromised of different chemicals (or analytes as they are known specifically in immune system testing). Low levels of antibodies such IgA and IgG (immunoglobulin A and G) can show greater levels of physical stress, whereas as high levels of cortisol (a stress hormone) can show greater levels of mental and physical stress (Gleeson et al., 2004). However, there are no universal norms for analytes such as IgA, which means that the only method of getting using results from measuring such an analyte in an individual is to chart its course over a period of time, thus finding normal ranges for the individual. A very important conclusion from Krustrup (2006) was that despite the lower than top level absolute intensity in the investigated friendly games by Danish 4th division players, the relative physiological strain of the footballers was similar to top level footballers. In general, the intensity of the lower level players' games in that study was considered similar to elite level average performances. Therefore, stress markers could probably be influenced in analogous patterns.

Evidence has supported that long training sessions with high intensity actions and insufficient recovery between games seem to sustain a depressed immune function for longer periods. A high incidence of infections is reported in individuals with

selective deficiency of IgA or poor saliva flow rates. In parallel, high levels of IgA are associated with low incidence of upper respiratory infection. Salivary IgA levels increase significantly immediately post-exercise sessions, while after a second same day training session salivary IgA outcomes do not appear to suppress (Sari-Sarraf, V., 2006).

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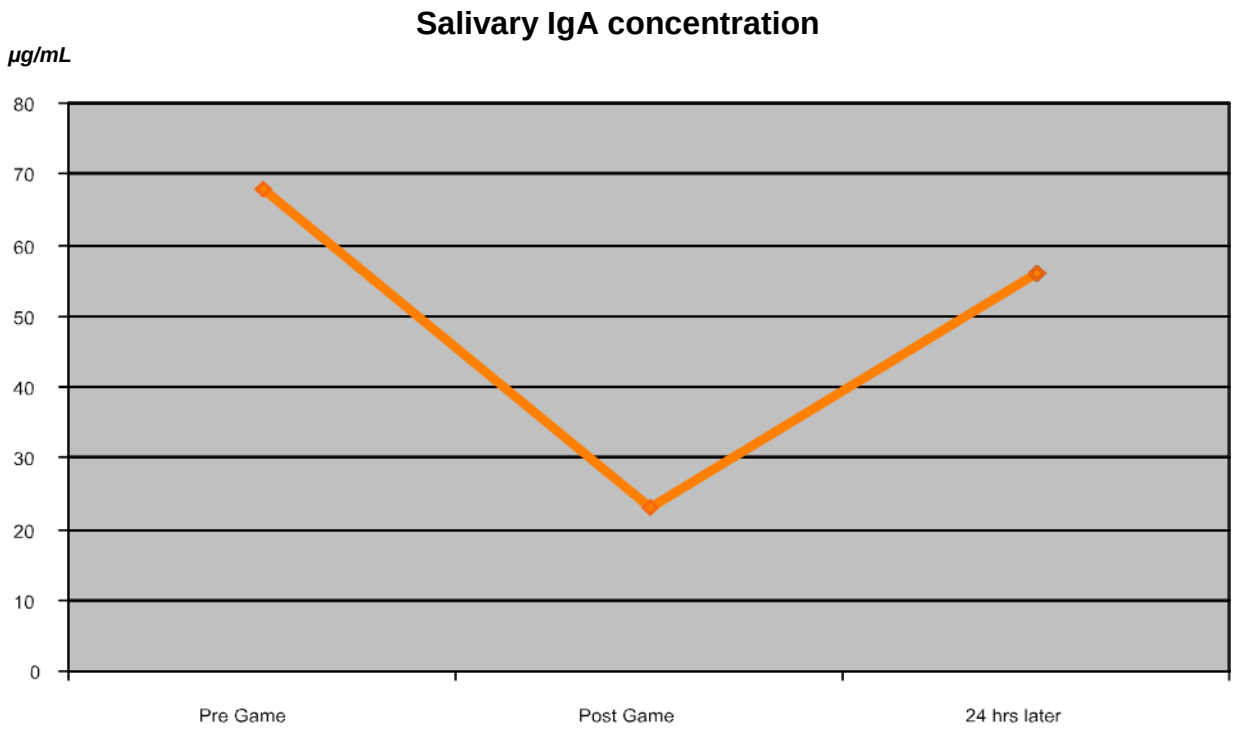

Figure 2.7a Typical changes in salivary immunoglobulin-A (µg/mL) concentration changes around a football match.

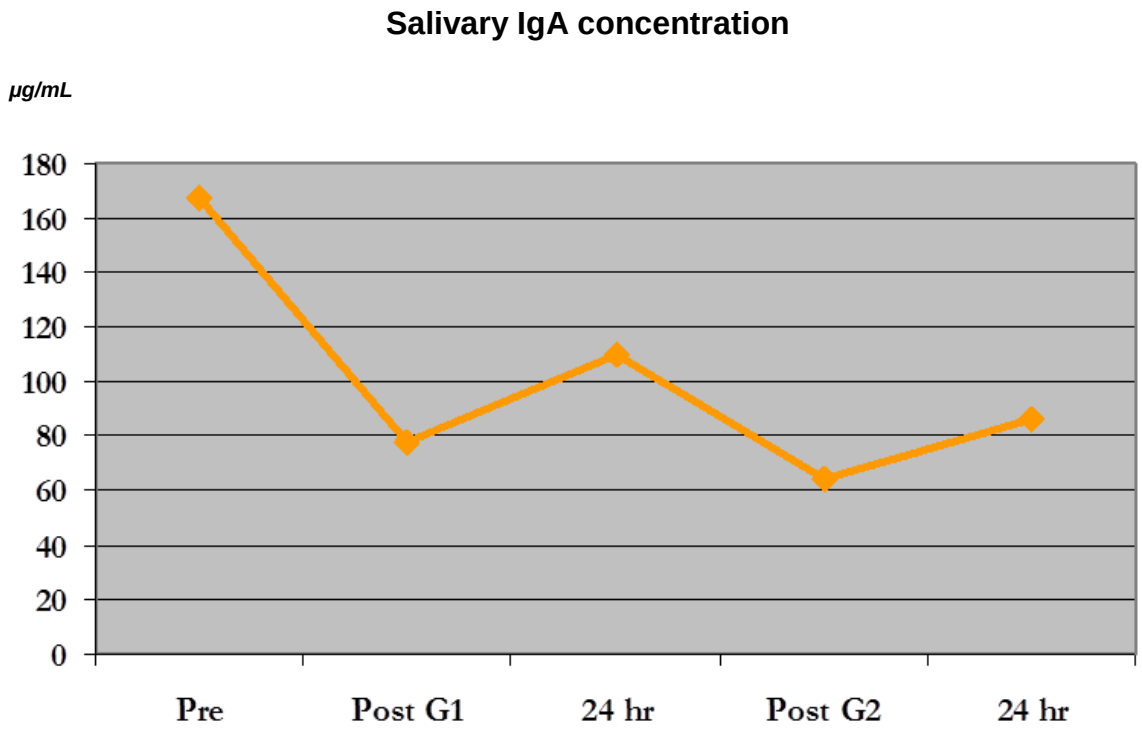

Figure 2.7b Two matches in three days, salivary immunoglobulin-A (µg/ml) concentration changes.

Oliver (2007) associated the consuming of a high carbohydrate diet throughout a 6 day period of increased training, with post-exercise reductions in s—IgA concentration. Unfortunately, there aren't any reports of data such as ingestion of fluids or supplements during exercise, which may had influence the salivary flow and therefore impact the levels of IgA concentration. This comes to agreement with previous studies, stating that carbohydrate ingestion during intense and prolonged exercise (~75-80% VO_{2max}) cannot halt the reduction of post-exercise salivary IgA output (Nieman et al., 2003a).

Some inconsistent findings of s-IgA concentration could be due to the different methods used to express IgA data, which makes comparison studies difficult. Also, if alternations in salivary flow rate are not taken into account, an apparent increase in s-IgA levels may actually reflect the concentrating effect on absolute s-IgA concentration. In contrast, stimulation of saliva flow, for example by chewing, could result in a diluting effect on the secreted s-IgA and thus a false impression of a

decrease in s-IgA concentration could be presented (Bishop and Gleeson, 2009). Probably the most important role to high incidences of infection, in high level footballers, would be the insufficient recovery time, leaving athletes vulnerable. Many confounders could influence immune function and this can make it difficult to identify the link between URTI and any exercise-induced alterations in cellular and mucosal immunity. Individuals' age, nutritional habits, psychology or which part of the athletic season can all play their role around the immunity function. Additionally, any kind of pathogen exposure could increase the possibilities for infection e.g. common training drinking bottles, closed changing rooms and gym areas with infected team mates.

Increased exercise stress is well established in physiological, biochemical changes and is often in conjunction with psychological alterations, all of which result from an imbalance in homeostasis. Hormone levels are influenced by the physical exercise especially of testosterone and cortisol level. Testosterone is considered as the main anabolic hormone because it stimulates protein synthesis mainly in muscle and skeletal tissues, accounting for more than half of the body's mass. Cortisol is the catabolic hormone involves in many functions such as gluconeogenesis via the proteolytic pathway, increases protein breakdown, inhibits glucose uptake and increases lipolysis. T/C (Testosterone/Cortisol) ratio is used as an indicator of balance between the anabolic and catabolic state of an athlete (Chang, Tseng, Tan et al., 2005).

Conclusively, training in nutritionally sub-optimal states has been further associated with elevations in stress hormones e.g. cortisol, with a longer term corresponding reduction in circulating testosterone levels. As a result, many professional clubs now utilise daily or weekly testing of salivary markers (including salivary immunoglobulin A, cortisol and testosterone) as an adjunct assessment of the effects of current training load and hence player status. The stress-free salivary collection for cortisol measurement has an advantage compared to plasma, since venipuncture for blood collection can lead to the increase of stress. Salivary-free cortisol concentrations do not seem to be dependent on salivary flow rate (Chiappin et al., 2007). Nutrients deficiency could be critical for preserve an effective immune system. Malnutrition has been identified as a major parameter of low immune defence against invading pathogens and therefore makes athletes more susceptible to infection (Gleeson et al., 2004).

2.7 Nutrition, training and immune system

Nutrition plays an important role to the immune system from the beginning of a person's life. Nutrients are also necessary for the immune response to pathogens so that cells can divide and produce antibodies and cytokines. Many enzymes in immune cells require the presence of micronutrients, and critical roles have been defined for zinc, iron, copper, selenium, vitamins A, B6, C, and E in the maintenance of optimum immune function.

Nutritional immunology is a rapidly growing area of scientific scrutiny, and according to Nieman, Nicolette and Bishop (2006), four key principles have emerged:

1. A well-balanced nutritional intake based on individual's needs, provides all the necessary "weapons" for immune system defencing lines. Also there is no absolute result stating that vitamin/mineral supplements improve immunity of healthy individuals above normal levels (Calder and Kew, 2002).

2. Serious reduction of immune defencing system along with mucosal areas becoming susceptible to pathogens can be cause to deficient nutritional intake. According to Keusch (2003), protein-energy-malnutrition (PEM) can be responsible for this defence line opening.

3. In cases of immunocompromised individuals and in situations of specific infections, several nutrients like glutamin, arginine and antioxidant micronutrients seem to provide further immune system defence called "immunonutrition" (Grimble, 2005; Calder, 2004).

4. Advanced supplements may prove useful in countering immune suppression for healthy adults during unusual mental and physical stress (Hamer et al., 2004).

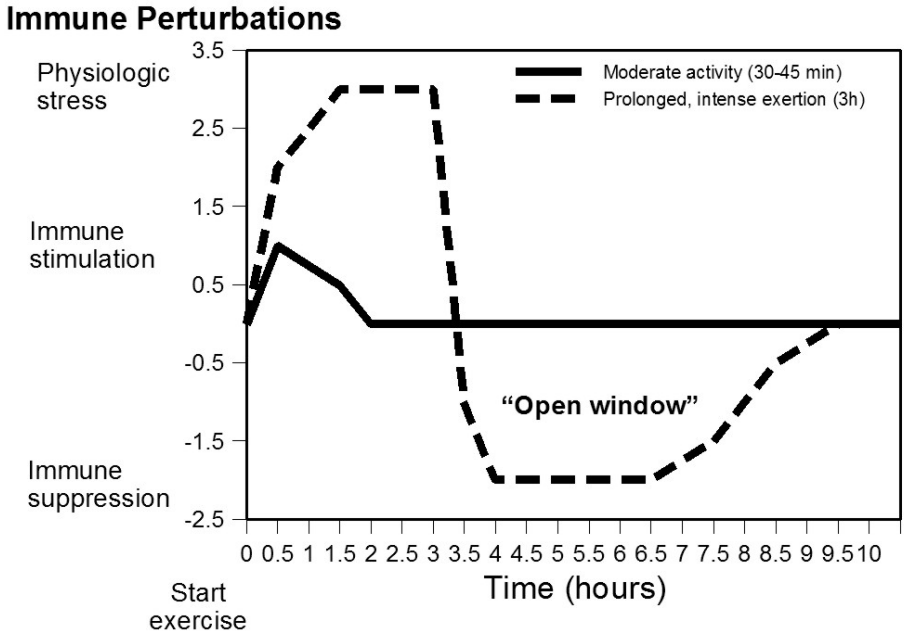

Figure 2.8 "open window theory": while moderate exercise causes mild immune changes, intensive exercise (90 minutes or longer) reduces immune defence levels and increases the possibility for opportunistic upper respiratory tract infections (Nieman and Bishop, 2006).

The maintenance of an ideal level immune response propositions a healthy diet which provides all the nutrients needed for the immune system to function appropriately. As Calder (2002) suggested, the immunity regular level cannot be exceeded by additional micronutrient consumption. On the other hand, deficient consumption of macronutrients and micronutrients may have negative consequences in immune status and susceptibility to pathogens. Kreusch (2003) has proved that protein-energy-malnutrition (PEM) causes a downturn in most aspects of immune function and strongly increases risk of various types of infection. Many supplements have been studied thus far in humans, but only carbohydrate beverages (30-60g.h^{-1}) have emerged as an effective countermeasure to exercise-induced immune suppression, while a fat-rich diet (62% of dietary energy) may be detrimental to immune function compared with a carbohydrate-rich(65% of dietary energy) diet (Gleeson et al., 2004). In agreement to Sari-Sarraf (2006) findings, antioxidants and glutamine have received much attention, but the data results have failed to support

their part in contradicting immune changes after heavy training. Supplements studied thus far in humans include zinc, dietary fat, plant sterols, antioxidants (e.g. vitamins C and E, beta-carotene, N-acetylcysteine, and butylated hydroxyanisole), glutamine, and carbohydrate. However, evidence support only the ingestion of carbohydrate, with the rest of the above supplements doubtfully countermeasure to exercise-induced immune suppression (Gleeson et al., 2004; Nieman, 2001).

The effect of fluid intake on salivary responses to exercise was investigated at rest and after exercise following a 48 h period of fluid and/or energy restriction (Oliver et al., 2007). Restricting fluid intake to ~200 ml/day was associated with a 64% decrease in saliva flow and a parallel increase in s-IgA concentration. Controlling energy intake to around 1200 Kcal/day resulted in a decrease in s-IgA secretion rate after 24 hours. The results were not impaired by performing a treadmill capacity test after 48 h of treatment and were reversed within 6 h of rehydration and refeeding.

In relation to post-exercise decrements of salivary IgA output, studies showed that carbohydrate ingestion has no significant effect. During laboratory based protocols that tried to stimulate the activity patterns and physiological demands of football match play (~ 70% VO_{2max}) have shown that carbohydrate ingestion compared to placebo can negate some of the immune responses associated with placebo ingestion (Bishop et al., 2005).

2.8 Testing and monitoring

Protocols employed to physiologically monitor or test athletes must be objective, reliable and repeatable as well as generally valid (Currell et al., 2008). The objectivity ensures the results remain unchanged with minimal influence from both the tester and the environment. The validity confirms that the selected test, effectively measures the values for which is assessed to measure. The reliability and repeatability of testing protocols used should provide similar results if applied on repeated days. Longitudinal monitoring must ensure those parameters, since repeatability can be affected by many factors. The sum of all factors needs to be

controlled and monitored correctly (e.g. time and day of test, fatigue status, temperature). The tests and measurements chosen by any sports scientist should be in place to effectively monitor health, physiological markers and performance of athletes for the duration of both training sessions and competition. Furthermore the choices of equipment, software and analysis methodology must be validated and assessed for reliability in advance of any research. In the case of a research that demands the use of different equipment to measure or evaluate same values, a pilot test comparing their results can investigate the relationship and subsequently results can be comparable.

In football world, for the physiological evaluation of athletes, sports scientists are balancing between laboratory and field testing protocols. Several researchers have investigated the advantages and disadvantages respectively. A significant correlation occurs between the values of some commonly used field tests and the related laboratory protocols. Nevertheless, the battery of tests used in every team has to coop with time limitations, players' psychology, cost of assessments and much more. Generally a careful choice of tests should be of interest for team fitness coaches in order to evaluate and improve the performance of the players (Currell and Jeukendrup, 2008).

The whole season's physiological training load should be divided in macrocycles (3-4 blocks). A parallel plan should be prepared for nutritional intake. Evaluation of individuals' diet needs might differ, but in general the different macrocycles change in terms of training load, therefore the energy needs to change as such. Following these macrocycles differences, in line with managers' training perspective, a battery of tests and monitoring methods should be assessed.

The corresponding evaluation of nutritional status (including food choices, meal timing etc.) not only within a team, but between different types of teams may provide an insight into potential factors that may influence player performance at different periods of a training season. The nutritional status of individual athletes or groups can be evaluated with several different approaches. Some common methods are food frequency questionnaires, food recall diaries or randomly selected days for estimated diet record. Methodological differences can affect the accuracy of results analysis. Important details if taken into consideration can minimise interpreting

results falsely like neglected adequacy of standard portions, misrepresentation of meals, supplements and individuals' habits that may be not reported by choice (e.g. alcohol, smoking, drugs).

2.9 Rationale for study

Nutritional energy intake among footballers has been well investigated in the literature and sufficient consuming amounts have been indicated for each macronutrient energy intake. However, there are no apparent findings to state recommendations regarding micronutrients ideal amount in footballers' diets. Furthermore, minimal research has been done in determining if and how the nutritional energy intake interacts with seasonal training load energy cost. Is nutritional periodization required in order to balance the energy cost changes across football season?

An additional parameter which needs to be investigated concerns the alterations of essential energy intake and training load energy cost between teams of different leagues, ranking positions and especially for teams from different countries. What is the influence of the above parameters regarding the stress markers and how could these findings practically assist training strategies?

CHAPTER 3

METHODOLOGY

3.0 Methodology

3.1 Pre-experimental Testing.

3.1.1 Study design and participant selection.

Following ethical approval from Life and Medical Sciences Ethics Committee, protocol number: LMS/PG/UH/00023

A total of 14 football players (age 25 ± 1.3 years; height 1.75 ± 2.8 cm; body mass 73.8 ± 1.5 kg) participated in the pilot study. This experimental phase of the study was designed to follow the protocols that would be assessed during main testing periods, but several different parallel methods where used to test and monitor the variances of all three categories. Through this comparison of several methods and with agreement to the literature, all steps were undertaken to ensure that the results were valid. Furthermore, it provided the opportunity to mark and reduce any practical or logistics errors regarding preparations needed prior to testing assessments, collection and analysis of data, safety and time for saliva samples to laboratory.

3.1.2 Pilot study work.

Previous researchers have established the evidence needed for the reliable use of all the candidate methods that are mentioned below, as valid indicators of nutritional intake, training load and stress markers responses in football. Furthermore, the practicality of the methods due to the nature of research was needed to be assessed prior the main experimental study. Where applicable pilot work involved the calibration of equipment to assess and compare the repeatability and accuracy of the data collected via different methods for:

- Nutritional intake comparing two recall diary methods (4 days v 7 days) to assess practicality of evaluating total energy intake and related macronutrient contribution as applied to football (Bates, Lennox and Swan 2010; Irazusta, Gil, Irazusta, Casis and Gil, 2005).

- Training load comparing several methods (Garmin GPS, Qtravel GPS, Lagalacolli GPS, METS and Pedometer) to assess practicality of evaluating total distance covered and Energy cost (Casamichana, Castellano, Calleja-Gonzalez, San Román and Castagna 2013; Aughey, 2011; Larsson, 2003).

- Saliva physiological stress markers (IgA, Cortisol, and Testosterone). Comparison of stress markers results between methods of standard laboratory analysis (ELISA) and Real-time analysis (Gatti and De Palo, 2011).

Pilot study questions

Qp1) is there a difference in nutritional results between acute (4 days) v chronic (7 days) assessment using a recall diary method?

Qp2) which reliable and valid methods could be used (or combined) for the quantification of football weekly training load, with respect to practical ease, field use and logistics limitations.

Qp3) is there a strong relationship between results from real-time saliva analysis kit (Ipro Interactive Ltd) and standard laboratory (ELISA) salivary analysis?

These pilot test questions directed the following **hypotheses:**

Hp1) There will be no difference in key nutritional variables between the two methods.

Hp2) There will be a strong significant relationship between training load monitoring methods; GPS Qstarz-Xtreme (10Hz) and analysis via Lagalacolli software (Florence-Italy), Garmin 1Hz and Compendium of physical activities using MET values (estimated intensity values), in regards to:

- Total distance covered
- Estimated energy cost (kcal)

Hp0a) There will not be a strong relationship between estimated training load from Pedometer and GPS Lagalacolli 10Hz, in regards to total distance covered.

Hp3) There will be a strong relation of stress markers results between real-time and standard laboratory analysis.

3.1.2 Pre experimental Procedures.

During the pilot and the main monitoring weeks:

- Equipment and nature of testing protocols were introduced to the participants.
- The rules that needed to be followed for each step of the research were explained to the participants.
- The time, date and sequence for each step were defined to the participants.
- The order of each test or monitoring procedure during research periods was determined in advance and was the same for every team.

Three main categories of data were collected during pilot testing:

- nutritional energy intake
- quantification of training load
- salivary biomarkers

This was also necessary due to the unique design of this research, since the entire procedure involved teams from two different countries (geographically, weather conditions), but also with different availability of scientific or even basic monitoring equipment. With respect to all the above, some methods were practical needed to be compared with more robust methods.

Assessment of nutritional intake

Food recall diaries were given out to all participants and collected data were then analysed byDiet Professional software, ScienceTech Diet 200A-VIII Professional (Greece). Results of 4-day and 7-day diaries were compared by dependant sample ttest using SPSS (version 22, SPSS, inc., Chicago, Illinois). For all measured variables there were no significant differences between a 4-day and a 7-day diary.

Therefore it was decided that the most appropriate method would be the 4-day food recall diary.

The nutritional energy intake method needed to be valid and reliable but at the same time logistically simple, with respect to the reluctant that might have occurred on behalf of the footballers. Minimum time to spend and the simplicity to record the meals in accurate quantities were essential for footballers' positive attitude towards the study. For this reason, a further effort or time consuming method even with higher accuracy than food recall diary could not be assessed in groups of footballers. Participants were first given the food diaries, guided verbally on how to complete them and then asked to follow the written example attached on the diary (Refer to Appendix 9.8.2). Additionally, some usual mistakes on reporting foods and liquids were indicated (e.g. espresso coffee instead of double shot espresso coffee with one sugar) in order to avoid if possible.

Assessment of training load

Eventually, the assessment method for estimating training load was the more difficult to choose, due to the complexity of football training sessions. An ideal monitoring assessment would include a valid GPS method in parallel to Heart Rate real-time system. However, the GPS system cost for purchase or rent was quite high and moreover it could not be used for indoor training sessions in field or in the gym. Therefore, along with the validity and reliability, the practicality of all candidate methods was assessed in parallel, through a reproduction block of the expected seasonal monitored blocks. For example, 3 field training sessions were organised with 10 participants all wearing: (i) a vest with GPS Qstarz-Xtreme monitoring unit on the back, (ii) Garmin watch on the wrist, (iii) HR belt around the chest and (iv) Pedometer on the shoe laces. The incoming data from the above equipment were used for estimating training load through the following methods:

- Lagalacolli GPS analysis (Florence-Italy) software
- Garmin (USA) GPS analysis
- Compendium of physical activities using MET values (estimated intensity values).
- Pedometer

Assessment of salivary stress markers

The Real-time method was compared to ELISA method for practical and valid use. A rather new technology was attractive to assess in the field (on-site) and therefore both collection and analysis of samples would be much more rapidly accomplished. Even though the costs for such assessment were considerably high, the possible advantages imposed to the inclusion of saliva stress markers methods into the pilot test to determine which one would be best to assess in main experimental period.

Saliva was collected by a device suitable for both uses with real-time (on-site) and ELISA (standard laboratory test) immunoassay sandwich type tests for immunoglobulins and hormones. The collection had to be fast, collected volume known and analyte recovery adequate. The device consists of a synthetic polymer-based swab material attached to a volume adequacy indicator stem and a dropper bottle with extraction buffer. The indicator stem changes colour upon the collection of about 0.5mL of saliva. The swab is placed in the dropper bottle containing known volume of extraction buffer. The bottle is shaken for 30-60 seconds and the sample is ready for on-site testing or to be sent for laboratory testing. The device was used to collect saliva at pre-experimental testing from 14 volunteers recording both collection time and volume. Enzyme immunoassays were used to determine the recovery of IgA and cortisol from saliva collected. The average collection time (as indicated by the colour change) was 27.7 seconds (STDEV: 8.47, range: 19.3-52 seconds) and collection volume was 0.55mL (STDEV: 0.06, range: 0.42-0.64 mL). Analyte recovery following 1 minute shaking was over 85%.

Table. 3.1.1a Ipro's Salivary IgA (sIgA) Enzyme Immunoassay reliability data.

Calibrator conc. [ug.mL $^{-1}$] of sIgA in the sample	Average Optical density (O.D.450nm) (n = 6)	Standard of Deviation	% Coefficient of Variation
0.0	0.018	0.00145	8.07
18.8	0.138	0.004	2.58
37.5	0.256	0.005	2.15
75.0	0.406	0.01	2.57
150.0	0.701	0.04	5.77
300.0	0.991	0.033	3.37
450.0	1.180	0.033	2.76
600.0	1.382	0.023	1.68

Samples giving values below18.75 ug.mL $^{-1}$ are classed as minimum or below quantitation limit (LOQ) of the assay. Samples giving values above 600ug.mL $^{-1}$ are classed as maximum and maybe diluted and re-assayed in order to obtain a value falling within the calibration curve.

Table. 3.1.1b Ipro's salivary cortisol EIA reliability data.

Calibrator conc. [ng.mL $^{-1}$] of cortisol in the sample	Average Optical density (O.D.450nm) (n = 6)	Standard of Deviation	% Coefficient of Variation
0.0	2.578	0.082	3.19
0.5	2.274	0.039	1.70
1.0	2.093	0.051	2.45
2.0	1.832	0.056	3.07
4.0	1.445	0.063	4.34
8.0	1.069	0.084	7.85
16.0	0.785	0.155	19.69
32.0	0.441	0.066	14.87

Samples with concentration below 0.5 ng.mL^{-1} are considered as minimum (below quantitation limit (LOQ) of the assay).

Key findings and decisions from pilot work

For nutritional energy intake, pilot testing found good relationship between the 4-day food recall diary and 7-day food recall diary (Refer to table 3.1.1d). Therefore, it was

decided to assess the specific method for establish nutritional intake data. Analysis of data was accomplished via Dietplan6 software.

The GPS method was attractive because regardless of the extra costs it was simple to use and the validity was well established in the literature. Hence it was decided to use GPS units of Qstarz-Xtreme (10Hz) for monitoring outdoor training sessions and choose in parallel a method which could estimate training load under any circumstances (Refer to table 3.1.1c). With mind that assessment of the selected final method should be valid, practical and reliable, 2011 Compendium of physical activities codes and MET values was preferred. In general, recommended estimation of the caloric cost of physical activity with the equation, kilocalories= MET x weight in kilograms x duration in hours.

Despite the strong relationship (Figures 3.1a and 3.1b) between standard laboratory analysis method (ELISA) and real-time analysis (LFD) on pilot test samples, Laboratory method was eventually preferred for the reason that Testosterone hormone could not be analysed at that time via real-kit equipment and T/C ratio was important for the justification of research hypothesis. Samples were therefore sent to the laboratory of Iprointeractive Ltd (London) for analysis.

Table 3.1.1c Comparison of methods estimating training load. Strong relationship for MET v Lagalacolli methods for estimated energy cost as calories (p = 0.217)

Method	Average Total distance covered (km)	Average Calories
Garmin	8.24	744
Q-travel	8.08	716
Lagalacolli	7.98	740
MET		710
Pedometer	6.09	472
	km (p =)	**kcal (p =)**
Garmin v Q	0.003	0.005
Garmin v Lagalacolli	0.009	0.175
Q v Lagalacolli	0.035	0.011
MET v Lagalacolli		0.217
Pedometer v Lagalacolli	0.003	0.010

Table 3.1.1d Comparison of 4-day v 7-day food recall diary at pre-experimental testing block

Nutrient	4-day	7-day	*p* =
Calories (kcal)	1728.91	1840.71	0.20
Protein (gr)	108.55	108.63	0.49
Fat (gr)	73.20	70.05	0.37
Carbs (gr)	180.61	193.95	0.29
Calcium (mg)	963.26	887.24	0.17
VitA (mcg)	554.89	809.71	0.09
Vit B12 (mcg)	5.31	5.05	0.29
B6 (mg)	2.70	2.78	0.25
C (mg)	122.40	146.58	0.18
D (mg)	173.33	197.44	0.23
E (mg)	8.71	8.72	0.50
K (Ug)	300.81	229.57	0.08
Thiamine (mg)	5.58	8.82	0.16
Magnesium (mg)	369.46	4014.80	0.18
Niacin (mg)	32.71	26.45	0.10
Riboflavin (mg)	29.09	2.01	0.09
Selenium (mcg)	115.92	125.03	0.19
Fe (mg)	15.53	14.48	0.21
Folic Acid (mcg)	416.05	430.02	0.46
Fiber (g)	232.56	15.82	0.17
Phosphorus (mg)	1245.37	1370.90	0.31
Zinc (mg)	12.66	11.56	0.06

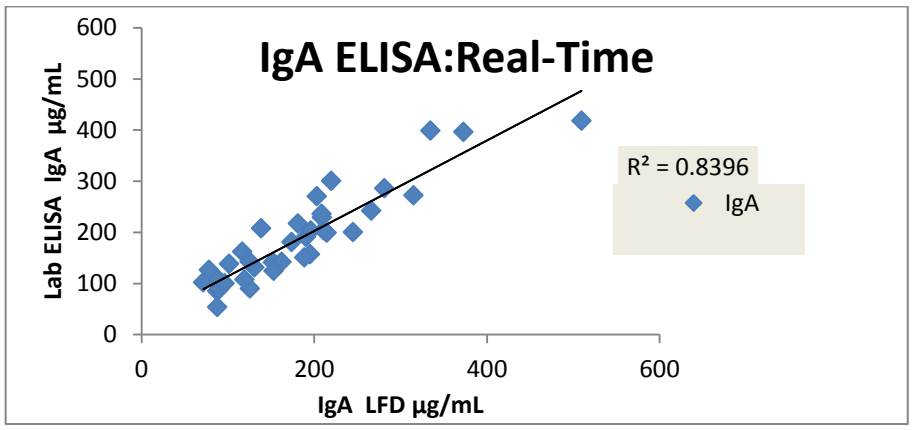

Figure 3.1a Relationship between salivary immunoglobulin IgA determinations from ELISA (standard laboratory test) & IPRO LFD (real-time analysis kit)

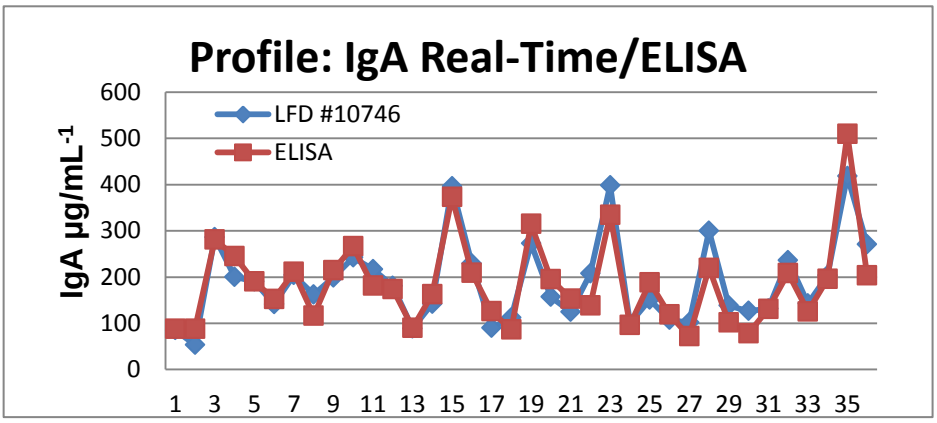

Figure 3.1b Relationship between salivary immunoglobulin IgA determinations from ELISA (standard laboratory test) & IPRO LFD (real-time analysis kit)

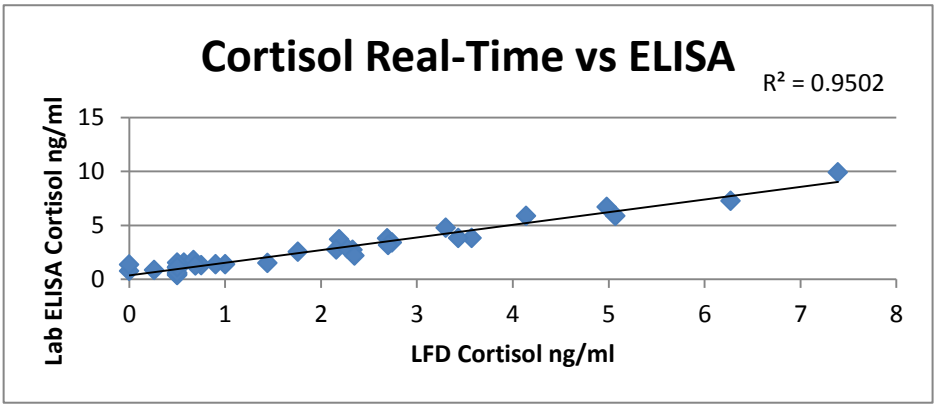

Figure 3.1c Relationship between Cortisol determinations from ELISA (standard laboratory test) & IPRO LFD (real-time analysis kit)

3.2 Main experimental work.

3.2.1 Participants/ Main Research design

Participants (n=45; age 24.47 ± 6.07 years; height 1.75 ± 0.08 m; mass 74.86 ± 9.57 kg) were male footballers from four different teams; one UK based professional team and two Cyprus based teams; semi-professional and recreational. Amateur participants volunteered to take part in the study and the professional participants were recruited with the assistance of their fitness coach in the team. Human participation ethical approval was granted by the ECDA for Hertfordshire University, with informed written consent obtained. Following pilot work, data were collected on participants over three periods of 14 days each, during habitual training periods over a typical season. Those were pre-season (July/ August), mid-season (November/ December) and end-season (March/ April). Testing was carried out both in Cyprus and UK at the selected phases, always with the same sequence. Hence, data were collected initially in the UK over a 7-day period and then repeated in Cyprus for the other two teams. Participants were professionals, semi-professionals and amateurs.

Table 3.2a Testing blocks Schedule for all three groups during football season.

Groups	Pilot-test	Pre-season block	Mid-season block	End-season block
UKpro		July 2013	November 2013	March 2014
CYpro		August 2013	December 2013	April 2014
CYrec	April 2013	August 2013	December 2013	April 2014

3.2.3 Procedures

Prior to the determination of monitoring dates (blocks for pre-season, mid-season, end-season), a number of steps were undertaken to ensure that the results were valid and reliable:

- Candidate teams were informed regarding all the protocols, weekly procedures and stipulations for each testing parameter.
- Due to the important role of each teams' fitness coaches, they were fully briefed on the nature of pilot testing and main testing procedures to ensure effective testing delivery.

The procedures were explained to the participants and all the necessary consent forms were signed before the first day of testing. All experimental testing and monitoring took place in each team's training ground. While collection of data was standardised for all the research procedures regarding the timing of collection, environmental conditions were noted during each session of the monitoring procedures. Upon arrival at the training centre of their team, anthropometric data was collected; participants' height was measured using a stadiometer (Seca mod 220, Germany) followed by body mass and body fat percentage recordings using a multi-frequency bioelectrical impedance analysis (BIA) with eight-point tactile electrodes (MF-BIA8; InBody 720, Biospace Ltd, Korea).

Nutritional intake methodology and analysis

Recall diaries were assessed to each participant before the first training session of the monitored week in order to record their food and activities (other than team's training) with standardised instructions for completing it, along with written examples attached on the diary. For example, the timing of each meals was requested, the consumed food name and if possible a detailed description with amount (g), cooking method and any detail that might assisted the accuracy of the report. Moreover, all consumed liquids had to be described in detail regarding the type, amount (ml), percentage of alcohol and added ingredients e.g. sugar. If supplements and medicines were taken, participants were requested to report them by brands and dosage. Due to the everyday personal contact with the participants, additional guidance was given if needed for completion of their diary, which reduced nutritional

intake miss-reporting (Refer to Appendix 9.8.2). Participants were requested not to change their current habitual eating patterns.

The food recall diaries analysis was carried out using the Diet Professional software, ScienceTech Diet 200A-VIII Professional (Greece). Average daily calorific intake and intake of macronutrients; carbohydrate, protein and total fat were evaluated. Average daily micronutrients intake was also evaluated and data were noted as percentage of RDA (Recommended Daily Allowance percentage).

Training load quantification and analysis

Participants training load was determined from pilot work analysis, where training sessions were recorded indirectly in terms of duration (minutes) and energy cost using the 2011 Compendium of physical activities codes and MET values (Ainsworth, Haskell, Herrmann, Meckes, Bassett Jr, Tudor-Locke, Greer, Vezina, Whitt-Glover and Leon, 2011). The intensity units as standard MET with the Resting Metabolic Rate (RMR) represented as 3.5 ml.kg.min. Most common specific activities used to describe the participants' training sessions included: resistance training (3.5 - 6.0 METS), running (6.0 - 23.0 METS) and soccer specific (7.0 - 10.0 METS).

In parallel, during outdoor training sessions direct training load quantification was assessed via Qstarz Xtreme 10-Hz GPS units. Participants were wearing specific t-shirts with the GPS units placed in the top back pocket. All units were turned on before the training sessions started and turned off immediately after the end of training. Using Lagalacolli software (Spinitalia, Italy), the data from GPS units were analysed individually for each participant. Total kilocalories were determined with respect to body mass. Among the numerous values given by the software analysis, total distance (kilometers) and sessions' parts duration (seconds) were noted for double recording the training load.

Table 3.2b Standard weekly training program requested to be followed by all groups and schedule for monitoring methods assessment. All the investigated parameters were assessed in relation to hard day training (Day 3).

Standard Week	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Training Intensity	Cool down	Off	Medium to hard	Medium to hard	Medium	Easy, steady pieces	Game
Training sessions	1	0	1	2	1	1	
Food recall diaries	Assessment of diaries	√	√	√	√	Return of diaries	
Training load	METS, GPS		METS, GPS	METS, GPS	METS	METS	
Saliva collection			1 at rest before training, 1 after training	1 at rest before training			

Saliva sampling procedure and stress markers analysis

Three different saliva samples were received from each participant during each monitored week. The sample was taken at rest before the second training session of the week (15-45 minutes prior training session). The second sample was taken immediately after the training session, before participants exited the pitch (< 15 minutes). The third sample was collected at rest before the next day's training session (15-45 minutes prior training session). Participants placed an Oral Fluid

Collector (IPRO OFC, Ipro Interactive, Oxfordshire, UK) consisting of a synthetic polymer based material on a polypropylene tube, on top of their tongue to collect saliva (Strazdins, Meyerkort, Brent, D'Souza, Broom and Kyd, 2005). The OFC has a volume adequacy indicator, giving a clear colour change when 0.5 mL (± 20%) saliva was collected. Analyte recovery from the OFC has previously been demonstrated to be in excess of 85% within 1 minute of gentle shaking (Jehanli et al., 2011).

The OFC swab was inserted immediately into a container with an extraction buffer containing sodium phosphate, salts, detergents and preservatives designed to prevent growth of microorganisms and facilitate extraction of proteins and small mass molecular analytes from the swab.

Secretory immunoglobulin A (sIgA), salivary cortisol and salivary testosterone concentrations were all determined, in duplicate, from the same sample using enzyme immunoassay (EIA) test kits provided by (Ipro Interactive Ltd., Oxfordshire, England). The EIA kits presented assay color intensity proportional to the concentration of each specific analyte and a dose-response curve, using standard solutions of each analyte, enabled determination of sample concentration of each analyte. The assay ranges were: sIgA 18.75-600 µg/mL; cortisol 0.25-32.0 ng/ml^{-1}: and testosterone 10-500 pg/mL. The intra-assay coefficient of variation (CV) was: sIgA < 5.77%; cortisol < 7.85%: and testosterone <7.94%. The inter-assay CV was: sIgA < 12.52%; cortisol <13.10%; and testosterone < 9.4%.

The principle of ELISA as described by Nomura (2012) is based on the antigen-antibody reaction which is for capturing a target substance and the enzyme reaction which is for detecting the mass of a target substance via optical density of reaction produced color. The brief description of ELISA (competitive method) is as follows: (1) Thaw saliva samples kept in a biological freezer by moving them into a biological refrigerator (4 Celsius). (2) Centrifuge each saliva samples for 10 minutes at 1500 rpm to precipitate mucins or other solid contents. (3) Add each saliva sample (or known samples for references) into antibody-coated 96-well micro-plate. (4) Add a constant amount of “enzyme conjugate” which is the target biomarker (antigen) combined with horseradish peroxidase (HRP) into the micro-plate and incubate for an hour. In this step, antigen-antibody reaction is occurred competitively between original target in the saliva sample and that in the enzyme conjugate. (5) Wash the

micro-plate to flush unbind target. (6) Add tetramethylbenzidine (TMB) solution to induce enzyme reaction with enzyme conjugate which is captured by antigen coated on the bottom of each well of the micro-plate in the step (4). The amount of the bind enzyme conjugate, which is there as a result of competitive reaction process, can be detected as the strength of optimal color (450nm) caused by enzyme reaction. Therefore this optimal density is inversely proportional to the concentration of target containing in the original saliva sample. (7) Finally, the target concentration in each sample is determined by referencing the optimal density of the reference samples. All analysis procedures take roughly about 3 to 5 hours for one microplate.

The regular secretion of all three biomarkers which are investigated, have a 24-hourly change (diurnal): the highest level is in the morning and gradually decreases afterwards to the lowest level in the night time. Therefore saliva sampling should be conducted depending on the objective of a study. Also as recommended, sample collection was assessed in the morning both for reasons of repetitive sampling by a distinct time point of a day (around 09:00-11:00am) and because the groups participating in the study were training around those hours.

Physical performance testing protocol

All participants followed the same testing sequence of the physical performance tests. Although all tests were conducted on grass surfaces in each team's training ground, the participants wore their soccer or running shoes depending on their choice. Due to the tight program of professional teams, physiological testing protocol was designed to allow participants to be tested during availability of time, with the maximum of 2 weeks ± from the assessment of the main monitoring week.

Testing sequence summary:

(*notice that none of the data below was included in the final results)

Standarised warm-up 5 minutes at 7.8km / hour. 3 minutes individual self-selected stretching.

Jump tests (3 x Counter movement jump no arms + 3 x Counter movement jump with arms). 5 minutes rest.

Intermittent multi stage running test (Yo-Yo IRTL1).

Jump testing protocol involved an additional preparation which involved 10 squats and five two foot counter movement jumps. The height of all jumps was recorded using a Globus Jumpmat (Vareze, Italy).

Counter movement jump without arms:

(*notice that none of the data was included in the final results)

The participants were instructed to stand with their feet shoulder width apart on the jump mat and keep their hands on their hips. Then the participants were instructed to perform a counter movement jump. With instructions to jump vertically as high as possible, the jump was invalid if the hands didn't remain on the hips or if the landing wasn't centred on the jump mat. All participants completed three valid jumps and the highest jump (cm) was then used for the purpose of data analysis.

Counter movement jump with arms:

(*notice that none of the data was included in the final results)

The participants were instructed to stand with their feet shoulder width apart on the jump mat. Then the participants were instructed to perform a counter movement jump using their arms to assist them during the jump. With instructions to jump vertically as high as possible, the jump was invalid if the landing wasn't centred on the jump mat. All participants completed three valid jumps and the highest jump (cm) was then used for the purpose of data analysis.

Yo-Yo IRTL1:

(*notice that none of the data was included in the final results)

Prior to the test, all participants were asked to give their maximum effort and attempt to reach the highest level possible. Individuals were withdrawn from the test if they were no longer complying with test regulations. If they failed to reach the line before the audio signal, individuals were given two verbal warnings. In case of a third failure, automatically individuals were withdrawn. Frequently, individuals withdrew voluntarily from the test. For the purpose of data analysis, the level and number of shuttles into

the level at which each subject withdrew from the test was recorded. An estimation of maximum oxygen uptake (VO_{2max}) was then obtained from a table of predicted VO_{2max} values.

***** During the planning of the present research, a physical performance testing protocol was considered as an important parameter for investigation with the aim of having a conclusive profile for all participants that would include their fitness level. Unfortunately, after accomplishing the research protocol in full for the two teams at pre-season testing block and by the time of assessing the protocol to the third team, the coach decided that due to important friendly games ahead, the players should not perform any kind of testing. Therefore, it was not possible to retain the physiological testing as part of the main research protocol during the next testing blocks.

Statistical analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS Inc,v19, Chicago, Illinois, USA). Data was assesed for normality using Shapiro-Wilks test for normal distribution and Levenes test for equal variances as appropriate. Data was then assessed using a two factor mixed design analysis of variance (ANOVA) for time and group interactions. Where pertinent, a one way ANOVA with Bonferroni post hoc adjustments was utilised to assess within or between group effects. An alpha level of 0.05 was employed for statistical significance. Data are reported as means ± SE.

CHAPTER 4

RESULTS

4.0 Results

4.1 Assessment of dietary intake from recall diary history

4.1.1 Assessment of total energy intake and macronutrients

Total energy intake

Average daily total energy intake (TEI)

Mean team daily intake per testing block data is shown as total energy intake (TEI) in Figure 4.1 below. From a two way mixed design anova assessment, where sphericity was not assumed, Greenhouse-Geisser test was used to assess for significant interactions. For TEI, a significant group v time interaction effect was found ($F_{2.43, 18.22}$=5.42; p = 0.011). Post hoc assessment found significant interaction within group 2 (CYsem) only. In this group, TEI increased from 1502.20 ± 143.95 kcal.d^{-1} pre-season to 1718.60 ± 142.87 kcal.d^{-1} at the mid-season point (p = 0.34). TEI for CYsem was also significantly different at end season (1789.80 ± 116.17 kcal.d^{-1}) compared to pre-season (p = 0.018) but not mid-season (p > 0.05).

Conversely, no significant effect was found between groups at all time-points. At pre-season for UKpro v CYsem (p = 1.00), UKpro v CYrec (p = 0.663) and CYsem v CYrec (p = 0.310). Likewise, at mid-season for UKpro v CYsem (p = 0.326), UKpro v CYrec (p = 0.138) and CYsem v CYrec (p = 1.00). Finally, at end-season FOR UKpro v CYsem (p = 1.00), UKpro v CYrec (p = 0.407) and CYsem v CYrec (p = 1.00).

Data for group 1 (UKpro) increased in a non-significant manner from 1772.67 ± 116.04 kcal.d^{-1} pre-season, to 1928.38 ± 112.83 kcal.d^{-1} mid-season and similarly to 1874.00 ± 64.34 kcal.d^{-1} at the end-season point (p >0.05). Interestingly, data for group 3 (CYrec) decreased from T1 (2006.50 ± 129.14 kcal.d^{-1}) pre-season to T2

(1554.40 ± 140.96 kcal.d^{-1}) mid-season and T3 (1694.00 ± 96.33 kcal.d^{-1}) end season, although this was not significant (p>0.05).

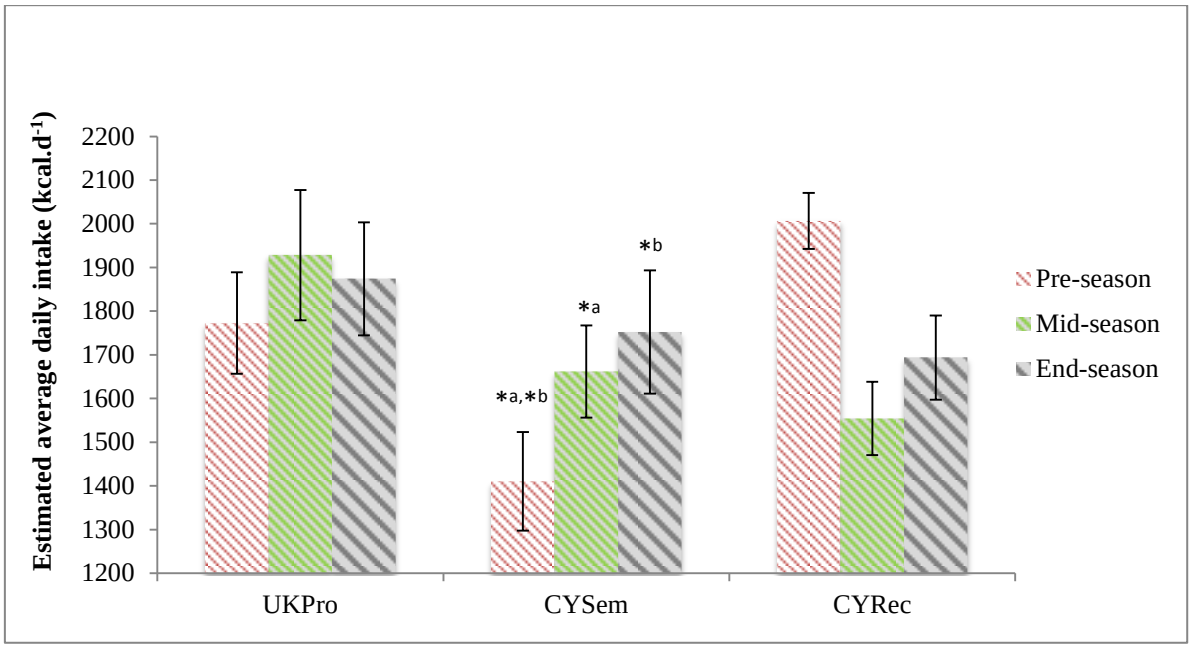

Figure 4.1a Average daily total energy intakes (TEI) for each cohort at each time point.

*^{a} = significant difference for CYsem increase from 1502.20 ± 143.95 kcal.d^{-1} pre-season to 1718.60 ± 142.87 kcal.d^{-1} at the mid-season point (p = 0.34).

*^{b} = significantly different at end season (1789.80 ± 116.17 kcal.d^{-1}) compared to pre-season (p = 0.018).

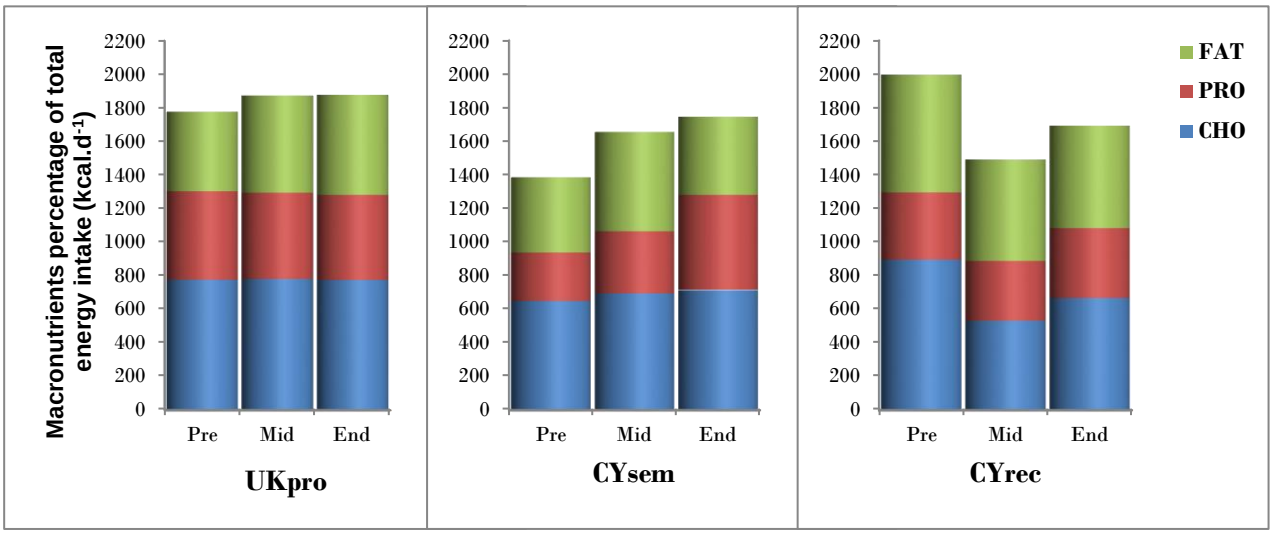

Figure 4.1b Macronutrients energy intake contribution in total energy intake at each testing block in the season

Macronutrients

Total carbohydrate intake

Average daily carbohydrate energy intake (CI)

Mean team daily carbohydrate energy intake per testing block data is shown in Figure 4.2 below as carbohydrate intake (CI). Carbohydrate intake was generally different between groups during the three testing (T) time-points. Two way ANOVA assessment, where sphericity was not assumed, Greenhouse-Geisser test showed a significant time interaction ($F_{1.44,\ 54.73}$ =9.08; p = 0.001) and significant group v time ($F_{2.88,\ 54.73}$ =5.86; p = 0.002). Assessment of Post-hoc found significant interaction between groups at pre-season ($F_{2,\ 40}$ =4.15; p = 0.024). Additionally, Post-Hoc Bonferroni test revealed significant difference at pre-season (T1) between CYsem 656.67 ± 35.05 kcal.d^{-1} and CYrec 902.67 ± 69.25 kcal.d^{-1}, (p = 0.031).

UKpro had high and near equal mean values at all time-points: pre-season T1 (784.43 ± 56.98 kcal.d^{-1}), mid-season T2 (789.00 ± 55.91 kcal.d^{-1}) and end-season T3 (782.00 ± 51.01 kcal.d^{-1}). Nevertheless, tests of within-subjects effects showed significant interaction for UKpro ($F_{1.14,\ 15.90}$ =6.71; p = 0.017). Significant within-subjects effects were also shown for CYrec ($F_{2,\ 28}$ =11.71; p = 0.001) which interestingly, at pre-season 902.67 ± 69.25 kcal.d^{-1} presented the highest value than any group at all time-points. On the contrary, at mid-season CYrec had the lowest value than any group at all time-points 538.67 ± 105.37 kcal.d^{-1} and in the end-season showed an upturn 647.67 ± 55.83 kcal.d^{-1}. CYsem data increased in a non-significant manner from 656.67 ± 35.05 kcal.d^{-1} pre-season to 702.86 ± 71.58 kcal.d^{-1} at the mid-season and moreover to 722.29 ± 37.18 kcal.d^{-1} at end-season point.

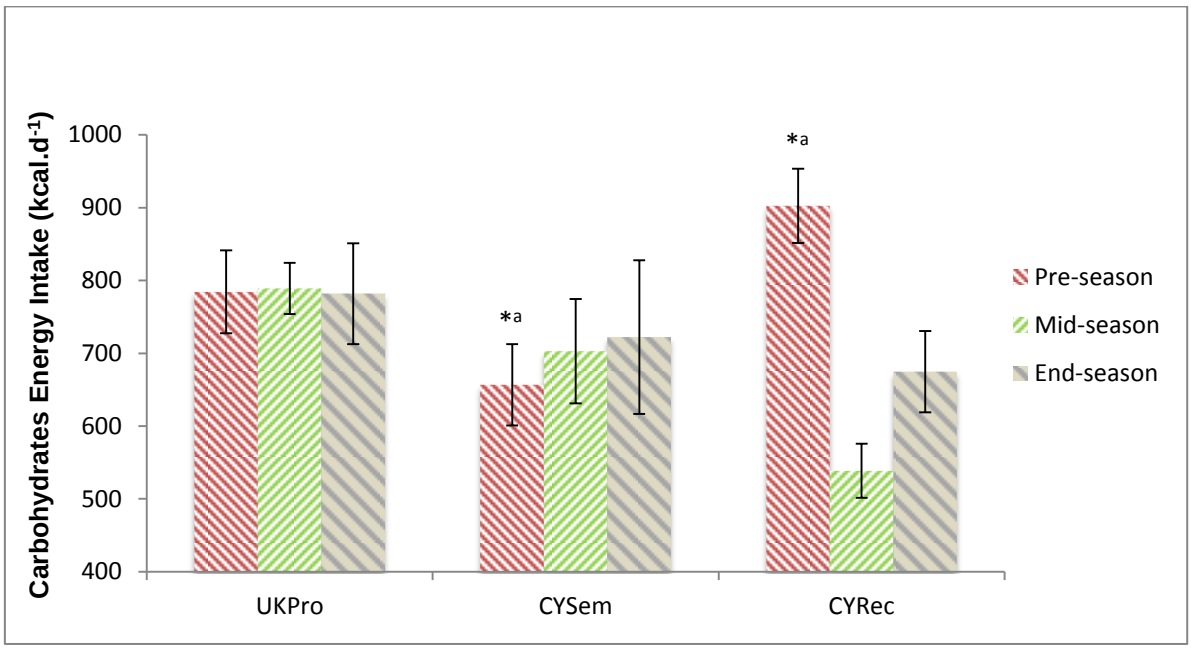

Figure 4.2 Average daily carbohydrates energy intake (CI) for each cohort at each time point.

*[a]= significant difference at pre-season between CYsem 656.67 ± 35.05 kcal.d^{-1} and CYrec 902.67 ± 69.25 kcal.d^{-1}, (p = 0.031)

*[b]= Significant within-subjects effects for CYrec, (p = 0.001).

Total Fat intake

Average daily fat energy intake (FI)

an team daily fat energy intake per testing block data is shown data in Figure 4.3 below as fat Intake (FI). Data revealed that groups did not have similar development during the three time (T) point tests. Two way ANOVA assessment, where sphericity was assumed, showed no significant interactions for time ($F_{2, 32}$=0.766; p = 0.473) or group v time ($F_{4, 32}$=2.43; p = 0.068).

It was found that CYrec group had higher fat intake in comparison to the other two groups. At pre-season (T1) mean teams data were different between CYrec 697.50 ± 70.71 kcal.d^{-1}, UKpro 471.45 ± 38.88 kcal.d^{-1} and CYsem 445.50 ± 102.64 kcal.d^{-1}. During the next testing time-points, UKpro showed an increasing trend from 576 ±

34.86 kcal.d^{-1} mid-season to end-season 592.88 ± 27.87 kcal.d^{-1}. Higher values were shown also at mid-season for CYsem (583.71 ± 60.52 kcal.d^{-1}), followed by a decrease at end-season 462.86 ± 34.18 kcal.d^{-1}, but no statistical significance was found (p > 0.05). Finally, CYrec had much lower values than baseline at 601.50 ± 80.21 kcal.d^{-1} mid-season and 607 ± 43.63 kcal.d^{-1} end-season.

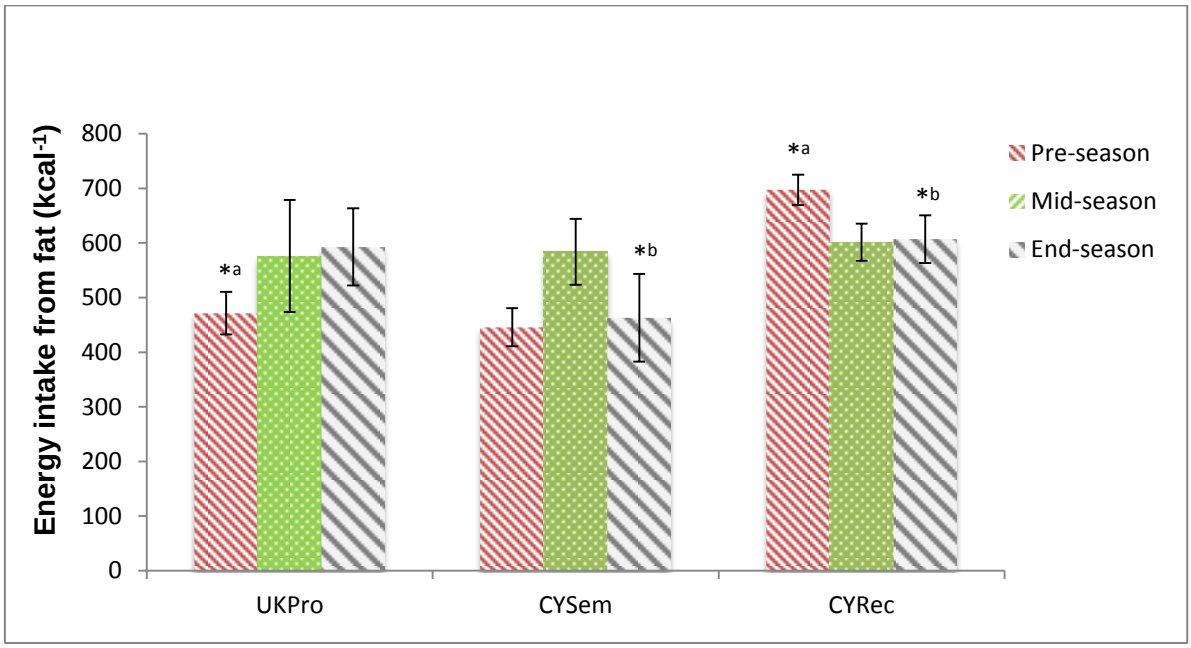

Figure 4.3 Average daily fat energy intake (FI) for each cohort at each time point.

*^{a} = significant interaction at pre-season for UKpro group v CYrec, (p = 0.040).

*^{b} = significant difference at end-season for CYsem v CYrec, (p = 0.037).

Total Protein intake

Average daily protein energy intake (PI)

Mean team daily protein energy intake per testing block data is shown in Figure 4.4 below as protein intake (PI). Results for protein intake (kcal) found that protein intake was different between cohorts. This was demonstrated via significant interaction effect for time ($F_{2, 32}$=5.72; p = 0.008) and group v time ($F_{4, 32}$=4.32; p = 0.007).

Pairwise comparisons revealed significant time interactions between Pre-season (T1) v Mid-season (T2), (p = 0.923). Pre-season (T1) v End-season (T3), (p = 0.023). Lastly, End-season (T3) v Mid-season (T2), (p = 0.005).

UKpro in general had high average values through all three testing time-points and presented near equal mean values at all testing time-points; baseline T1 (524.00 ± 44.82 kcal.d^{-1}), T2 (511.00 ± 36.05 kcal.d^{-1}) and T3 (505.00 ± 11.00 kcal.d^{-1}). CYsem had an increasing trend during testing time-points with low values at T1 (289.33 ± 43.14 kcal.d^{-1}), increased at T2 (368.00 ± 27.09 kcal.d^{-1}) and presented the highest value than any group at all time-points during T3 (565.14 ± 52.69 kcal.d^{-1}). CYrec values were at T1 (399.00 ± 17.81 kcal.d^{-1}), at T2 (357.33 ± 55.82 kcal.d^{-1}) and showed the highest values at T3 (414.67 ± 35.02 kcal.d^{-1}).

Assessment of Post-hoc tests found significant interaction between groups at T1 for UKpro (524.00 ± 44.82 kcal.d^{-1}) v CYsem (289.33 ± 43.14 kcal.d^{-1}) (p = 0.001) and UKpro v CYrec (399.00 ± 17.81 kcal.d^{-1}), (p = 0.041). Also at T2 for UKpro (511.00 ± 36.05 kcal.d^{-1}) v CYsem (368.00 ± 27.09 kcal.d^{-1}), (p = 0.049) and UKpro v CYrec (357.33 ± 55.82 kcal.d^{-1}), (p = 0.042). Finally, at T3 for CYsem (565.14 ± 52.69 kcal.d^{-1}) v CYrec (414.67 ± 35.02 kcal.d^{-1}), (p = 0.019)

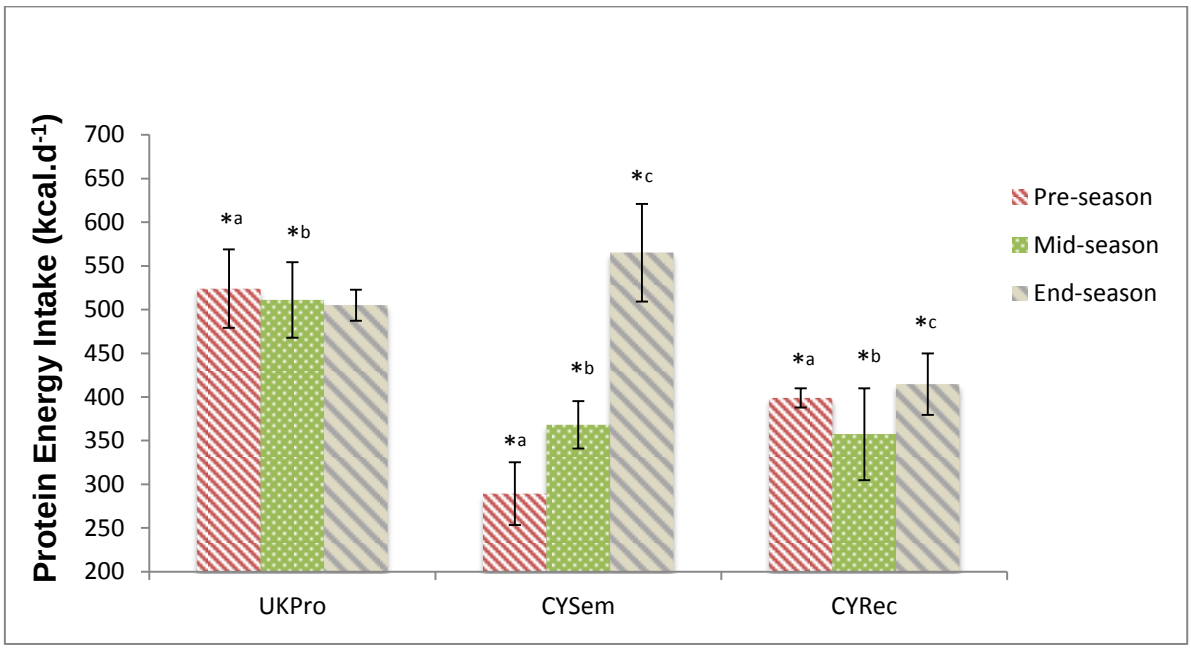

Figure 4.4 Average daily protein energy intake (PI) for each cohort per testing block.

*[a] = significant interaction at pre-season between UKpro (524.00 ± 44.82 kcal.d^{-1}) and CYsem (289.33 ± 43.14 kcal.d^{-1}), (p = 0.001) and UKpro v CYrec (399.00 ± 17.81 kcal.d^{-1}), (p = 0.041).

*[b] = significant difference at mid-season for UKpro (511.00 ± 36.05 kcal.d^{-1}) v CYsem (368.00 ± 27.09 kcal.d^{-1}), (p = 0.049) and UKpro v CYrec (357.33 ± 55.82 kcal.d^{-1}), (p = 0.042).

*[c] = significant difference at end-season for CYsem (565.14 ± 52.69 kcal.d^{-1}) v CYrec (414.67 ± 35.02 kcal.d^{-1}), (p = 0.019)

Total Fibre intake

Average daily fibre intake (FI)

Mean results for daily fibre intake per testing block data is shown in Figure 4.5 below as fibre intake (FI). Results found that fibre intake UKpro had much higher values at all three testing time-points in comparison to the other groups. Repeated measures analysis did not find any significant interactions for time ($F_{1.22,\ 18.29}$ =1.87, p = 0.189), or for time v group ($F_{2.44,\ 18.29}$ =0.763, p = 0.505). It was found that UKpro had the higher values at both pre-season 20.80 ± 2.39 $g.d^{-1}$ and mid-season 20.87 ± 2.32 $g.d^{-1}$, followed by a decrease at end-season 18.95 ± 1.84 $g.d^{-1}$. CYsem similarly retained equivalent values between pre-season 14.02 ± 3.18 $g.d^{-1}$ and mid-season 14.05 ± 2.84 $g.d^{-1}$, followed by the highest group values 17.13 ± 1.91 $g.d^{-1}$ at end-season. Finally, CYrec from starting point 16.55 ± 2.86 $g.d^{-1}$ pre-season was visibly decreased 13.13 ± 1.89 $g.d^{-1}$ to mid-season and showed a slight rise 14.85 ± 1.72 $g.d^{-1}$ at end-season. Pairwise comparisons for groups acknowledged that there were differences but not significant (p > 0.05) for UKpro v CYsem, p = 0.081. UKpro v CYrec, p = 0.99. CYsem v CYrec, p = 0.920

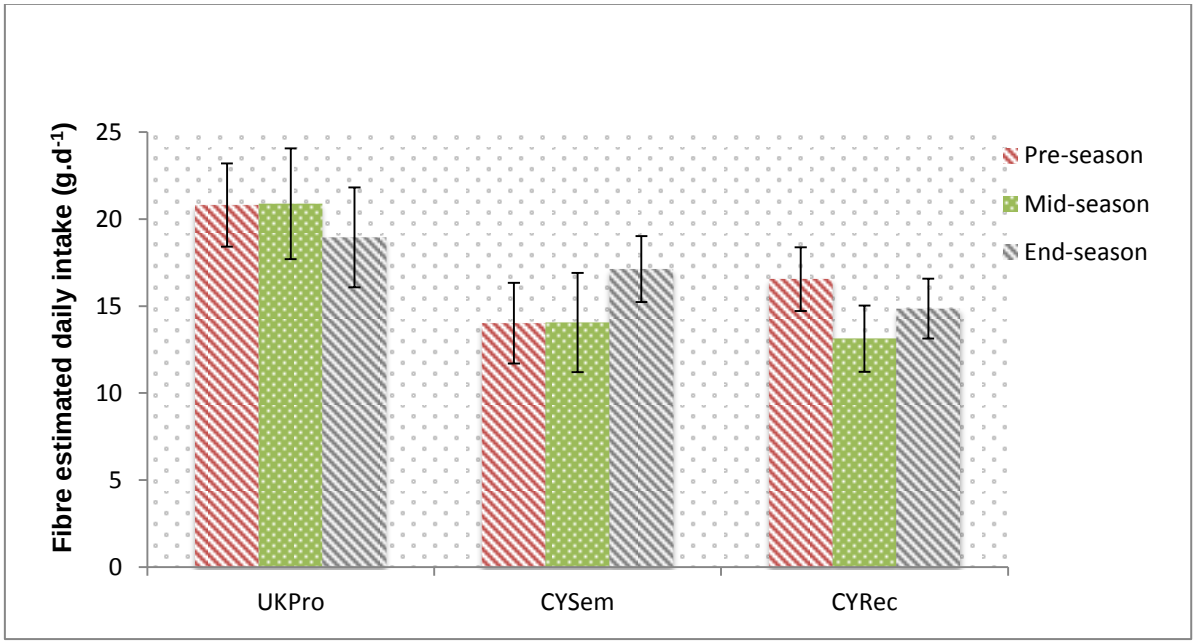

Figure 4.5 Mean results for average daily fibre intake for each cohort per testing block, showing higher and near to equal values during all three testing- blocks for UKpro group.

4.1.2 Micronutrients

Average estimated daily intake of selected macronutrients

Vitamin C

Average daily Vitamin C intake

Mean results for daily vitamin C intake per testing block data is shown in Figure 4.6 below as vitamin C Intake (Vit.C). Results found that vitamin C intake for UKpro had significantly higher values at all three testing time-points compared to the other two groups. Two way ANOVA showed significant interactions for Time (p = 0.045) and group v time (p = 0.039). Analytically, UKpro had the highest values during 185.14 ± 25.99 $mg.d^{-1}$ end-season, to 151.22 ± 29.00 $mg.d^{-1}$ mid-season and started at 159.14 ± 32.59 $mg.d^{-1}$ pre-season. On the contrary, CYsem had the highest values during 132.52 ± 45.34 $mg.d^{-1}$ pre-season, then the lowest at 73.15 ± 9.63 $mg.d^{-1}$ mid-season and finally 98.82 ± 9.30 $mg.d^{-1}$ end-season. Also CYrec had the highest values at pre-season 121.66 ± 16.73 $mg.d^{-1}$, from 104.80 ± 27.86 $mg.d^{-1}$ mid-season and 95.66 ± 13.50 $mg.d^{-1}$ end-season. In agreement, the assessment of post-hoc tests showed significant interaction between groups at pre-season for UKpro v CYsem, p = 0.016, UKpro v CYrec, p = 0.004 and CYsem v CYrec, p = 0.001.

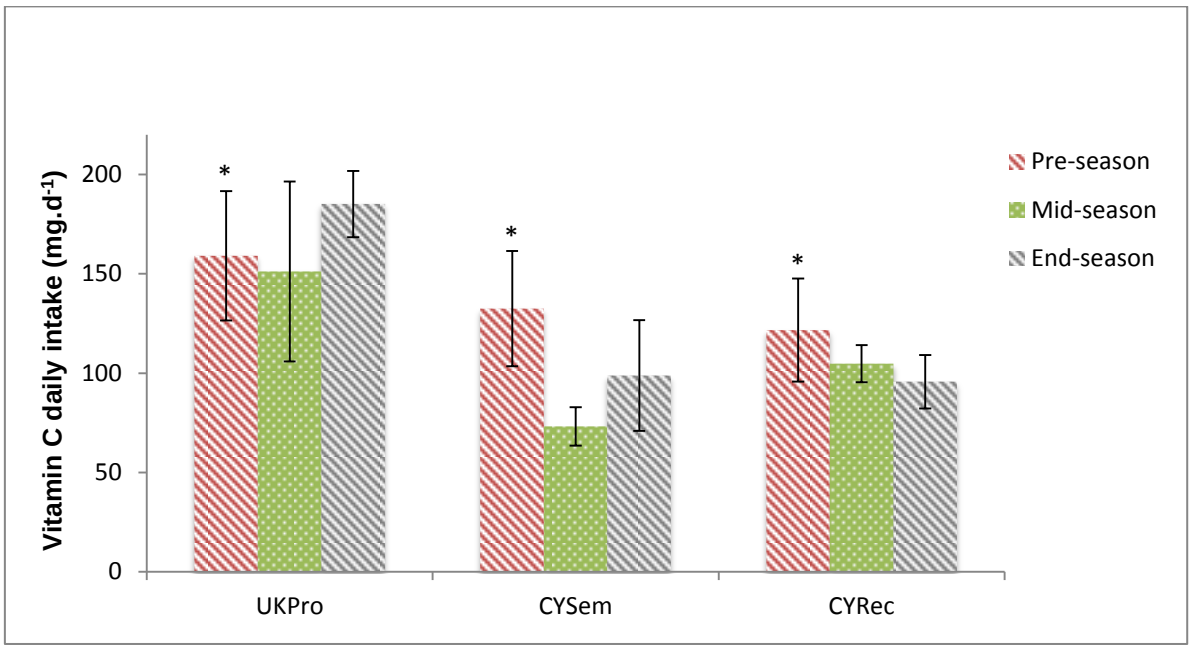

Figure 4.6 Average daily vitamin C intake for each cohort per testing block. Higher values were shown for UKpro at all testing blocks, with the differences being *significant at pre-season.

Vitamin D

Average daily Vitamin D intake

Mean team average daily vitamin D intake per testing block data is shown in Figure 4.7 below as vitamin D intake (Vit.D). Results showed that at pre-season UKpro 4.34 ± 0.88 $\mu g.d^{-1}$ and CYrec 5.16 ± 1.05 $\mu g.d^{-1}$ had the highest values while in contrast CYsem showed the highest values at end-season 3.72 ± 0.71 $\mu g.d^{-1}$. Through mid-season 3.39 ± 0.84 $\mu g.d^{-1}$ and end-season 3.32 ± 0.80 $\mu g.d^{-1}$ UKpro values were decreased and very equal. For CYsem at pre-season 1.69 ± 0.65 $\mu g.d^{-1}$ and mid-season 1.63 ± 0.52 $\mu g.d^{-1}$ were the lowest of all groups at all testing blocks. On the other hand, CYrec values decreased importantly at mid-season 2.13 ± 0.37 $\mu g.d^{-1}$ and finally showed an increase to end-season 2.90 ± 0.65 $\mu g.d^{-1}$.

Two way ANOVA showed no significant interactions for Time ($F_{2, 14}$=1.15; p = 0.345) or group v time ($F_{2, 14}$=0.178; p = 0.839).

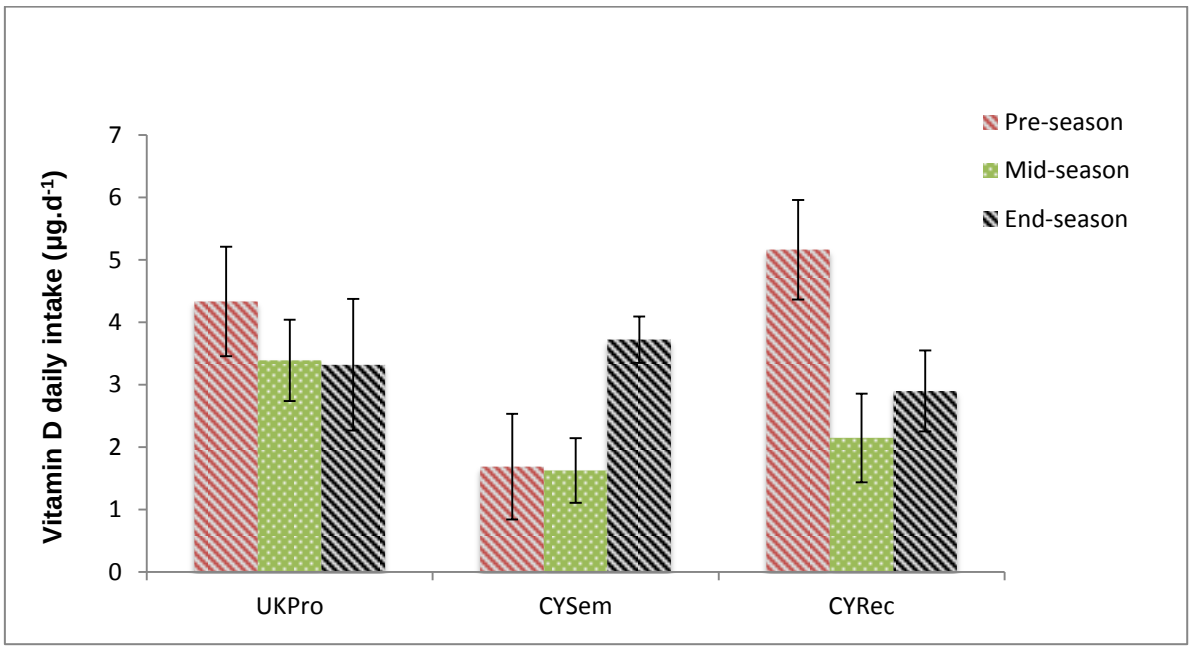

Figure 4.7 Average daily vitamin D intake for each cohort per testing block. During pre-season UKpro and CYrec groups had their highest values, while on the contrary CYsem showed the highest values at end-season.

Vitamin E

Average daily Vitamin E intake

Mean team average daily vitamin E intake per testing block data is shown in Figure 4.8 below as vitamin E intake (Vit.E). Results showed that during all three testing blocks CYsem group had the lowest values with 4.59 ± 1.15 $mg.d^{-1}$ pre-season, at 4.24 ± 0.61 $mg.d^{-1}$ mid-season and at 5.44 ± 0.30 $mg.d^{-1}$ end-season. UKpro started with 7.99 ± 1.47 $mg.d^{-1}$, increased at 8.25 ± 1.74 $mg.d^{-1}$ mid-season and had the lowest values at 6.85 ± 1.15 $mg.d^{-1}$ end-season. Similarly, CYrec started low with 5.39 ± 0.71 $mg.d^{-1}$ pre-season, increased importantly but not significantly at 9.19 ± 2.70 $mg.d^{-1}$ mid-season and decreased at end-season 6.10 ± 0.30 $mg.d^{-1}$. Results from 2-way ANOVA repeated measures found significant differences for time v group ($F_{4,\ 30}$= 2.83, p = 0.042). However, assessment of Post-hoc tests did not find any significant interaction (p > 0.05).

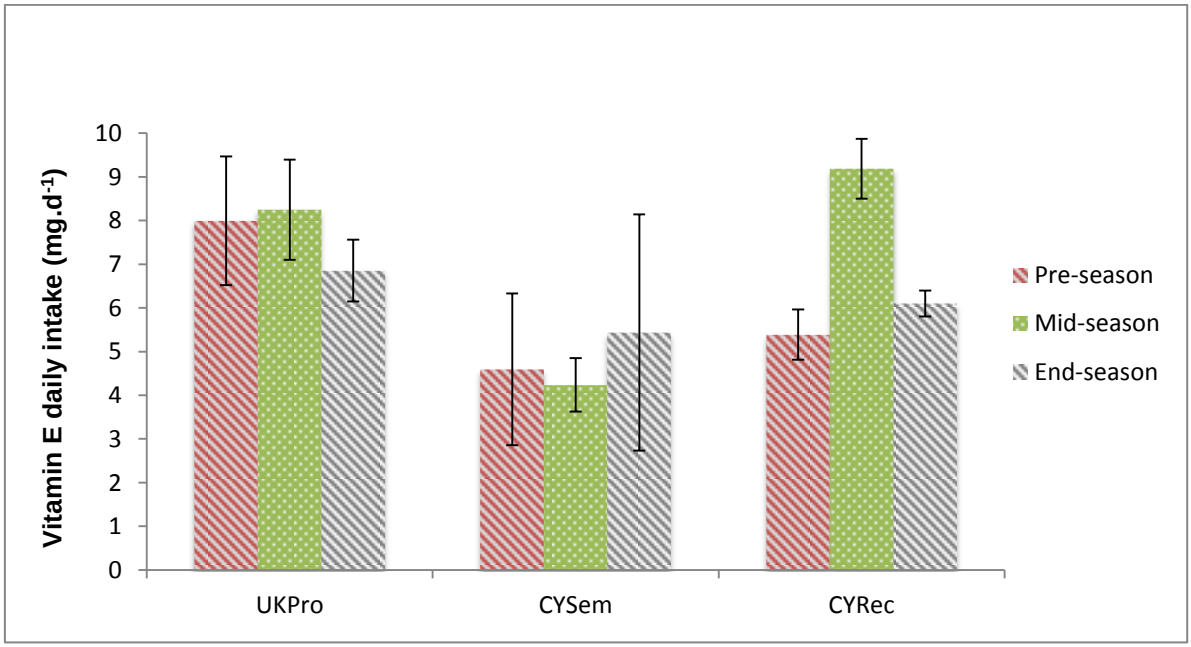

Figure 4.8 Average daily vitamin E intake for each cohort per testing block. CYsem group showed lower values than the other two groups during all testing-blocks even though these differences were not significant.

Magnesium

Average daily Magnesium intake

Mean daily magnesium intake data is shown at Figure 4.9 below. Data across all testing blocks showed high quantities for UKpro group particularly in comparison to the other two groups. During T1, UKpro (359.90 ± 45.04 mg) and CYrec (290.45 ± 20.66 mg) groups had their highest values of the testing season, while CYsem (266.83 ± 11.57 mg) had higher values at T3. A decreasing trend is apparent for UKpro and CYrec mean values, from first to last time points. However, statistical results from 2-way ANOVA repeated measures did not show any significant differences for neither time ($F_{1.29,\ 16.61} = 0.28$, $p = 0.67$) or time v group ($F_{2.59,\ 16.61} = 0.80$, $p = 0.49$).

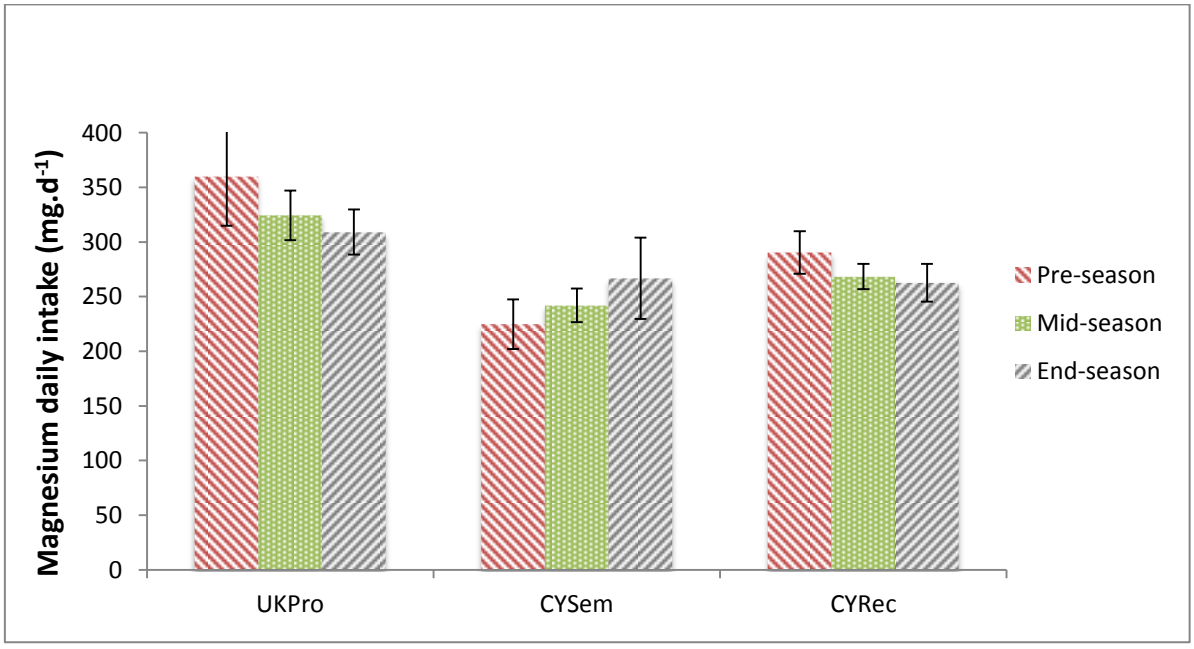

Figure 4.9 Average daily Magnesium (Mg) intakes for each cohort per testing block. The highest values are shown for UKpro at all testing blocks, but no significant differences were found when compared to the other groups.

Zinc

Average daily Zinc intake

Mean team daily Zinc intake per testing block data is shown in Figure 4.4 below as Zinc intake (ZI). Results for Zinc intake ($mg.d^{-1}$) found that groups did not have similar trend during the three testing time-points (T). Generally, the lowest values were found for CYsem with 8.66 ± 1.19 $mg.d^{-1}$ pre-season increased to 9.55 ± 0.47 $mg.d^{-1}$ mid-season and to 10.10 ± 0.56 $mg.d^{-1}$ end-season. In contrast, the highest values were found for UKpro with 13.32 ± 1.45 $mg.d^{-1}$ pre-season to 13.41 ± 1.39 $mg.d^{-1}$ mid-season and later to 12.71 ± 1.17 $mg.d^{-1}$ end-season. Finally, CYrec had initially 11.60 ± 0.84 $mg.d^{-1}$ pre-season, a decrease to 9.84 ± 1.17 $mg.d^{-1}$ and over to 10.75 ± 0.86 $mg.d^{-1}$ end-season.

Assessment of a two way mixed design anova assessment, where sphericity was assumed showed no significant interactions for Time ($F_{2,\ 30}$=0.171; p = 0.844) or group v time ($F_{4,\ 30}$=0.474; p = 0.754).

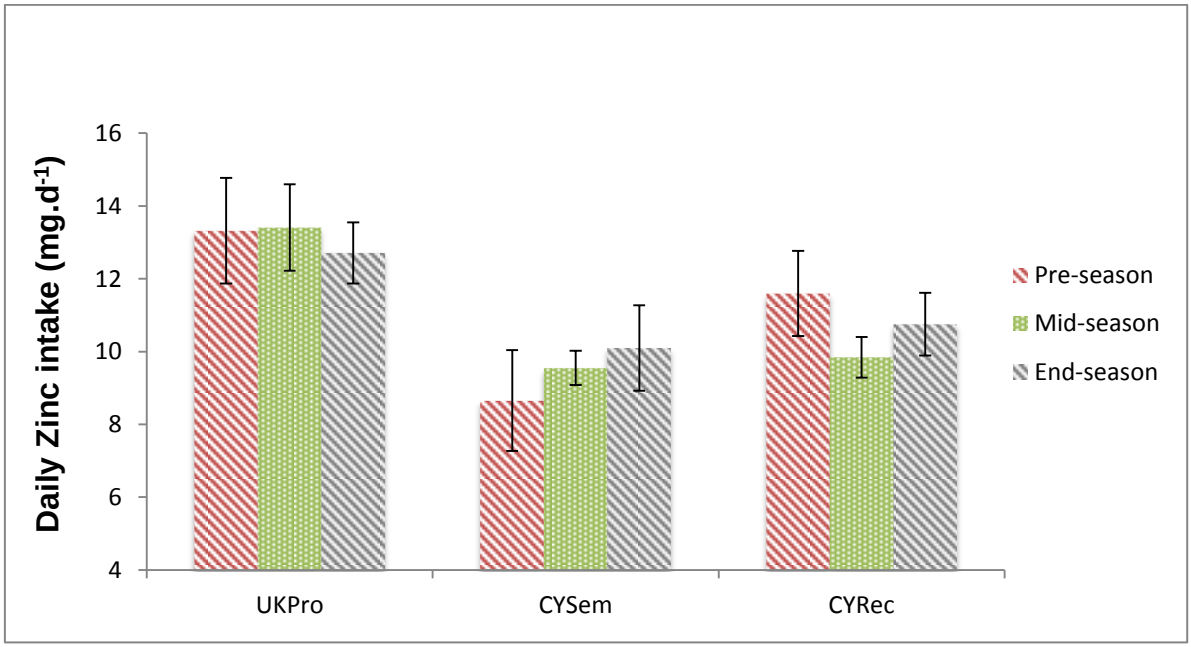

Figure 4.10 Average daily Zinc (Zn) intake for each cohort per testing block. The UKpro group showed higher values at all testing-blocks.

4.2 Training load

Training load is expressed as energy cost in estimated kilocalories (kcal).The caloric cost of physical activity was estimated using the following equation:

Kilocalories= MET x weight in kilograms x duration in hours.

Metabolic equivalent (MET), is a physiological measure expressing the energy cost of physical activities and is defined as the ratio of metabolic rate (and therefore the rate of energy consumption) during a specific physical activity to a reference metabolic rate, set by convention to 3.5 ml $O_2 \cdot kg^{-1} \cdot mins^{-1}$.

The liability and repeatability of the method was examined via pilot testing in parallel to Global positioning systems Garmin (Kansas, USA) and Lagalacolli (Florence, Italy) software.

Average Daily Training Load

Average estimated energy cost (kcal) per day of the week

Mean results of the three different time points (T) indicate that on average all groups increased their training load between baseline pre-season UKpro (2584.10 ± 51.90 $kcal.d^{-1}$), CYsem (2172.99 ± 53.53 $kcal.d^{-1}$), CYrec (2104.267 ± 66.69 $kcal.d^{-1}$) and end-season UKpro (2614.89 ± 63.32 $kcal.d^{-1}$), CYsem (2110.90 ±51.43 $kcal.d^{-1}$), CYrec (2197.61 ± 49.02 $kcal.d^{-1}$). In general, UKpro had higher training load during all three testing points of the season. The other two groups did not have significant differences between any of the testing time-points. A slight decrease from pre-season assessment was found during mid-season for UKpro 2544.62 ± 61.19 $kcal.d^{-1}$ and CYrec 2087.86 ± 66.83 $kcal.d^{-1}$. Results for CYsem during mid-season (1991.13 ± 45.45 $kcal.d^{-1}$), indicate an increase of the training load compared to pre-season and end-season monitored blocks.

Repeated measures 2 way anova found significant interaction between time points ($F_{1.49, 57.90}$ =7.642, p = 0.003) and time v group ($F_{2.97, 57.90}$ =7.13, p = 0.001). Pairwise comparisons between groups, revealed that these differences were significant for UKpro v CYsem, (p = 0.001) and UKpro v CYrec, (p = 0.001). Time pairwise comparisons also found significant interaction for pre-season v mid-season, (p = 0.007) and pre-season v end-season, (p = 0.004).

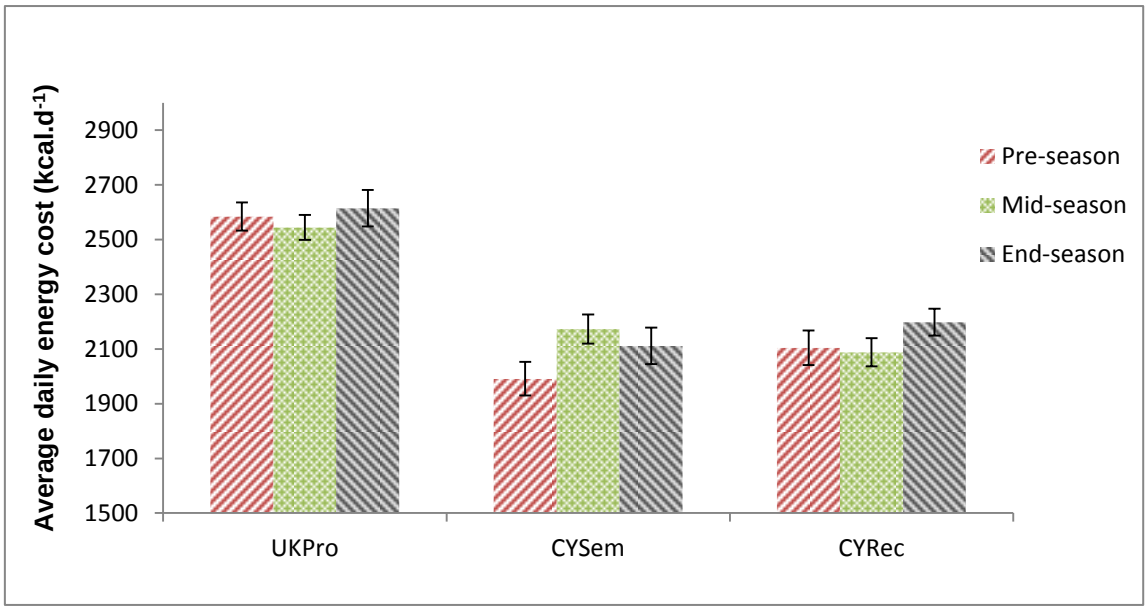

Figure 4.11 Average estimated energy cost (kcal) per day of the week. The UKpro group showed higher and near to equal values during all testing-blocks, compared to the other two groups.

Training Day energy cost

Average estimated energy cost (kcal) per training day

Mean data for Training Day estimated energy Cost (TDC) is demonstrated in Figure 4.12 below. UKpro had considerably higher TDC in comparison to CYsem and CYrec groups at all three testing time-points. In addition, assessment of two way anova reported significant interaction between the time points ($F_{1.48, 57.71}$=25.48; p =0.00) as well as significance group v time interaction ($F_{2.96, 57.71}$=10.56; p =0.00).

The highest energy cost of training days was observed at mid-season for UKpro group (3021.48±77.59 kcal) and at end-season for both CYsem (2417±61.88 kcal) and CYrec (2527.22±71.20 kcal) respectively. Pairwise comparisons found statistically significant differences between groups UKpro v CYsem, (p = 0.001) and UKpro v CYrec, (p = 0.001).

An increasing trend was obvious for CYsem and CYrec TDC through the three testing blocks. Also the mean values did not differ greatly between those two groups. The overall trend showed an important increase in training load per daily training of all groups during pre-season in comparison to mid-season. While both CYsem and CYrec groups retained an increasing trend between mid-season and end-season, UKpro group training load showed a decrease at end-season, even lower than mean values of pre-season.

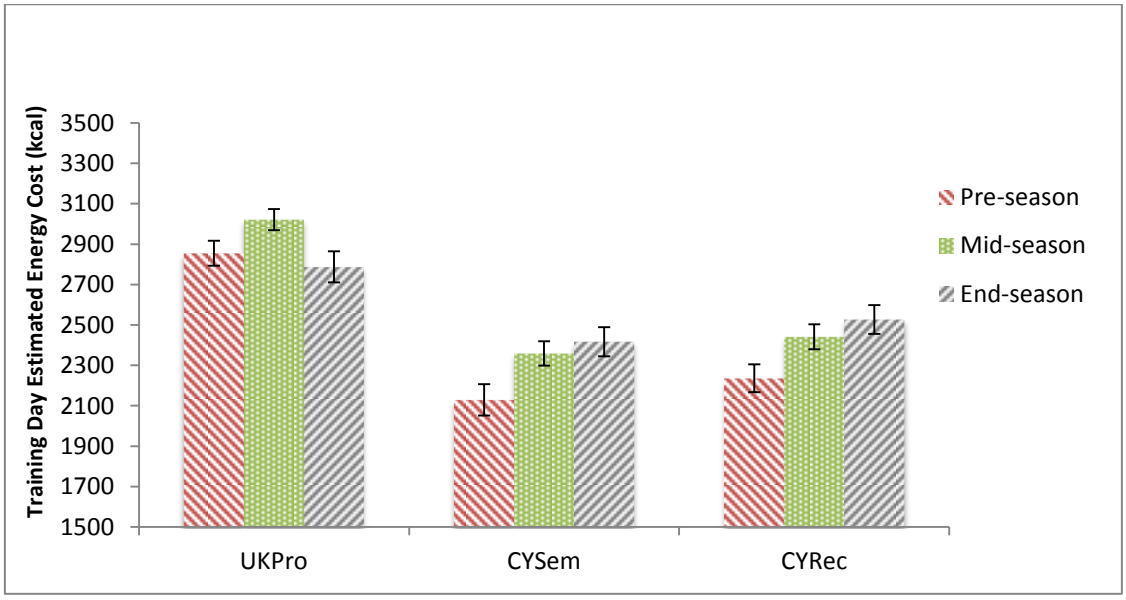

Figure 4.12 Average estimated energy cost (kcal) per training day. Different trends through the season between UKpro and Cyprus groups. The UKpro showed higher values than the other two groups at all testing points.

4.3 Physiological stress markers

Salivary IgA

Salivary Secretory Immunoglobulin A pre training

Mean team data per testing block for salivary secretory immunoglobulin A (s-IgA), collected before training session is shown in Figure 4.13 below as IgA pre-training (IgA-pre). It was found that UKpro had considerably lower values of IgA-pre in comparison to CYsem and CYrec groups at all three testing time-points. However, assessment of two way anova did not report any significant interaction for time ($F_{2, 22}$=0.13; p =0.987) or any significance for group v time interaction ($F_{4, 22}$=0.545; p =0.705).

In general, CYrec compared to the other groups showed in average the highest values from pre-season 267.53 ± 46.40 (µg/ mL) to mid-season 368.21 ± 45.85 (µg/ mL) and later to end-season 318 ± 28.42 (µg/ mL). On the contrary, UKpro had the lowest values at all testing points with pre-season 180.07 ± 18.94 (µg/ mL), which increased at mid-season 209.46 ± 65.71 (µg/ mL) and decreased at lowest level by end-season 166.68 ± 25.03 (µg/ mL). Lastly, CYsem retained very similar average values through pre-season 279.77 ± 85.88 (µg/ mL) to mid-season 274.57 ± 42.85 (µg/ mL) and later to end-season 274.86 ± 28.96 (µg/ mL).

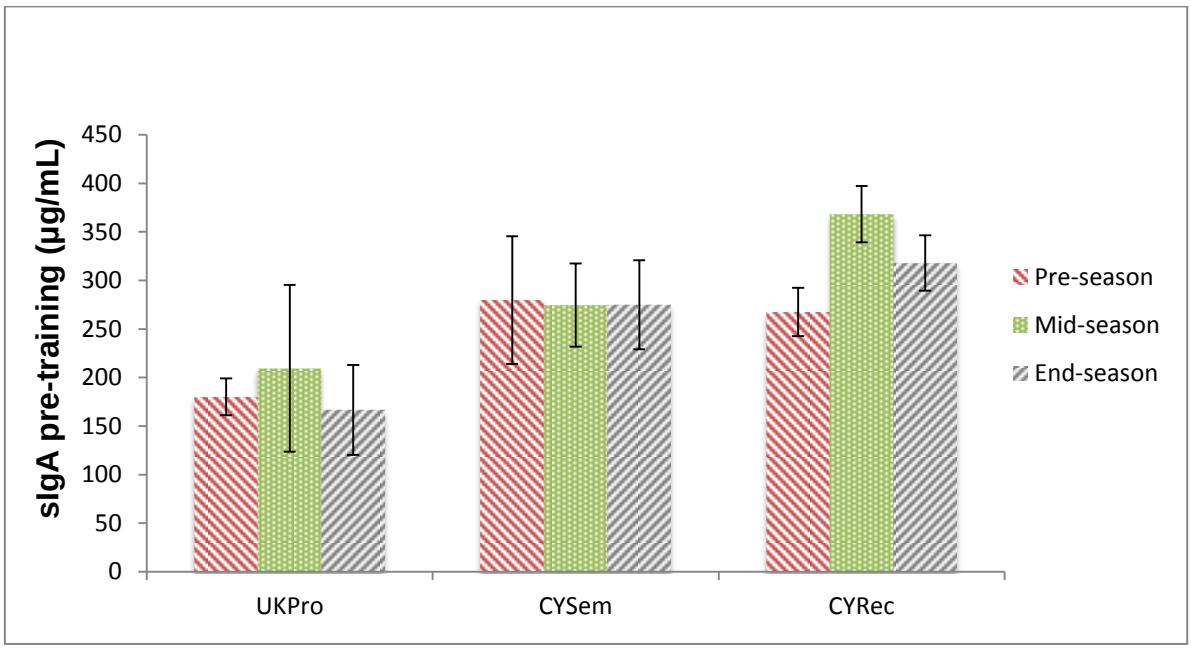

Figure 4.13 Average Salivary IgA concentrations before hard training session for each cohort per testing block. CYsem showed values near to equal at all testing-blocks UKpro had the lowest values and CYrec the highest values.

Salivary Secretory Immunoglobulin A after training

Mean team data per testing block for salivary secretory immunoglobulin A (s-IgA) collected after training session is shown in Figure 4.14 below as s-IgA after-training (IgA-after). It was found that UKpro had considerably lower values of IgA-after in comparison to CYsem and CYrec groups at all three testing time-points. However, assessment of two way anova did not report any significant interaction for time ($F_{2, 32}$=0.911; p =0.412) or any significance for group v time interaction ($F_{4, 32}$=0.430; p =0.786). Assessment of Post-hoc Bonferroni test, revealed significant interaction at mid-season between UKpro (172.28 ± 42.17) and CYrec (489.41 ± 77.57) (p = 0.003) and at end-season between UKpro (174.23 ± 36.25) and CYrec (419.78 ± 49.03) (p = 0.001). Despite visible differences within CYrec pre-season 298.40 ± 70.30(µg/ mL) v mid-season 489.41 ± 77.57(µg/ mL) and end-season 419.78 ± 49.03(µg/ mL) there was not any statistically significant interaction (p > 0.05). Also CYsem did not have any statistically significant differences from pre-season 320.13 ± 48.87(µg/ mL) v mid-season 307.57 ± 38.51(µg/ mL) and end-season 269.86 ± 49.03(µg/ mL).

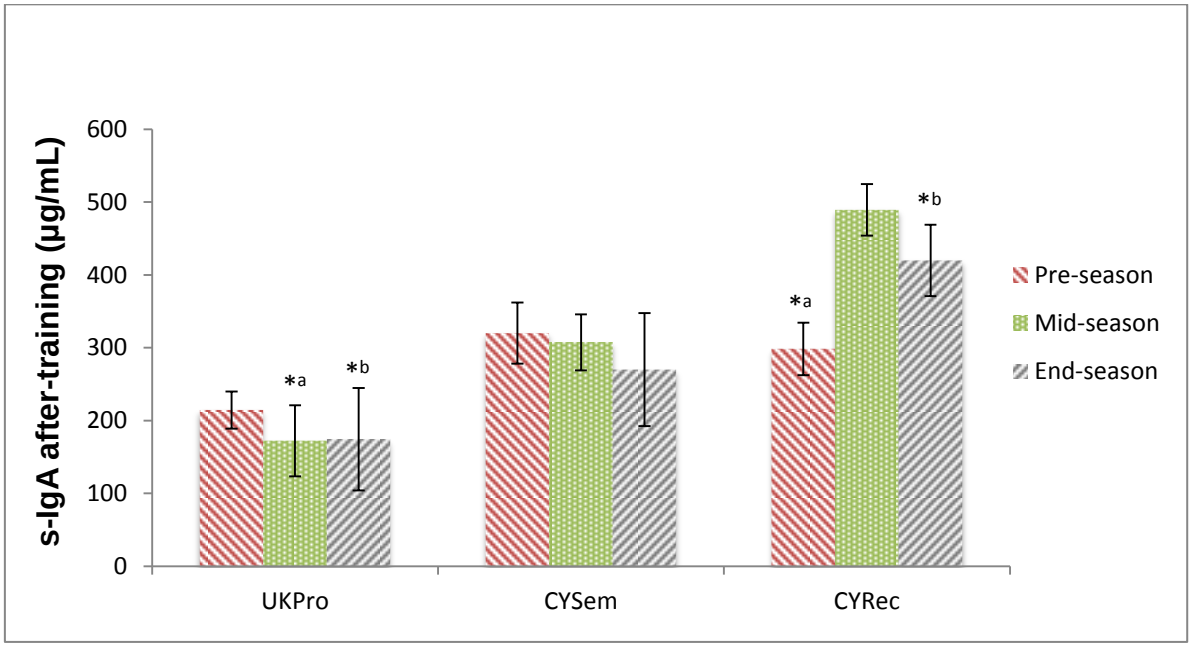

Figure 4.14 Salivary secretory immunoglobulin-A concentrations after training session. Results showed UKpro with the lowest values at all testing points.

$*^{a}$, $*^{b}$ = significant differences

Salivary Secretory Immunoglobulin A next day training

Mean team data per testing block for salivary secretory immunoglobulin A (s-IgA) collected at the next day of training session is shown in Figure 4.15 below as s-IgA next-training (IgA-next). It was found that UKpro had considerably lower values of IgA-next in comparison to CYsem and CYrec groups at all three testing time-points. Interestingly, CYrec retained much higher values than the other groups during all testing points and had the highest concentrations at pre-season 413 ± 100.51 (µg/ mL). However, assessment of two way anova did not report any significant interaction for time ($F_{2, 32}$=0.177; *p* =0.838) or any significance for group v time interaction ($F_{4, 32}$=1.311; *p* =0.287). Assessment of Post-hoc Bonferroni test, revealed significant interaction at mid-season for UKpro (107.50 ± 27.82) v CYrec (397.09 ± 45.53) (*p* = 0.002) and UKpro v CYsem (329 ± 42.06) (*p* = 0.001) at end-season between UKpro (117.45 ± 15.29 µg/ mL) and CYrec (375.22 ± 47.25 µg/ mL) (*p* = 0.004) and UKpro v CYsem (293.71 ± 25.26 µg/ mL) (*p* = 0.001). UKpro had high values only at pre-season 294 ± 44.92(µg/ mL). On the contrary, CYsem showed a balanced constancy from pre-season 303.01 ± 44.23(µg/ mL) v mid-season 329.00 ± 42.06(µg/ mL) and end-season 293.71 ± 47.25(µg/ mL).

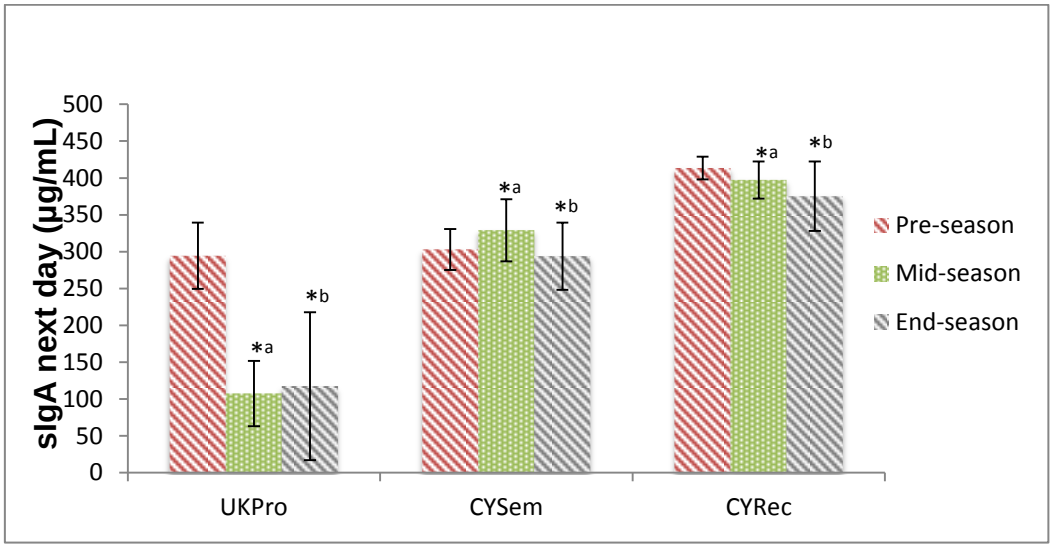

Figure 4.15 Salivary secretory immunoglobulin-A concentrations, next day of training session. The CYrec group showed the highest values and UKpro showed the lowest at all testing-blocks.

*a, *b = significant interactions between groups

Testosterone/ Cortisol Ratio

Testosterone/ Cortisol Ratio Pre-training

Mean team data per testing block for Testosterone/ Cortisol Ratio collected pre-training session is shown in Figure 4.19 below as T/C-Pre. Results showed different leanings between the three groups during time. Interestingly, CYsem group had considerably the highest levels at all-time testing blocks, starting with values at pre-season with 0.20 ± 0.06 µg/ mL and decreased later with near to equal at 0.17 ± 0.04 µg/ mL mid-season and 0.17 ± 0.02 µg/ mL end-season. On the contrary, CYrec started significantly lower with 0.05 ± 0.01 µg/ mL pre-season, to 0.11 ± 0.04 µg/ mL mid-season and finished to 0.09 ± 0.01 µg/ mL end-season. Finally, UKpro had low values at pre-season 0.08 ± 0.02 µg/ mL and increased later with 0.09 ± 0.01 µg/ mL mid-season and 0.12 ± 0.03 µg/ mL end-season. Assessment of two way ANOVA where sphericity was not assumed, Greenhouse-Geisser test showed significant time v group interaction ($F_{2.95,\ 39.88}$ =4.12; p = 0.013). Assessment of Post-hoc Bonferroni test found significant interaction at pre-season between CYsem and CYrec (p = 0.28).

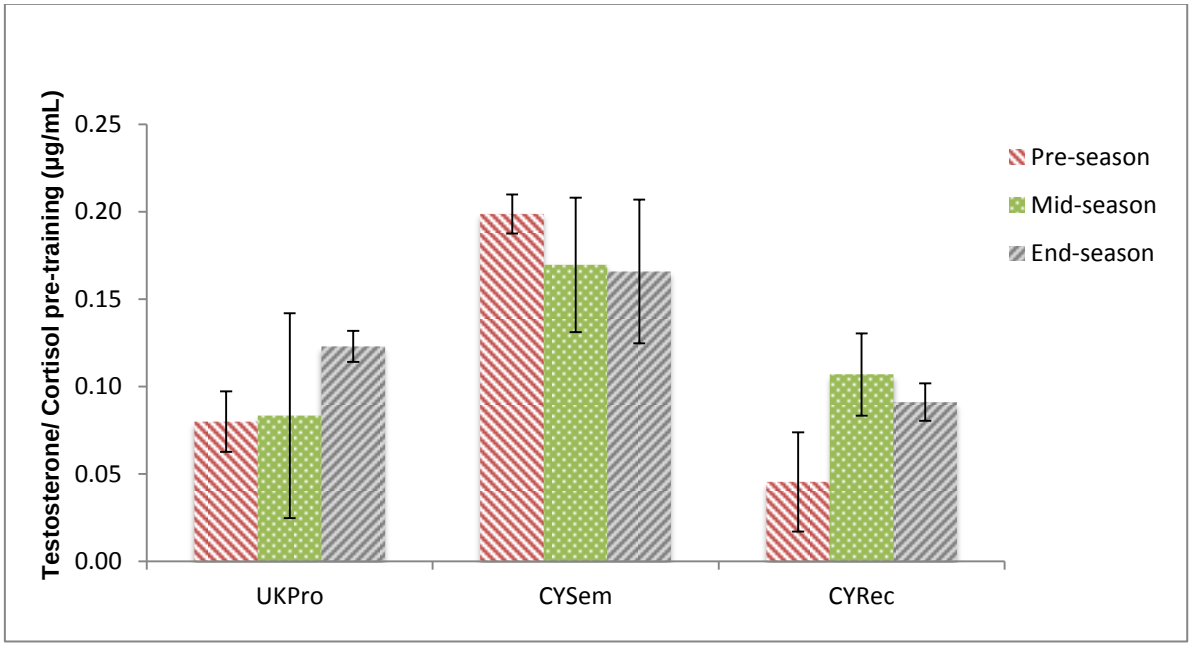

Figure 4.19 Average pre-training T/C concentrations for each cohort per testing block.

Testosterone/ Cortisol Ratio After-training

Mean team data per testing block for Testosterone/ Cortisol Ratio collected after-training session is shown in Figure 4.20 below as T/C-After. Results showed different leanings between the three groups during time. Higher values were found at all three testing blocks for CYsem with 0.17 ± 0.05 µg/ mL pre-season, decreased later at 0.16 ± 0.05 µg/ mL mid-season and 0.12 ± 0.04 µg/ mL end-season. UKpro started with low values at pre-season 0.07 ± 0.01 µg/ mL, increased later with at 0.16 ± 0.05 µg/ mL mid-season and low again at 0.08 ± 0.02 µg/ mL end-season. Finally, CYrec had low pre-season values with 0.08 ± 0.02 µg/ mL and increased later with near to equal values at 0.14 ± 0.05 µg/ mL mid-season and 0.14 ± 0.04 µg/ mL end-season

Assessment of 2way anova where sphericity was not assumed, Greenhouse-Geisser test did not find any significant interactions for time ($F_{1.42,\ 21.27}$ =3.20; p = 0.075) or time v group ($F_{2.84,\ 21.27}$ =2.20; p = 0.120).

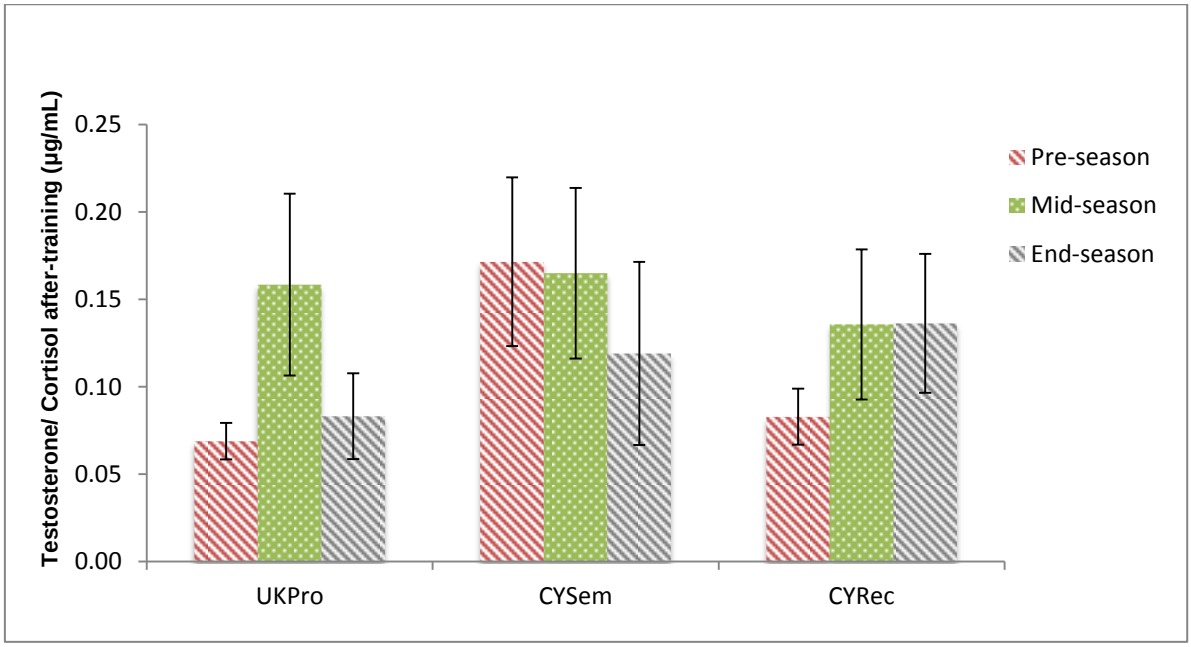

Figure 4.20 Average after-training T/C concentrations for each cohort per testing block.

Testosterone/ Cortisol Ratio Next-day

Mean team data per testing block for Testosterone/ Cortisol Ratio collected Next-day before training session is shown in Figure 4.21 below as T/C-Next. Results showed different leanings between the three groups during time.

Higher values were found at all three testing blocks for CYsem with 0.14 ± 0.03 µg/ mL pre-season, increased later at 0.14 ± 0.04 µg/ mL mid-season and 0.15 ± 0.03 µg/ mL end-season. CYrec had high pre-season values with 0.11 ± 0.03 µg/ mL and decreased later at 0.05 ± 0.01 µg/ mL mid-season and 0.06 ± 0.01 µg/ mL end-season. UKpro started with 0.06 ± 0.02 µg/ mL pre-season, decreased at 0.06 ± 0.01 µg/ mL mid-season and had its highest values at 0.10 ± 0.01 µg/ mL end-season.

Assessment of 2way anova where sphericity was assumed, did not find any significant interactions for time ($F_{2, 28}$ =2.13; p = 0.137) or time v group ($F_{4, 28}$ =2.28; p = 0.085).

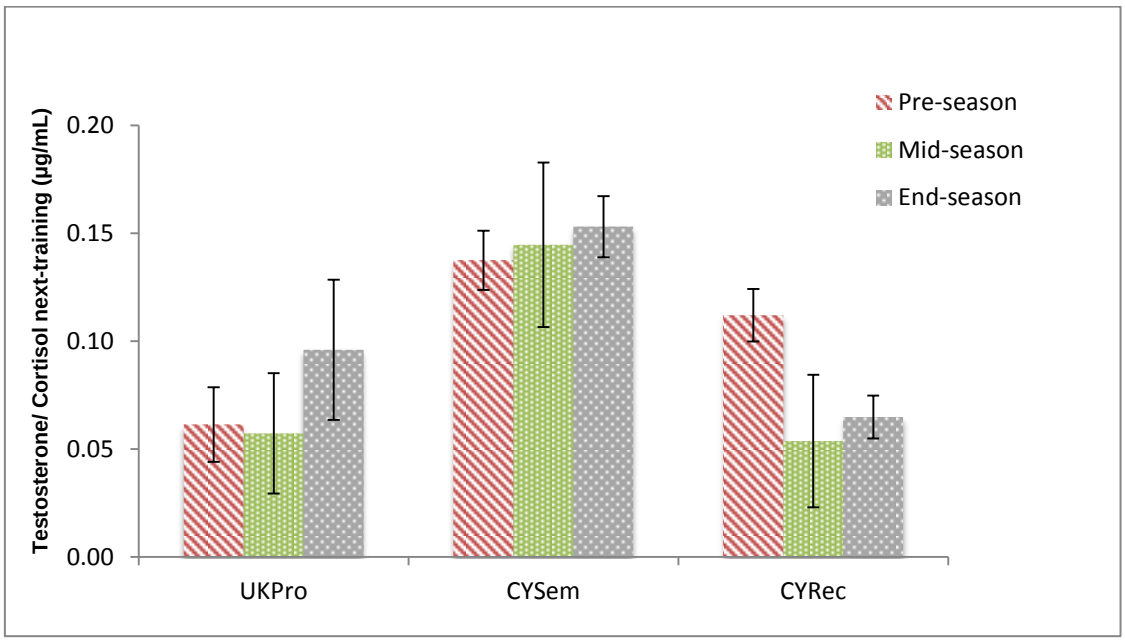

Figure 4.21 Average T/C concentrations during next day of hard training for each cohort per testing block.

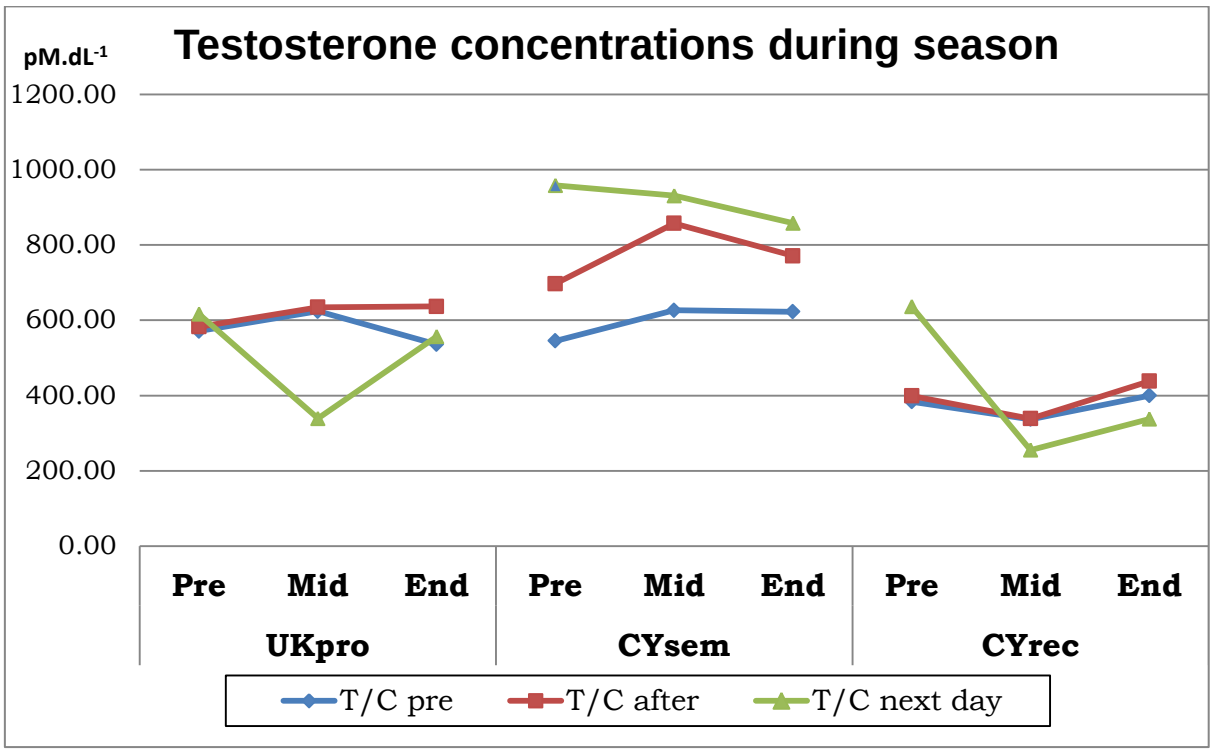

Figure 4.22 Average Testosterone concentrations during season for each cohort per testing block.

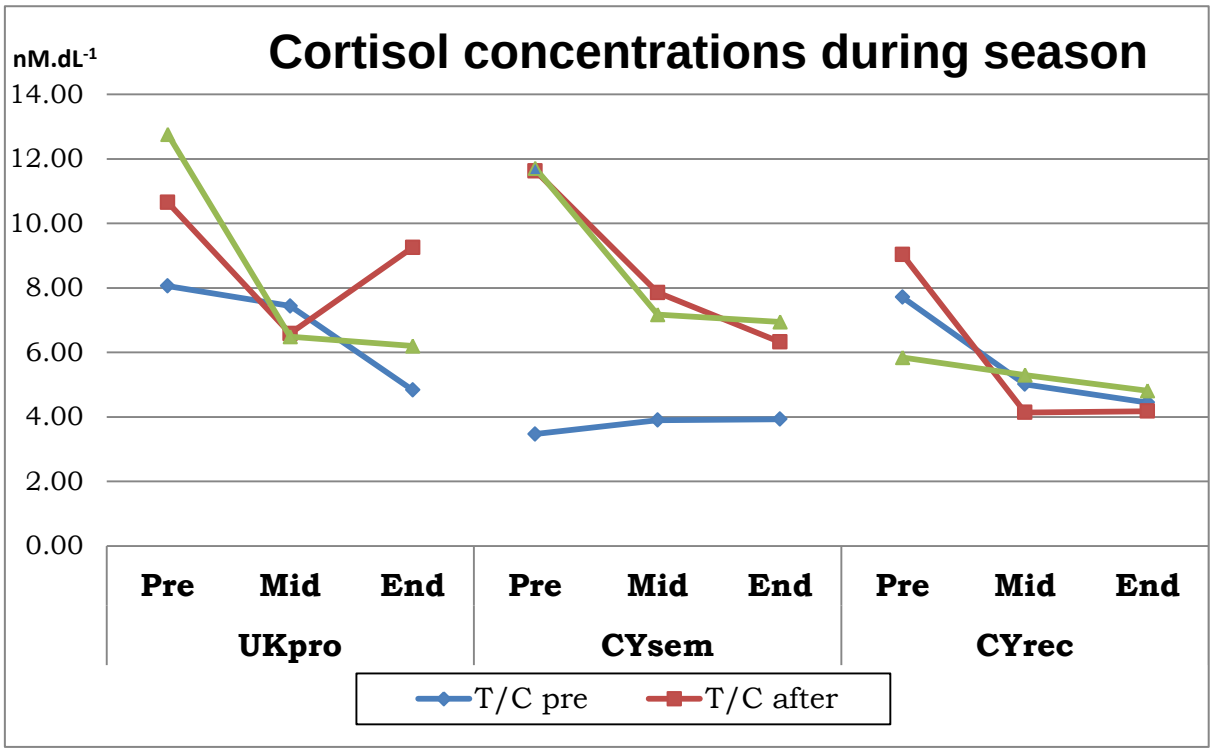

Figure 4.23 Average Cortisol concentrations during season for each cohort per testing block.

Body Fat Percentage

Mean team data per testing block for body fat percentage collected pre training session is shown in Figure 4.22 below as fat percentage (Fat%). Results showed that UKpro had considerably lower levels at all testing blocks in comparison to the other groups with 7.91 ± 0.42 at pre-season, 8.10 ± 0.40 at mid-season and 7.81 ± 0.32 at end-season. On the contrary, CYsem results show higher values at all three testing blocks 17.24 ± 1.11 % at pre-season, 16.14 ± 0.92 % at mid-season and 15.04 ± 0.72 % at end-season. . Interestingly, CYrec results with 13.32 ±.1.05 % at pre-season, 13.33 ±0.83 % at mid-season and 13.17 ± 0.87 % at end-season, had near to equal values during all testing blocks and lower than semi-professional group. Significant differences were found via two way ANOVA assessment, where sphericity was not assumed, Greenhouse-Geisser test showed no significant time interaction ($F_{1.51, 63.59}$ =7.72; p = 0.003) or group v time ($F_{3.03, 63.59}$ =5.70; p = 0.002).

Also the assessment of Post hoc Bonferroni test confirmed that there were significant interactions at all testing blocks. Firstly, at pre-season for UKpro v CYsem (p = 0.001), UKpro v CYrec (p = 0.001), CYsem v CYrec (p = 0.014), later at mid-season for UKpro v CYsem (p = 0.001), UKpro v CYrec (p = 0.001), CYsem v CYrec (p = 0.038), and at end-season for UKpro v CYsem (p = 0.001), UKpro v CYrec (p = 0.001).

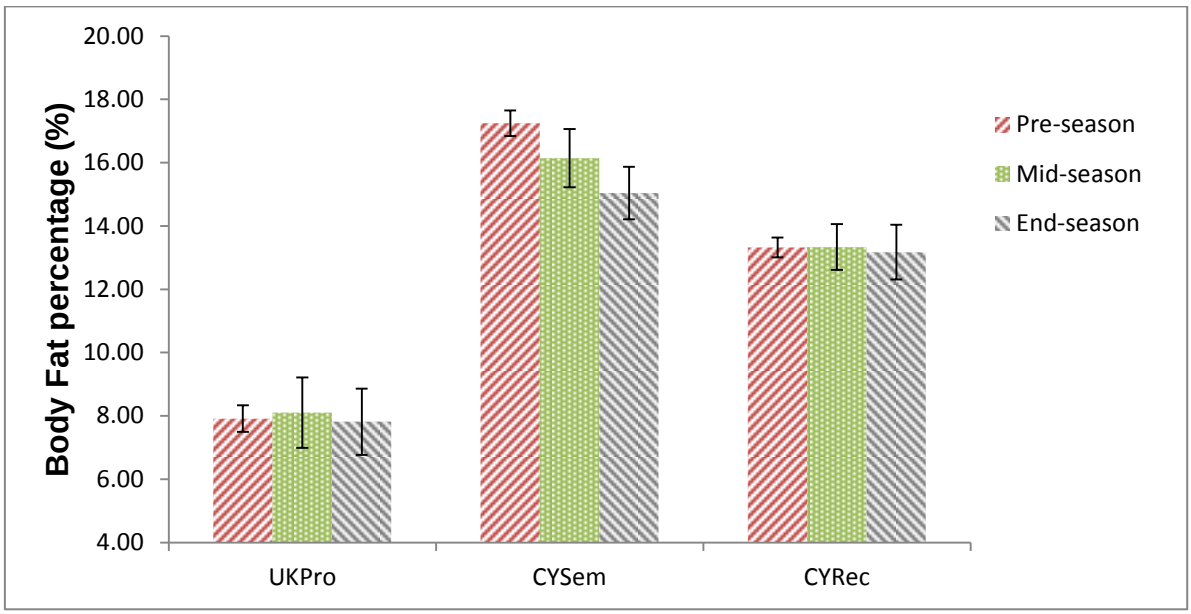

Figure 4.24 Average Body fat percentages for each cohort per testing block. The highest values are shown for CYsem and the lowest for UKpro at all testing-blocks. End result significant differences at all three testing groups and between all groups except between Cyprus groups at end-season.

Environmental conditions

Recording of environmental conditions during training sessions was assessed in parallel with the main parameters of this research. From a scientific view these findings should be added in order to justify some of the main study results. For example some Cyprus based teams training sessions took place at environment of 35^0C and 65% humidity, while the same testing block for UK was at 10^0C and 35% humidity (Refer to figures 4.25 and 4.26).

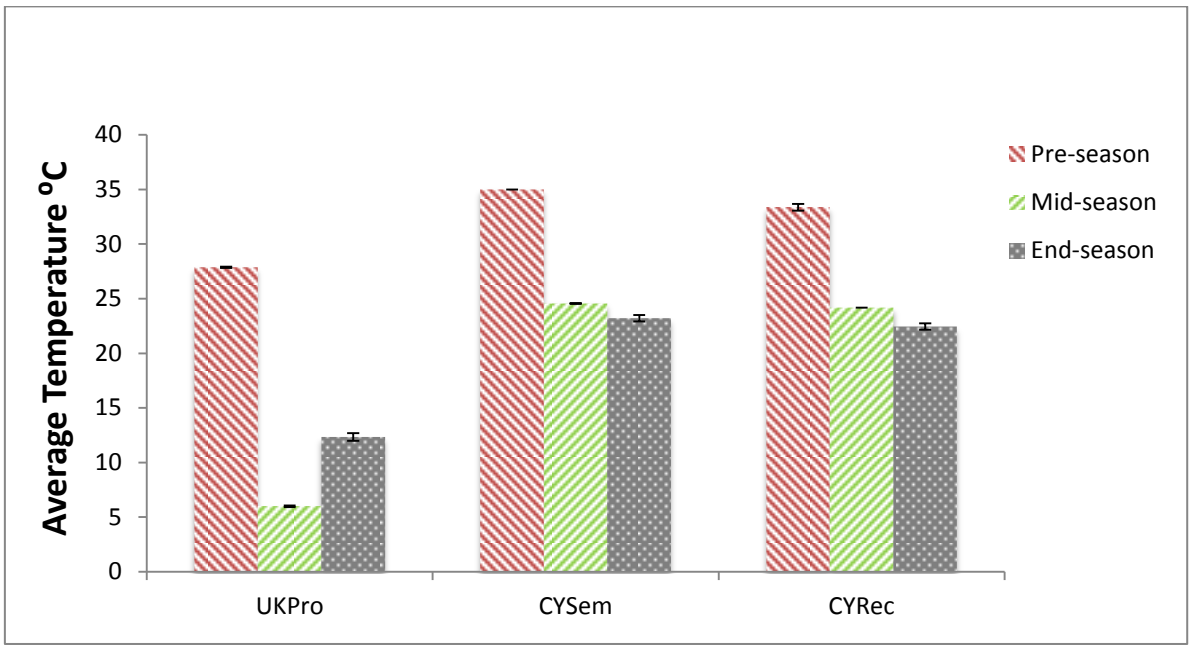

Figure 4.25 Average temperature Celsius (^{0}C), through season testing-blocks for all three groups. In average, high temperatures were observed for Cyprus groups.

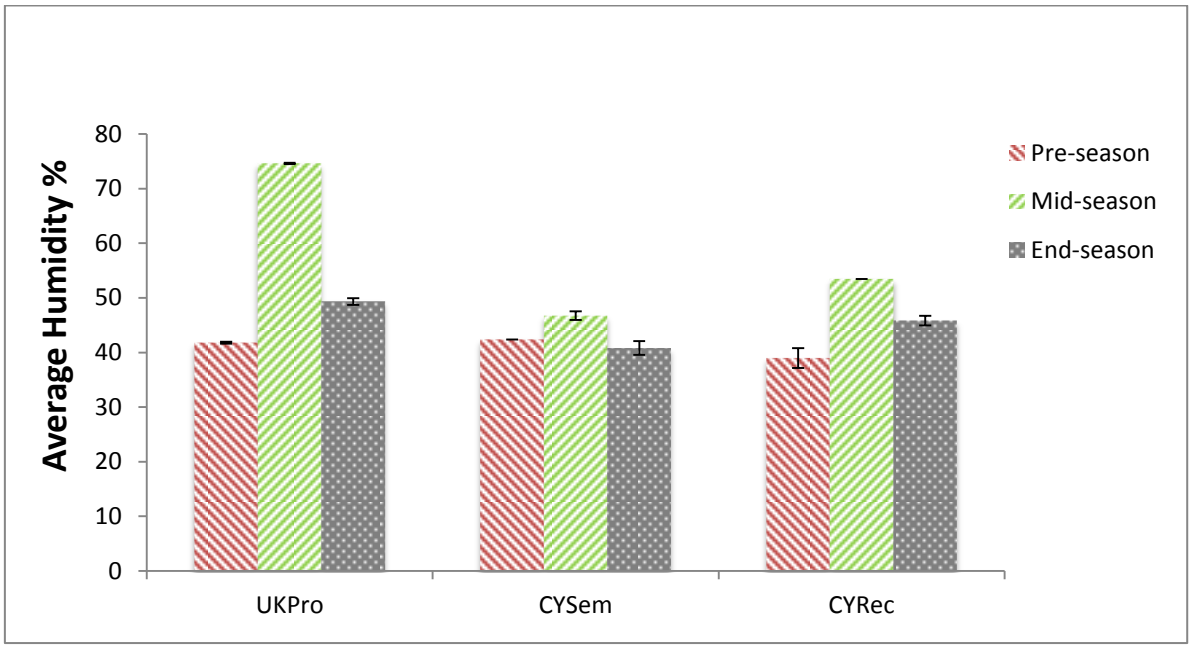

Figure 4.26 Average relative humidity through season testing-blocks for all three groups. Generally, similar values for all groups and testing-blocks, except UKpro mid-season with higher values.

CHAPTER 5

GENERAL DISCUSSION

5.0 General Discussion

5.1 Introduction

The objective of this chapter is to assimilate the findings of the respective studies which constitute this thesis on the differences in nutritional intake and training load between football teams from different countries, and how that influences training stress markers at various points of the training season. To facilitate this process the hypotheses that were tested are either accepted or rejected based upon the results of the research.

5.2 Nutritional Energy Intake

Findings for the average daily total energy intake (TEI) were highlighted in relation to the three different testing blocks which represented a typical football season. Evidently, across all three testing blocks none of the groups received inadequate energy intake, below daily energy expenditure and according to their professional level as expected from the conclusions of previous studies. Furthermore, at end-season all groups had near to equal values and despite the fact that there were differences between groups, these were not statistically significant. Notably, pre-season for semi-professional group (CYsem) had the lowest energy intake. The hypothesis expected to find significantly higher values for all groups at pre-season compared to mid- and end-season, which however was not the case and therefore hypothesis (H1i) was rejected. In comparison to recreational footballers the professional footballers were receiving higher energy intake during mid- and end-season, but lower intake during pre-season.

In general, UKpro group showed higher energy intake through all three testing blocks with 1772.67 ± 116.04 kcal.d^{-1} pre-season, to 1928.38 ± 112.83 kcal.d^{-1} mid-season and to 1874.00 ± 64.34 kcal.d^{-1} at the end-season block (p >0.05). On the contrary, CYsem group showed lower values 1502.20 ± 143.95 kcal.d^{-1} pre-season to 1718.60

± 142.87 kcal.d^{-1} mid-season and to 1789.80 ± 116.17 kcal.d^{-1} end-season block. Also and CYrec group showed low values at 1554.40 ± 140.96 kcal.d^{-1} mid-season and 1694.00 ± 96.33 kcal.d^{-1} end season, while interestingly this group had the highest values of all groups at any testing block with 2006.50 ± 129.14 kcal.d^{-1} at pre-season.

A probable explanation could be found by observing the training load (energy expenditure) at the same testing period where values appear to be near to equal with the other two testing blocks. Evidently, across all three testing blocks none of the groups received inadequate energy intake according to their professional level as expected from the findings of previous studies (Hassapidou, 2001; Maughan, 1997). For all groups, training sessions generally as raw data were categorized in aerobic, anaerobic and resistance training, with reference to intensity, volume and intervals of each at all sessions. While UKpro retained a similar training schedule across season in terms of training duration, volume and intervals, the other two groups did not keep similar analogy between any testing blocks. This could be an explanation why UK group by means of professional team retained a structured fraction of macronutrients' energy intake, while on the opposite the other two groups presented altered distribution between testing-blocks. The fractional contribution of the macronutrients to total energy intake was similarly analogous to that of the general population.

In the literature energy requirements for footballers are indicated between the range of 3819 – 5185 kcal.d^{-1} for professionals and over 2000kcal.d^{-1} for lower levels. These estimations were based on the average training and game energy expenditure (Leblanc, 2002). Previous studies stated that the energy intake of their participants had similar fractional contribution of the macronutrients to total energy intake of the general population (Kalapotharakos, 2006; Rico-Sanz et al., 1998; Maughan, 1997). Mediterranean' dietary practices principally include high percentage of CHO in total energy intake. According to a dietary survey with Italian national athletes results indicated that the apparent contributions of CHO and fat in their diets, was different to the intakes reported in other dietary surveys of athletes from other countries. In line to the current study, it was stated that athletes have difficulties to achieve significant dietary changes that conflict with the eating practices of the general community (Ono et al., 2012; Schena, Pattini, Mantovanelli, 1995). Most professional clubs have adapted Italian cuisine as a football specific diet, but basically only pasta is regularly

included in real life daily diet. That makes footballers unwilling to decisively follow such diet program on a weekly basis, which results to inadequate carbohydrate intake. Generally, besides personal taste and background, traditional ideas and each country's football philosophy restrict nutritional choices of individual footballers.

Overall total energy intakes and relative macronutrient contribution appear to vary between studies and groups with similar professional status. Mainly, these reports suggest that individual food preferences are responsible for the differences found regarding nutritional total energy intake and macronutrient percentages. Consequently, the differences found in the current research regarding the macronutrients different fraction in total energy intake among groups and season testing-blocks, could be the result of various personal food choices between footballers of any professional status. In agreement, studies have indicated that whilst football athletes share broadly similar nutritional targets (i.e. energy intake, macronutrient intake), there would appear to be larger variance between athletes in terms of food choices, food combinations and nutrient timing as a means to achieve such targets. Through a food recall diary, similar to this research method of nutritional intake assessment, Burke (2006) reported dietary intakes of male soccer players during training from players of different national leagues.

More recent studies, agree to a nutritional intake plan according to energy estimated energy cost as the result of basic metabolic rate plus training energy expenditure as well as the personal preferences and experience of the individual athlete (Burke et al., 2011). However, the complexity of estimating total energy expenditure and requirements of each footballer are based on the individualisation principle, subject to individual's metabolic rate, precise training cost estimation, thermic effect of food and perhaps several other parameters (Manore & Thompson, 2006). Moreover, nutritional tactics have been used by athletes as an instrument in order to enhance their performance and in line the scientific sports nutrition research is investigating the amount and the timing of the food that should be consumed (Ono et al., 2012). Furthermore, the nutritional needs across season develop in parallel to training energy expenditure, but also food choices and macronutrients percentage of total intake may vary. The importance of nutritional periodization arises regarding weekly programs, micro- and macro-cycles. Modifications of total energy intake and moreover carbohydrate loading seem to occur in modern football, depending on

seasonal phase targets. For example, pre-season was associated in previous studies statements, as a period targeted with higher aerobic training and also higher carbohydrate energy intake. Moreover, guideline for football nutrition suggests a carbohydrate pre-game loading (3 days prior).

Footballers through the season, take part in several training sessions plus a match in a week with the energy cost being considerably high. For the purposes of the current research, a standard easy to assess method was used (MET values and Compendium of physical activities codes) and a secondary control method was assessed when possible for more accurate energy cost estimations (GPS 10Hz, via Lagalacolli). Undoubtedly, monitoring the training load is an unconditional need in modern football even though other parameters, such as nutritional interaction and physiological stress markers must be monitored in parallel, in order to extract accurate and useful conclusions.

Despite the successful use of 4-day food recall diary in previous studies with professionals athletes (Paschoal and Amancio, 2004), the inaccuracy of reported nutritional intakes via food diary and the probability for underreporting on behalf of participants could explain lower intake values could be a possibility why there are low intake values. This conclusion is based on the steadily low reported total energy intakes for all groups, which did not balance against energy cost of training sessions. There are several reasons why participants could underreport, including the limited comprehension of energy balance importance or various changes in footballers' psychological moods depending on personal performance and team results. Also, there is always the possibility of not reporting particular foods or drinks because of lacking trust to researchers collecting data. Finally, some participants could have not understood how to describe correctly portions sizes and ingredients.

Additionally, the body fat percentages did not show alterations that could possibly support the low energy intakes. It seems likely that any underreporting of intake among these players was inadvertent rather than deliberate. Despite the challenge to minimise this probable error by interviewing players and re-explaining how to properly record their nutrition in diaries almost every day, the possibility of missing meals and wrong portion sizes cannot be excluded. The selected food recall diary method for monitoring energy intake was examined for inaccurate reports according

to previous studies comparing diet assessment methods (Brunner, Stallone, Juneja, Bingham and Marmot, 2001). The likely reason for this error could be that food-diary completion and analysis are time consuming. It was estimated that food diaries take a minimum of 30 min for the subject to complete and a further 45 min/day for researchers to analyze.

A very important parameter that may have influenced participants' energy intake in terms of food choices and also consumed quantities, could have been that UKpro group was taking on a daily basis breakfast and lunch at team's restaurant where guidance for foods recipes, cooking methods and quantities were under experienced chefs. On the other hand, footballers in Cyprus groups had to eat on their own, at home or at local dinning places. Moreover, professional practitioners were employed in UKpro team with important roles regarding nutrition advices and additional supplementation on individual needs whereas CYsem and CYrec did not have such ease by their clubs, perhaps due to financial matters and generally team available budgets.

The UKpro restaurant head chef shared some major targets which were pursuit through teams' daily cooked recipes:

- Healthy recipes, no fat just a bit of olive oil
- Recovery nutrition after game
- Red meat when there was no game next day
- Vegetable soups before lunch, to cover the players' needs if they did not consume vegetables.
- Special daily attention to macronutrients percentages included in menus

5.2.1 Macronutrients

In parallel to total energy intake, carbohydrate percentage of total energy intake was much lower than recommended values given from previous studies (2000 – 2400 $kcal.d^{-1}$). In more detail, carbohydrates energy intake was constantly higher for UKpro compared to the other groups through all three testing blocks. Noticeably at all three blocks, UKpro presented values near to equal with pre-season 784.43 ± 56.98 $kcal.d^{-}$

[1], mid-season 789.00 ± 55.91 kcal.d^{-1} and end-season 782.00 ± 51.01 kcal.d^{-1}. Despite the fact that at mid- and end-season UKpro had the highest values among all groups these differences were not statistically significant ($p > 0.05$). It was interesting to note that carbohydrate energy intake was the highest, as expected for the UKpro group, even though a significantly higher intake was not found at pre-season as hypothesized. Therefore hypothesis (H1ii) for carbohydrate energy intake was rejected and null hypothesis (H0a) was accepted. The carbohydrate energy intake in parallel to total energy intake was of considerable lower amounts to those displayed in a previous study (Maughan et al., 1997). During all three testing blocks, the carbohydrate energy intake is very similar to total energy distribution. At the moment, it should be noted that energy intake seasonal trend similarly to training load, was near to equal for UKpro between testing blocks. This, nevertheless, represents a good level of nutritional guidance for the UKpro footballers.

In detail for CYsem, data from 656.67 ± 35.05 kcal.d^{-1} pre-season increased to 702.86 ± 71.58 kcal.d^{-1} at the mid-season and moreover to 722.29 ± 37.18 kcal.d^{-1} at end-season point. Finally, CYrec at pre-season 902.67 ± 69.25 kcal.d^{-1} presented the highest value than any group at all time-points. On the contrary, at mid-season CYrec had the lowest value than any group at all time-points 538.67 ± 105.37 kcal.d^{-1} and in the end-season increased at 647.67 ± 55.83 kcal.d^{-1}. Conclusively, there was not significantly high**er** carbohydrate intake by UKpro group, since pre-season values compared to the following groups were higher only for CYrec and therefore hypothesis (H1ii) was rejected and null hypothesis (H0a) was accepted.

In agreement with previous researchers carbohydrate should meet the energy requirements of their training programs and optimize restoration of muscle glycogen stores (Burke *et al.,* 2004). Despite the generally low reported total energy intake, the percentage contribution from carbs was high and near to equal across season testing-blocks. Generally, an important underreporting energy intake is apparent. According to the training load energy cost and the energy intakes findings of the current research, if nutritional intake reporting was precise, participants of all groups should have either been exhausted with all the consequences arising from such state, or through season a substantial body muscle and fat mass loss should have been reported. Nonetheless, the composition analysis did not reveal any significant changes.

In the literature, the recommended daily intake of carbohydrates to maintain muscle glycogen stores during continuous days of intense training is 500 – 600 g or 60 – 70% of total energy intake (Maughan, 1997; Clark, 1994). Although some lead researchers tried to form a structured guideline for daily carbohydrate intake, more football specific studies are needed in advance to practical application to footballers. Furthermore, the interpretation of specific formulas including anthropometric status and food energy analysis could create various misleading guidelines. While evidence generally indicates the importance of high carbohydrate availability during the competition and glycogen availability contributes to optimal performance, similar findings are still needed regarding the training session demands (Burke et al., 2011). Assessments of the required energy intake for training sessions should consider the mass of the footballer and the metabolic requirements arising from each session.

Protein energy intake results, in the same way to carbohydrate and fat energy intake, showed values for UKpro near to equal through all three testing blocks. The probability of a well-structured nutritional program by professional practitioners through season could explain this consistency among UKpro footballers. These values were higher than the other groups at pre- and mid-season. The research hypothesis (H1iii) was expecting to find significantly higher values for UK group based group. Indeed, via statistical analysis significant interaction between groups was found and UKpro was the group with superior values with the exception of end-season. Therefore hypothesis was accepted. For example, at pre-season UKpro with (524.00 ± 44.82 kcal.d^{-1}) v CYsem (289.33 ± 43.14 kcal.d^{-1}), (p = 0.001) and UKpro v CYrec (399.00 ± 17.81 kcal.d^{-1}), (p = 0.041).

The recommended dietary allowance (RDA) for protein is 56g (224kcal) or 0.80g.kg^{-1}.day^{-1} for adult males (DRI, 2002). However protein for footballers has been recommended in higher quantities by Lemon (1994), at 1.4-1.7 g.kg^{-1}.day^{-1} with respect to resistance training sessions that are part of modern football training. Therefore expectations of protein energy intake as calories for the footballers should have been around 360-500 kcal.d^{-1}. Generally, studies agree that protein availability is critical for optimizing many of the adaptations that take place in muscle in response to both endurance and resistance training. On the other hand, Hawley (2006) identified that there was not enough evidence to suggest that soccer players need to consume greater daily protein than most athletes. Actually, a high-protein diet could

actually constrain the response of muscle protein synthesis to exercise and therefore intermittent or high intensity training would not benefit from high levels of protein intake. Mainly the muscle protein synthesis differ with initial evidence proposing that high protein intake leads to lower levels of protein synthesis and declined muscle protein breakdown follows, a disruption of net muscle protein balance could presumably occur. A study by Bolster (2005) indicated that consumption of higher amounts of protein (3.6 g/kg) by endurance trained males resulted to increase post-exercise free amino acid availability. Muscle protein synthesis rate was decreased post-exercise, while essential amino acids (EAA) and branched amino acids plasma concentrations of were high. Increased amino acid availability could either higher protein synthesis post-exercise or limiting the body's reliance on endogenous sources of these free amino acids, thereby limiting proteolysis during exercise.

Most confirmed benefits regarding protein ingestion concern the post-exercise meals. Accordingly, a combination of protein and carbohydrate mixture was evidently beneficial for faster muscle recovery. The importance of protein balance is improved via the ingestion of a post-training mixture including carbohydrate and amino acids, leading to an enhanced protein synthesis. Karlsson (2004) recommended that branched-chain amino acids (BCAA) supplementation enhances protein synthesis during recovery from resistance training. In footballers training regime, resistance training is usually included in 1-2 sessions per week depending on the season time-point. However, immediate post exercise protein intake is not as important as total energy intake and protein intake over a 24hr window. It also depends on when the athlete is training again. It could therefore be of assistance to footballers, a nutritional strategy that included protein supplementation based on individual needs in parallel to the training type.

The results for energy contribution from fat intake showed that during all testing blocks, CYrec group was consuming higher quantities of total fat than the other two groups. Generally these differences were not significant, even during the highest difference at pre-season CYrec 697.50 ± 70.71 kcal.d^{-1}, UKpro 471.45 ± 38.88 kcal.d^{-1} and CYsem 445.50 ± 102.64 kcal.d^{-1}. Notably, the current research mean results for CYrec fat decreased by 96 kcal.d^{-1} between pre-season and mid-season while total energy intake showed the highest values at 2006.50 kcal.d^{-1} pre-season and decreased by 452kcal.d^{-1} at mid-season. Also during the same testing blocks,

average mean training load as energy cost was decreased only by 17 kcal.d^{-1}. On the opposite as hypothesis (H1iv) expected, there is an obvious higher fat energy intake at all testing blocks for CYrec in comparison to UKpro that was not significant (p > 0.05) and therefore hypothesis (H0a) was rejected and the null hypothesis was accepted.

Fat energy intake fraction of total energy intake is analogically high for both Cyprus groups. Similarly, previous researches have stated that Mediterranean based players' food choices of macro and micronutrients may differ to other countries. Generally, research has indicated that athletes tended to have inadequate carbohydrates intakes and higher than recommended fat intake. A comparison of Greek elite female athletes from different sports with a non-athletic group reported inadequate total energy intakes compared to daily energy expenditure and fat energy intake ranged from 36.6 to 41.6% of energy intake (Hassapidou and Mastrantoni, 2001). Some differences between the current and a previous study which included professionals from a Mediterranean country with very similar cuisine (Greece) were found to exist. The study showed fat intake near to 40% of total energy intake from participants records (Hassapidou et al., 2000). The researchers stated that their findings were characteristic of Mediterranean nutrition. The Mediterranean diet often is cited as beneficial for being low in saturated fat and high in monounsaturated fat and dietary fiber. One of the main explanations is assumed to be the health effects of olive oil included in the Mediterranean diet. In a more recent review, Burke and Kiens (2006) concluded that potential investigation of the probability that chronic adaptation to high fat diets during the training phase could be beneficial to athletic performance by offering alternative exercise energy fuel for the muscle than its often inadequate glycogen stores.

Other previous studies raised the suggestion that availability of muscle glycogen due to fat-adaptation may possibly be due to diminished glycogenolysis. Therefore, in anaerobic sports similar to football this modification may not offer metabolic advantages (Stellingworth et al., 2006). Professional footballers have been recommended to consume less than 30% of their total energy needs from fat (Clark, 1994). The Acceptable Macronutrient Distribution Range (AMDR) for total fat intake is 20-35 g.d^{-1} for adult males (DRI, 2002).

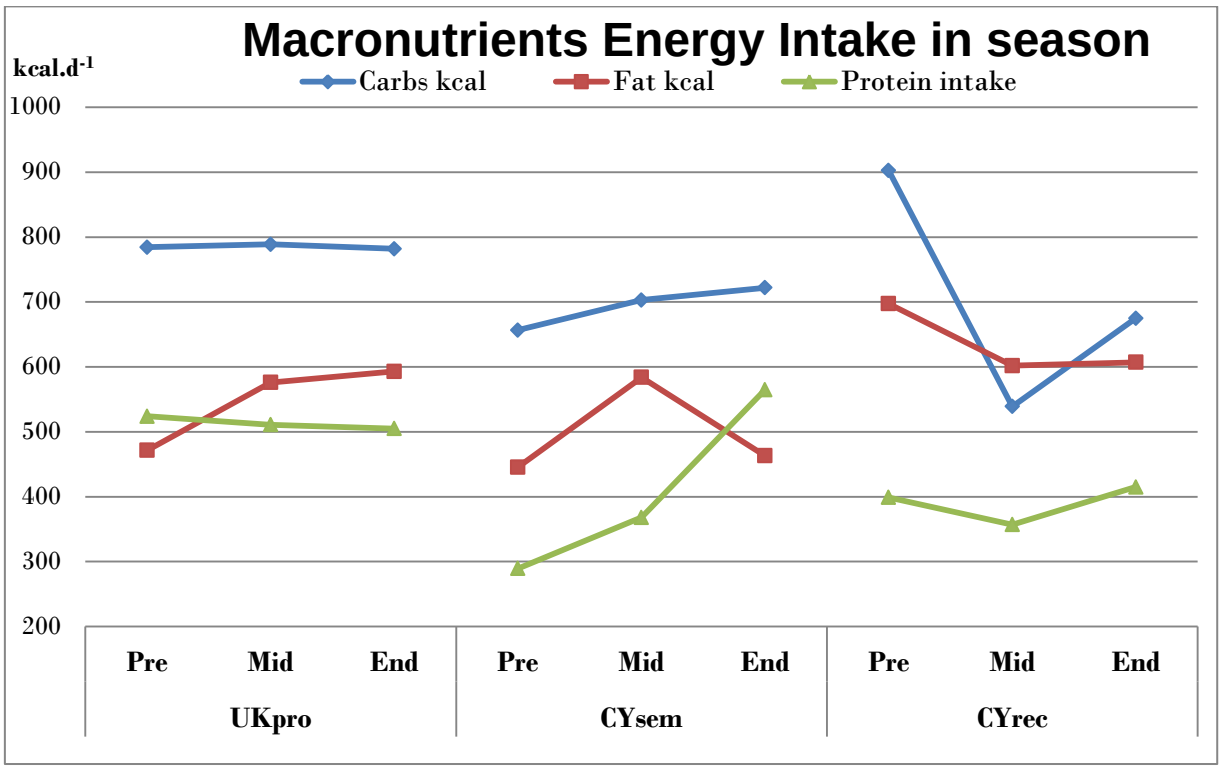

Figure 5.2.1 Fraction of carbohydrate, fat and protein energy intake across testing blocks in the season for all groups. The UKpro shows near to equal total energy intake across testing points, almost by similar contribution from each macronutrient.

In line with nutritional strategies supporting optimum performance in football, the understanding of the energy cost seems to be the most important parameter for getting the appropriate quantities of energy intake. Mainly, there are two major theories establishing the requirements of daily energy intake. Some researchers (Shephard, 1999), suggested that energy intake should be calculated in relation to kilograms of fat-free mass, while a different approach is suggested by Burke (2011) for providing the athletes with absolute amounts of CHO rather than scaled to body mass, always with training and competition energy cost regime in mind.

5.2.2 Selected Micronutrients

Findings for selected micronutrients indicated high quantities for UKpro across monitored season testing-blocks. Despite the fact that conclusively, across testing points UKpro presented in average the highest micronutrients intake, only vitamin C was found in significantly higher quantities. As regards to hypothesis (H1v), it was

expected that UKpro would have reported significantly higher quantities for all nutrients including the micronutrients. Therefore, while for vitamin C the null hypothesis (H0a) was rejected and alternate hypothesis was accepted, for vitamins D and E, magnesium and Zinc H0a was accepted. When linked to the findings of higher energy intake for UKpro in comparison to the other groups, an expectation was raised for similar results among micronutrients. Interestingly, at pre-season when CYrec total energy intake was the highest of all groups at any testing point, similar findings were found for vitamin D and E.

Previous researchers suggested that average requirements of vitamins and minerals for athletes should be higher than the RDA for healthy (non-exercisers) individuals (Magkos et al., 2003; Clark et al., 2003). Conversely, the literature seems to support the theory that if energy intake is sufficient, balanced and varied, supplementation for vitamins and minerals may not be necessary. Findings of the current study can only be reported in relation to recommendations for average healthy adults, since there are not any reports in literature or Dietary Reference Intakes (DRIs) for footballers or sportsmen more generally. Findings of the current research, suggest that footballers largely consume more than RDA% quantities of micronutrients, maybe because they believe it could be of assistance to a better performance or recovery. This however, cannot be supported before further investigation in the future.

As results showed, vitamin C intake for UKpro had significantly higher values at all three testing time-points compared to the other two groups. Interestingly, this group had the highest values at 185.14 ± 25.99 $mg.d^{-1}$ end-season, while the other two groups had their highest values at pre-season with CYsem132.52 ± 45.34 $mg.d^{-1}$ and CYrec 121.66 ± 16.73 $mg.d^{-1}$. The recommended dietary allowance (RDA) for vitamin C is 75-90 $mg.kg^{-1}.day^{-1}$ for adult males (DRI, 2002). While it is admitted that the needs of athletes are higher than those of less active individuals, similarly a well-balanced diet which covers athletes higher energy requirements should be able to provide the necessary vitamin C supply (>200mg/d). Naturally though, higher energy demands from training are met with higher nutritional intakes, which consequently offer greater ingestion of micronutrients as well. In general, the background literature agrees that athletes' individual nutritional goals should be established by a qualified professional. Similarly to healthy (non-exercised) individuals, some athletes who are involved in heavy exercise while facing problems with upper respiratory tract

infections may benefit from supplementation of vitamin C. On the other hand, supplementation of vitamin C has generally failed to show consistent results in improving oxidative burst activity. Consequently, stress period after high- intensity training sessions could be important periods for vitamin C deficit awareness in order to avoid susceptible immunity.

Results for vitamin D showed that at pre-season UKpro with 4.34 ± 0.88 $\mu g.d^{-1}$ and CYrec with 5.16 ± 1.05 $\mu g.d^{-1}$ had the highest values while in contrast CYsem showed the highest values at end-season 3.72 ± 0.71 $\mu g.d^{-1}$ after lower near to equal pre- and end-season. Later at mid-season 3.39 ± 0.84 $\mu g.d^{-1}$ and end-season 3.32 ± 0.80 $\mu g.d^{-1}$ UKpro values were decreased and very equal. Also CYrec was decreased after the highest pre-season, with 2.13 ± 0.37 $\mu g.d^{-1}$ mid-season and 2.90 ± 0.65 $\mu g.d^{-1}$ end-season. The recommended dietary allowance (RDA) for vitamin D, is 15 $\mu g.d^{-1}$ (600 $IU.d^{-1}$) for adult males 18-50 years old (DRI, 2002).

Results for vitamin E showed that during all three testing blocks CYsem group had the lowest values with 4.59 ± 1.15 $mg.d^{-1}$ pre-season, at 4.24 ± 0.61 $mg.d^{-1}$ mid-season and at 5.44 ± 0.30 $mg.d^{-1}$ end-season. UKpro started with 7.99 ± 1.47 $mg.d^{-1}$, increased at 8.25 ± 1.74 $mg.d^{-1}$ mid-season and had the lowest values at 6.85 ± 1.15 $mg.d^{-1}$ end-season. Similarly, CYrec started low with 5.39 ± 0.71 $mg.d^{-1}$ pre-season, increased importantly but not significantly at 9.19 ± 2.70 $mg.d^{-1}$ mid-season and decreased at end-season 6.10 ± 0.30 $mg.d^{-1}$. The recommended dietary allowance (RDA) for vitamin E, is 10 $mg.d^{-1}$ for adult males 18-50 years old (DRI, 2002).

Data for magnesium across all testing blocks showed high quantities for UKpro group particularly in comparison to the other two groups. The highest values were found at pre-season for UKpro (359.90 ± 45.04 mg) and CYrec (290.45 ± 20.66 mg), and at end-season for CYsem (266.83 ± 11.57 mg). A decreasing trend was apparent for UKpro and CYrec mean values, from first to last time points. In the footballers' food recall diaries, additional supplementation was reported mainly regarding magnesium in liquid form. This could have been suggested by a professional practitioner or it was purely individuals' choices. Magnesium's benefits can include reduced symptoms from conditions such as chronic pain, fatigue and insomnia. Footballers take magnesium because it is involved in neuromuscular transmission and activity and

muscle relaxation. The recommended dietary allowance (RDA) for magnesium is 400-420 $mg.d^{-1}$ for adult males 19-50 years old (DRI, 2002).

Results for zinc intake showed that the lowest values were found for CYsem with 8.66 ± 1.19 $mg.d^{-1}$ pre-season increased to 9.55 ± 0.47 $mg.d^{-1}$ mid-season and to 10.10 ± 0.56 $mg.d^{-1}$ end-season. In contrast, the highest values were found for UKpro with 13.32 ± 1.45 $mg.d^{-1}$ pre-season to 13.41 ± 1.39 $mg.d^{-1}$ mid-season and later to 12.71 ± 1.17 $mg.d^{-1}$ end-season. Finally, CYrec had initially 11.60 ± 0.84 $mg.d^{-1}$ pre-season, a decrease to 9.84 ± 1.17 $mg.d^{-1}$ and over to 10.75 ± 0.86 $mg.d^{-1}$ end-season. The recommended dietary allowance (RDA) for Zinc, is 11 $mg.d^{-1}$ for adult males 19-50 years old (DRI, 2002). Zinc, being an important mineral, plays a vital role in protein synthesis and helps regulate the cell production in the immune system of the human body. Generally, zinc can be found in the strongest muscles of the body and is found in especially high concentrations in the white and red blood cells. Similarly to magnesium, nutritionists and football practitioners often advice footballers to take additional supplementation, in order to sustain higher training load and recover faster.

In relation to this study, additional ingestion for footballers could be considered in parallel to performance targets for individual e.g. footballers with increased body fat or special eating habits. Evidently, previous researchers stated gap in research regarding the probable advantages for sportsmen regarding their performance and health (Margaritis, 2008). In addition, studies involving energy intake analysis of footballers and probable higher antioxidants intake needs are still to be established. Urso (2002) stated that antioxidants and trace element intakes are necessary to allow endogenous adaptation and to avoid excessive stress as a result of heavy exertion. More importantly, antioxidant-rich foods have bioactive properties which may support training adaptations and individuals in Mediterranean countries including athletes may naturally consume higher quantities of polyphenol rich compounds. As Cypriot teams' findings showed, training energy cost did not have negative impact on physiological stress markers, which could be an indication that nutritional energy intake was more of assistance to footballers recovery. The long-term risks of deficiency, as well as excessive intakes of antioxidant micronutrients, are largely unknown especially in football because they are still poorly investigated in athletes (Nieman et al., 2006). The combination of low energy and yet higher than RDA%

micronutrient intake and probable meal quantities irregularities shows the need for long-term studies evaluating dietary intake and nutritional status among athletes of all levels. Moreover, an indication for additional micronutrients supplementation may occur, which was not reported as instructed in the food recall diaries.

5.3 Training load

Training load findings revealed that energy expenditure for UKpro was not significantly different neither regarding the average daily or training day energy cost between all three testing blocks, in contrast to previous research that included professional footballers, where higher training load was found at pre-season compared to the mid- and end-season. While pre-season training varies according to specific strategies of microcycles, it is commonly accepted that the physiological demands of this stage of training are higher than at other times in the football season (Jeong et al., 2011; Svensson et al., 2007).Also, CYsem and CYrec did not change significantly the average daily or training day energy cost between testing blocks. Therefore, hypothesis (H2) regarding training load for all groups between testing blocks was rejected, since pre-season was not significantly higher for none of the teams. Notably, CYrec had higher but not significantly training load during pre- and end-season than CYsem, which could be an indication of similar if not better physiological status for CYrec.

Average energy expenditure per day of the monitored block (week) was expected to be higher in response to pre-season training plan, which is traditionally endurance based, and would decline throughout the season due to the imminent demands commencing from repeated games. However, this particular group of professionals (UKpro) seemed able to maintain and even increase training load but not in a significant manner, throughout the year. The periodization (macrocycles) plan maybe was strategically structured with similar training load in terms of energy expenditure. A different explanation could be that footballers did not have sufficient rest from previous football season and therefore a more moderate pre-season loading was assessed. Also semi-professional group (CYsem) and recreational group (CYrec) had higher training load at end-season than at pre-season. For these two groups,

periodization in terms of macrocycles was not assessed, mainly because the season testing blocks did not differ greatly in terms of team targets with one game per week. Moreover, the lower pre-season could be according to the lower physical condition of footballers caused by a long break before starting the new football season. As hypothesis stated, UKpro had significantly higher training load than the lower level groups. However, the hypothesis regarding training load between testing blocks was rejected since pre-season was not significantly higher. Notably, CYrec had higher training load in a not significant manner, during pre- and end-season than CYsem.

A typical week for UK professional team included six training sessions in five days and the day after the match free. Through the week, 'tapering' with higher intensity and session duration was met typically on the third and fourth day after game. In agreement, a similar training plan was stated in earlier researches in which professional footballers had participated (Bangsbo et al., 2005). On the other hand, CYsem and CYrec groups did not have a steady number of training sessions across the three weeks but these were not less than three sessions plus a game, across all testing points. By estimating the energy expenditure from training sessions through a standardised methodology (MET) and double monitored in parallel GPS 10Hz units, results from estimated training load cannot be suspected for major flaws.

Unfortunately, in order to understand more of the training impact on the participants, a battery of fitness tests including an intermittent high intensity protocol should have been assessed at every testing block (Bangsbo, 2006). Although this was the initial aim, assessment for data collection was not concluded due to assessment sequence interruption, after one of the participating club manager's considerations regarding probable team fatigue. Therefore it was not possible to establish the training impact individually on participating footballers' fitness level. Had the players been training harder at the start of the pre-season period, one might have expected a significant increase in training load due to higher percentage of aerobic training sessions through the pre-season and playing season periods.

Analogous results were found for the training day average energy cost, with UKpro showing significantly higher energy cost per training day. Therefore hypothesis (H2) suggesting higher training day energy cost between groups was accepted. Yet, pre-season training days energy cost was not higher than the next two testing blocks. On

the contrary, the highest energy cost of training days was observed at mid-season for UKpro group (3021.48±77.59 kcal) and at end-season for both CYsem (2417±61.88 kcal) and CYrec (2527.22±71.20 kcal) respectively.

These findings are not similar with previous studies which indicated that most seasonal training programs are designed, with heavier training load during pre-season in terms of weekly training sessions' number and duration although this may differ between clubs with regards to player standards (Bangsbo, 2005; Jeong, 2011). Nevertheless, with respect to the principle of individualisation and the theory of supercompensation adaptations following different exercise volume, training planning for short or long periods is anything but simple. Finally, only the outcome performance can be recognized as favourable physiological adaptation at the right timing (Lambert and Borresen, 2006).

Unfortunately, performance by means of official games could not be monitored during this research due to official matches regulations that would not allow the use of GPS devices. As previous researchers found important performance differences between high-level and moderate or recreational footballers, comparable results were expected for the training load in this research. Additional studies had previously found significant differences in performance parameters between teams from different countries (Dellal et al., 2011; Rampinini et al., 2009). Indeed, by using GPS devices (10Hz) as a parallel to the main method (MET) of training energy cost estimation, it was found that distances covered at training sessions, were notably different regarding total distances and high-intensity running distances. It should be noted that these assessments were only used as a control assessment for a better accuracy of MET estimated values.

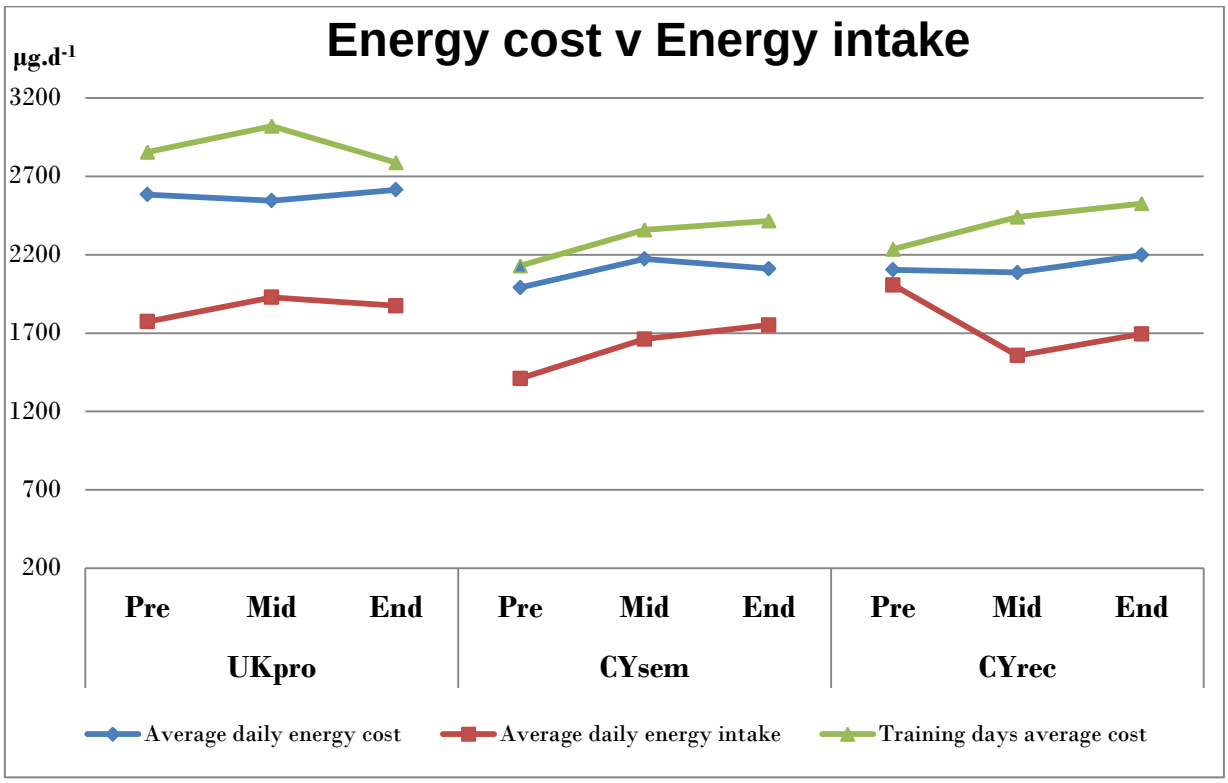

Figure 5.3 Energy cost (Average daily energy cost and Training days average cost) parallel to Average daily energy intake, at all three testing blocks. An visible similar trend is presented between energy cost and energy intake for CYsem.

5.4 Physiological stress markers

5.4.1 Salivary IgA

Findings for sIgA-pre training concentrations as reported from the analysis of collected samples did not change significantly for none of the groups between testing blocks. The IgA-after training with pre training levels as reference, developed differently between all groups. For example, at mid-season UKpro was slightly decreased while CYsem and CYrec were increased by 33 μg/mL and 121 μg/mL respectively. Interestingly, IgA-next day when compared with after training levels, it was increased at pre-season for UKpro by 80 μg/mL and 115 μg/mL for CYsem (Figure 5.4.1). The insignificant differences across season for pre-training samples and the after-training increase could be an indication for very high-intensity training sessions for the footballers of both teams.

According to previous researchers, salivary IgA levels increase significantly immediately post-exercise sessions, while after a second same day training session salivary IgA outcomes do not appear to suppress (Vahid Sari-Sarraf et al., 2006). More recently, a study by Henson (2007) was conducted in a group of ultra-marathon runners and during the two weeks post-race, about 25% of the runners reported URTI episodes. It was found that pre and post plasma cortisol as post-race values were almost doubled in comparison to pre-race values (412 ± 29 $ug.d^{-1}$, 806 ± 97 $ug.d^{-1}$). The antibody of s-IgA which was also examined at the same study followed the opposite than cortisol trend and its secretion rate was decreased for more than 54% compared to pre-race levels. Though, the findings of the current research support such statement (Figure 5.4) via Cyprus groups' results at all testing blocks, plus UKpro pre-season block. Also it should be noted that the testing protocol was designed for collecting saliva samples as reference to a day with hard training load in the weekly program.

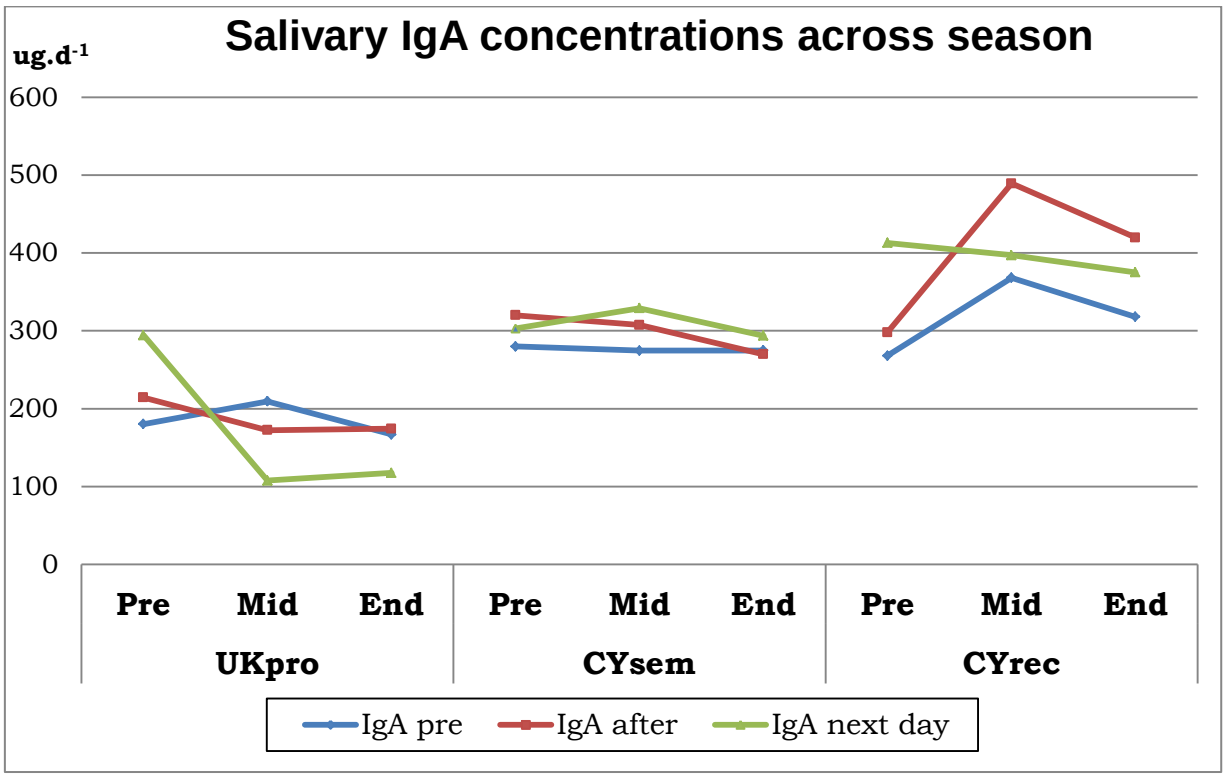

Figure 5.4.1 Salivary Secretory Immunoglobulin A across season for all groups (i) pre-training, (ii) after-training and (iii) next-day training. A very similar CYsem IgA concentration changes reflecting training impact on stress marker at all three testing points.

Salivary Secretory Immunoglobulin A pre training

In general, within groups salivary IgA pre-training concentrations retained nearly similar values between the three testing points. Only CYrec showed relatively higher values at mid-season but no significant difference was found.

Pre-training s-IgA concentrations were higher but not in a significant manner for CYrec compared to the other groups from pre-season 267.53 ± 46.40 (ug.ml^{-1}) to mid-season 368.21 ± 45.85 (ug.ml^{-1}) and later to end-season 318 ± 28.42 (ug.ml^{-1}). On the contrary, UKpro had the lowest values at all testing points with pre-season at 180.07 ± 18.94 (ug.ml^{-1}), which increased at mid-season 209.46 ± 65.71 (ug.ml^{-1}) and decreased at lowest level by end-season 166.68 ± 25.03 (µg/ mL). Interestingly, CYsem retained very similar average values through pre-season 279.77 ± 85.88 (ug.ml^{-1}) to mid-season 274.57 ± 42.85 (ug.ml^{-1}) and later to end-season 274.86 ±

28.96 (ug.ml^{-1})., Since, significantly different concentrations were not found for groups between testing blocks, consequently null hypothesis (H0c) for IgA was accepted.

Salivary Secretory Immunoglobulin A after training

Salivary secretory immunoglobulin-A concentrations after training session found that UKpro had the lowest values at all testing points. On the contrary, CYrec group had high values at all three testing time-points, particularly at mid- and end-season. Despite visible differences within CYrec pre-season 298.40 ± 70.30(ug.ml^{-1}) v mid-season 489.41 ± 77.57(ug.ml^{-1}) and end-season 419.78 ± 49.03(ug.ml^{-1}) there was not any statistically significant interaction (p > 0.05). Also CYsem did not have any statistically significant differences from pre-season 320.13 ± 48.87(ug.ml^{-1}) v mid-season 307.57 ± 38.51(ug.ml^{-1}) and end-season 269.86 ± 49.03(ug.ml^{-1}). Consequently, hypothesis (H3i) for IgA can be rejected.

The findings for s-IgA after training could be interpreted in line to individuals professional status and accordingly to fitness profile. In theory, the fitness level of the participants was analogous to team status and therefore a higher impact of high-intensity training session would have been expected for semi-pro and recreational group footballers. As such, lower values are reported for UKpro group and similarly higher values for semi-pro and recreational groups even though these were not significant. However, these values alone could only be substantial if were supported by physiological profiles from participants, an assessment which however was not completed due to unpredicted limitations. Furthermore, it is well established in literature that there are not any norms but instead individual profiles should be adapted via several samples through time. Moreover, the initial pre-training levels of concentrations should be taken in account when observing the differences that could give the impression of training impact on this stress marker (Refer to table 5.4.1).

Salivary Secretory Immunoglobulin A prior to next day training

It was found that UKpro had considerably lower values of IgA-next in comparison to CYsem and CYrec groups at all three testing time-points. Interestingly, CYrec retained much higher values than the other groups during all testing points and had the highest concentrations at pre-season 413 ± 100.51 (ug.ml^{-1}). UKpro had high

values only at pre-season 294 ± 44.92 (ug.ml^{-1}), while following testing blocks were very low at 107.50 (ug.ml^{-1}) and 117.45 (ug.ml^{-1}). On the contrary, CYsem showed a balanced constancy from pre-season 303.01 ± 44.23(ug.ml^{-1}) v mid-season 329.00 ± 42.06(ug.ml^{-1}) and end-season 293.71 ± 47.25(ug.ml^{-1}).

Salivary secretory immunoglobulin-A concentrations for the next day of selected training session showed CYrec group with the highest values and UKpro with the lowest values at all testing-blocks. Interestingly, UKpro at pre-season had higher values than the next testing blocks which were near to equal. A probable explanation for the higher pre-season IgA concentrations of UKpro could be the post-game insufficient time for recovery, inadequate energy intake or even the higher stress due to starting with expectations for the new season. Regarding the s-IgA concentrations for both the Cyprus teams, the average near to equal values that they present during all testing blocks could be an indication for their fitness level. For example if the participants training sessions were considered as high intensity and volume for recreational footballers, but their physiological levels were similar to higher level footballers (e.g. VO_{2max}), the s-IgA concentration would therefore react to a moderate training load impact. Furthermore this could be the result of a balanced training load and nutritional intake strategy. Selected micronutrients findings, generally showed that footballers consume foods rich in antioxidants or additional supplements to cover any probable deficiency.

5.4.2 Testosterone/ Cortisol Ratio

There have been several researches on the responses of cortisol and testosterone to exercise, but not much data on the subject of their patterns of response to different exercise methods. Moreover, while these anabolic and catabolic hormones have been studied as exercise related physiological stress biomarkers, each has a unique response to physical exercise due to their different origins. The increase of free testosterone to cortisol ratio in this study may indicate lower physiological stress in response to performing these exercises.

Testosterone/ Cortisol Ratio Pre-training

Findings of Testosterone/Cortisol Ratio collected pre-training showed that values between groups varied across time. Considerably higher levels were demonstrated

for CYsem group at all-time testing blocks as results showed, while on the opposite, CYrec and UKpro demonstrated generally lower values throughout testing blocks. However, only at pre-season statistical analysis found significant difference between CYrec with 0.05 ± 0.01 μg/ mL and CYsem with 0.19 ± 0.06 μg/ mL. Interestingly and on the opposite of IgA results, pre-training values differed significantly for T/C ratio between CYsem and CYrec and therefore null hypothesis (H0c) was rejected.

Testosterone/ Cortisol Ratio collected after-training session (T/C-After)

Results showed different trends between the three groups across time. Higher values were found at all three testing blocks for CYsem with 0.18 ± 0.05 μg/ mL pre-season, decreased later at 0.16 ± 0.05 μg/ mL mid-season and 0.12 ± 0.04 μg/ mL end-season. An interesting point was UKpro change through time, since it started with low values at pre-season 0.07 ± 0.01 μg/ mL, increased more than double later at 0.16 ± 0.05 μg/ mL mid-season and low again at 0.08 ± 0.01 μg/ mL end-season. Finally, CYrec had low pre-season values with 0.08 ± 0.02 μg/ mL and increased later with near to equal values at 0.14 ± 0.05 μg/ mL mid-season and 0.14 ± 0.04 μg/ mL end-season. These findings for post training sessions are in line to IgA results which did not show a major negative impact from training load. The T/C ratio was not always increased after training, but instead a variation was observed on the results among groups and across the testing points. Firstly, for UKpro an increase compared to pre-training values was found only at mid-season and for CYsem a decrease appears at all three testing blocks. Secondly, on the other hand CYrec retains higher post training values across testing points.

Findings for Testosterone/ Cortisol Ratio collected Next-day before training session (T/C-Next) showed different leanings between the three groups during time. For Ukpro and CYsem next-day, values at all three testing blocks were decreased at lower than pre-training levels. Interestingly, CYrec at pre-season had T/C ratio next-day higher than pre- and after-training values, while at mid and end-season next-day levels were lower than both the other testing times. More analytically, higher values were found at all three testing blocks for CYsem with average mean values at ± 0.15 μg/ mL. The CYrec had high pre-season values with 0.11 ± 0.03 μg/ mL and decreased later at 0.05 ± 0.01 μg/ mL mid-season and 0.06 ± 0.01 μg/ mL end-

season. UKpro started with 0.07 ± 0.01 µg/ mL pre-season, decreased at 0.06 ± 0.01 µg/ mL mid-season and had its highest values at 0.10 ± 0.01 µg/ mL end-season.

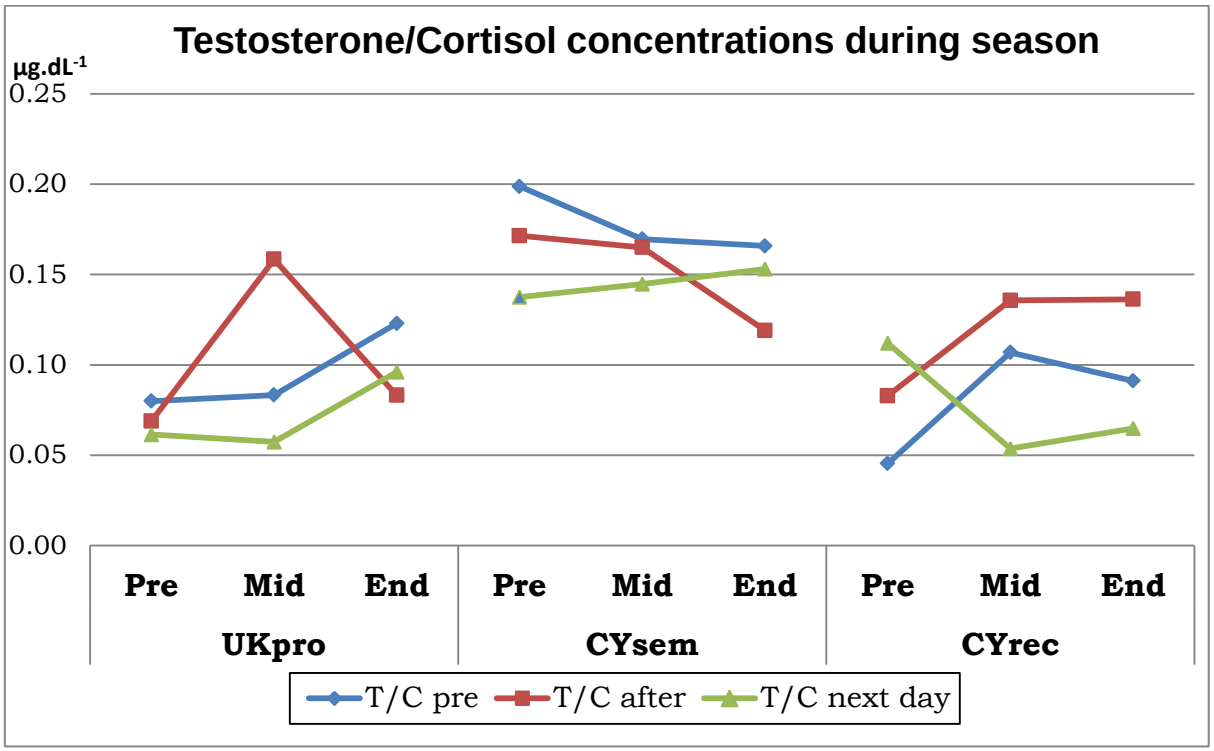

Figure 5.4.2 Testosterone/ Cortisol Ratio across season for all groups (i) pre-training, (ii) after-training and (iii) next-day training.

Evidence from the literature indicates that the increased susceptibility to URTI in athletes actually arises from a depression in immune system, usually caused from limited functioning of the immune system, influenced by stress and heavy training overloading. Mucosal immune system produces IgA which provides the first line of defence against pathogens and antigens presented at mucosal surfaces. Nevertheless, the prediction of URTI episode cannot be assessed simply by using this antibody alone, particularly for infections among sportsmen. Furthermore, there are no universal norms for analytes such as IgA and the interpretation of the results can only be done with respect to the principle of individuality and after considering the normal ranges for the individual through several repeated testing points (Gatti and De Palo, 2011). Also researchers supported that long training sessions with high intensity actions and insufficient recovery between games seem to sustain a depressed immune function for longer periods. A high incidence of infections is

reported in individuals with selective deficiency of IgA or poor saliva flow rates. In parallel, high levels of IgA are associated with low incidence of upper respiratory infection. However, the connection of immune markers with URTI was not an aim of this research.

The salivary testosterone-to-cortisol (T/ C) ratio has been widely used, because this avoids the stress caused by venepuncture. Investigation of psychophysiological stress during high-intensity training can use the salivary testosterone-to-cortisol (T/ C) ratio to observe the impact of training and performance on footballers (Gatti et al., 2011). Cortisol is considered to be the main catabolic hormone, by means of reducing protein synthesis, surges protein degradation and prevents the inflammatory process and immunity. Salivary cortisol hormone circulates freely through the salivary gland and shows little change in the flow rate variation, therefore has been recommended as an index of training stress. Interestingly, Doan (2007) found that cortisol was significantly lower throughout the competition compared with the baseline levels while in contrast testosterone remained the same. On the contrary, Cadore (2009) observed a moderate increase in salivary free testosterone after strength resistance exercise training in males, but no significant correlation was found between the concentrations of serum and salivary free Testosterone (sT), either at rest or after exercise. Depending on the intensity and extent of training sessions, the anabolic or catabolic hormones including testosterone and cortisol, show significant changes indicating a catabolic state. In agreement Jacks (2002), previously stated that type, duration, and intensity of exercise were related linearly with the sT concentration.

The anabolic and catabolic salivary components do not all differ due to physiological stress and various factors such as salivary flow rate and time of day (diurnal course) have different effects on them. As a rule, the salivary flow rate at highest values in the afternoon and linearly decreases to minimum during sleep. Regarding the testosterone and cortisol concentrations, the highest values take place in the morning and drop to their lowest concentrations levels in the afternoon and late at night. With these parameters considered, a previous study identified that testosterone to cortisol ratio was increased significantly only after exercise on treadmill was above intensities of 70 % and 85 % of maximum heart rate (Fatolahi, Rasaee and Peeri, 2011). Despite that all groups in the current research were training in the morning, the

probability of different awaking time in relationship to the arrival for training time, could have influenced mainly the concentrations of cortisol. Despite the fact that the food recall diary included a specific section for noting down the hours of sleep, going to bed time, quality of sleep and various other details, these raw data were used more for observation purposes than for direct connection to any result. Additionally, training load was estimated only in terms of energy expenditure (calories.day^{-1}) and different exercises intensities were not directly investigated. Therefore, the findings of T/C ratio development among groups in the current research could have been influenced from sessions of higher than 70% of maximum HR intensity.

Through testing blocks, UKpro had a visible higher concentration level at mid-season which could be an indication of different training intensities than those during pre- and end-season. Interestingly at mid-season, while average daily energy cost (AD) seemed to be the lowest between the three testing-blocks, both the average training day energy cost (TD) and the average energy intake (AI) had the highest values, even though with not significant differences. Across season the CYsem retained considerably harmonized balance between values of training load and energy intake. The decrease of after training T/C ratio through the testing blocks could be suggesting that correspondingly the footballers were training in lower intensities. In line, findings for salivary immunoglobulin A, showed to be unaffected from training intensities as the concentration levels for all samples were near to equal across season. Finally, regarding CYrec T/C ratio the findings showed that after training concentration levels had increased in similar analogy across the three testing blocks. The higher next day concentration that was found at pre-season was probably more of an insignificant difference due to sample collection time than connected to training session intensity.

The reduction in the testosterone concentration in parallel to cortisol increase appears after strenuous exercise. Currently, it is believed that the concentration of testosterone into cortisol (T/C ratio) would be a physiological indicative of overtraining in which the individual is exposed to, but it does not necessarily means overtraining syndromes. Studies found that psychological conditions of true competition can induce greater hormonal responses than exercise in controlled laboratory environment and a game for training purposes cannot be compared to emotional stress caused at real conditions (Moreira et al., 2009; Haneishi et al.,

2007). Through a study with top-level professional soccer players, findings revealed minimal influence on salivary cortisol from intensive competitive match training. Obviously when the components of a real competition are removed, the physiological stress did not seem cable of inducing essentially the impact on the endocrine parameters in all athletes. The findings of the current research interestingly, showed that CYrec group at all three testing blocks increased T/C ratio post-exercise which could be an indication of a lower physiological stress in response to training load assessment.

5.5 Body fat percentage (%)

Regarding body fat percentage (%), hypothesis (H4) was accepted since values were significantly higher for CYsem and CYrec groups, compared to UKpro. In all three testing blocks, UKpro had significantly lower values than the Cyprus based groups with 7.91 ± 0.42 at pre-season, 8.10 ± 0.40 at mid-season and 7.81 ± 0.32 at end-season. Interestingly, UKpro showed near 50% and near 40% lower values in comparison to CYsem and CYrec groups respectively. Despite the improvement through season, values remained at high levels for CYsem at all three testing blocks with 17.24 ± 1.11 % at pre-season, 16.14 ± 0.92 % at mid-season and 15.04 ± 0.72 % at end-season. . Interestingly, CYrec results with 13.32 ±.1.05 % at pre-season, 13.33 ±0.83 % at mid-season and 13.17 ± 0.87 % at end-season, had near to equal values during all testing blocks and lower than semi-professional group.

Regardless of the fact that many studies have previously examined the changes in fitness levels between the start and finish of the pre-season, few have examined continued responses through the playing season as well. Carling (2010) examined the effects of positional group (goalkeepers, defenders, midfielders, and forwards) and exposure time to play (participation time in training and matches) in relation to in-season variations. Significant variation of fat percentage and fat-free mass percentage was recorded between different positional groups during the seasonal testing points. The research demonstrates that pre-season fat percentages and muscle mass of players, respectively improved during mid-season and end-season. This comes in agreement to current research findings for CYsem seasonal body fat

percentage improvement. Similarly, previous researchers (Kalapotharacos, 2006; Rico-Sanz, 1998; Maughan, 1997), indicated that body fat percentages results differed between teams from countries according to international ranking levels. As such, UK teams with much higher rank as national league when compared to teams from Cyprus national league, was naturally expected to show significantly lower body fat percentages. Reports for important body fat percentage and physiological differences among teams of different ranking from the same national league were indicated (Kalapotharakos et al., 2006; Mohr et al., 2002; Wisloff et al., 1998; Apor, 1988).

On the topic of footballers' body fat percentages, UKpro showed near to 50% and 40% lower values in comparison to CYsem and CYrec groups respectively. CYsem improved through season but values remained at high levels. On the contrary, results from the current research are not in agreement with the findings of Kalapotharacos (2006), regarding the body fat% between teams of different ranking in national league. Thus, the CYrec team showed at all testing points lower values than CYsem team. Unexpectedly, despite the fact that testing time-points differences were not significant, values during pre-season were the lowest for all groups. It was noted that several players reported for pre-season training in good body fat% condition in response to self-selected base running programmes in the close-season period. For UK based footballers this represents a current change in culture, where many footballers voluntarily remain active after the end of official league season, as opposed to previous decades, where players reported for pre-season training in a de-conditioned state (White et al. 1988).

Other factors may also account for the apparent lack of change in body fat% through the playing year. There could be a possible influence from injuries and different individual training effect in form of players. Although none of the players included in the final sample had any long term injuries (> 1 week), they may have had minor problems which could have influenced any of the parameters at any given testing time. Furthermore, footballers may have various training regimes according to whether they are playing as starters or substitutes. Body composition of each athlete during a typical season reflects their current form with significant influence on performance as physiological markers improve while muscle increases and fat mass decreases. A reduction in body fat is achieved through a negative relation between

nutritional energy intake and daily energy expenditure. Training contribution is essential to the increase of energy cost and thus managing and negative energy balance over the total daily energy intake. In the literature, there is considerable evidence that a low availability of energy, previously defined as total energy intake minus the energy cost of the athlete's exercise programme, has serious consequences on the hormonal, immunological, and health status of the athlete (Loucks, 2004).

5.6 Discussion Summary

In the present study, nutritional energy intake, training load energy cost and saliva stress markers were investigated across three time testing-blocks and between the three groups: (i) UKprofessional, (ii) CYsemi-professional and (iii) CYrecreational. Important findings pointed out the inadequate nutritional energy intake for all groups across season all testing blocks. Interestingly and opposite to hypotheses, both nutritional energy intake and training load energy cost did not differ significantly between testing-blocks. On the contrary, regarding the total season findings, they lead to the assumption that an analogous harmonized development seems to occur among the two parameters, especially regarding the CYsem group. Finally, CYrec during pre-season had the highest energy intake and in line the highest carbohydrate and fat energy intake. Remarkably, at the same testing block CYrec had the lowest training load across season.

Also the macronutrients fraction across season could probably be accordingly to teams' levels of professionalism, with all the corresponding parameters that may influence the footballers regarding their food choices, portion sizes and probably the ingestion of additional supplements. For example, the UKpro showed near to equal total energy intake between all testing blocks and a very concern fraction of each macronutrient energy intake. Definitely, this indicates higher knowledge of individual footballer energy needs on behalf of professional group, but also confirms the important role of qualified scientists and practitioners around the footballers.

Nutritional findings for CYsem and CYrec are also analogically related to training load changes between testing-blocks. Generally, the results from both groups direct to a

more unstable macronutrients fraction between testing blocks, with analogically high-fat percentage. However, this cannot be assumed as an unhealthy diet pattern only because of total fat energy intake. In line, the findings on selected micronutrients indicated that even with the low quantities of reported macronutrients, all teams were ingesting higher quantities than recommended daily allowance percentages. Consequently, it is imperative that footballers receive proper guidance how to report the types of food, cooking methods, portion sizes, extras like sauces and drinks with special reference to alcoholic percentages. Extra attention should be given to off-training periods, when it is more possible to change every day habits and food patterns (Volpe, 2007).

Both the findings for average daily energy expenditure (AD) and average training day energy expenditure (TD) of the monitored blocks (week) indicated lower than expected pre-season values, since generally traditional periodization training plan is endurance based and with more training sessions per week. Also the following two testing blocks retained or even increased their training load. Likewise the particular group of professionals (UKpro) seemed able to maintain and even increase training load but not in a significant manner, throughout the year. Notably, CYrec had higher but not significantly training load during pre- and end-season than CYsem. These findings could be an indication that periodization in football is largely focused on volume and intensities of training sessions, probably depending on the interval duration between seasons. Moreover, the balanced relationship between nutritional total energy intake, daily energy cost and salivary IgA for the CYsem, point out that salivary stress markers could be an accurate monitoring method that can help sport scientists and nutritionists to control the variables that influence training impact.

In the current study, the GPS receiver units of 10Hz and data analysis via Lagalacolli software, delivered various parameters of training performance including total distance covered, several running intensity classifications, estimated metabolic power, energy cost (calories) and many others. Some basic findings of GPS (total distance covered and energy cost) during pre-experimental study were considered as a gold standard for choosing a valid and reliable primary method to use at main experimental study. The energy cost estimation via MET values has been previously been validated and cross tested with other methods estimating energy cost by using HR, like flex heart rate (FlexHR) and simultaneous heart rate-motion sensor (HR+M)

methods. Accordingly, the validity of the current research results regarding training load in terms of energy cost should be considered definite. Global positioning systems are great technological assistance for creating the activity profile of athletes from team sports. New studies and literature information reintroduce from GPS new potentials and field applications (Aughey, 2011). Therefore, the two training monitoring methods can be assessed in real-training conditions in order to estimate the training load impact on footballers.

Generally, previous studies in the literature identified that concentrations of these stress markers act differently in response to high-intensity and strenuous training signals. Evidence has supported that salivary IgA levels increase significantly immediately post-exercise sessions and long training sessions with high intensity actions and insufficient recovery retain depressing immune functions (Vahid Sari-Sarraf et al., 2006). A paradigm or sustained suppress would be s-IgA concentration levels during two football games in three days.

For the purposes of the current research, salivary stress markers were investigated in parallel to training load estimation and nutritional energy intake. Interestingly, important findings from a previous study showed that physiological stress caused via football performance, was similar between elite and lower level footballers regardless the different game intensities (Krustrup, 2006). Therefore, stress markers were expected to be influenced in analogous patterns during the current research. Findings for CYsem revealed a harmonic relationship across testing blocks between total energy intake, training energy expenditure and salivary IgA concentrations at pre-training, after-training and next-day training samples (Table 5.3 and Table 5.4.1). The prospect of a smooth effect on stress markers like s-IgA, rising from energy balance between nutritional intake and training load, could therefore be the desired result from both training and nutrition planning. In previous studies, the assessment of salivary immunoglobulins and antimicrobial proteins has been shown to successfully represent the effects of exercise on mucosal immunity (Papacosta and Nassis, 2011).

Conclusively, the ability of football practitioners to appreciate the energy cost during training and performance is decisive in order to follow a cost-effective nutritional strategy. Both based laboratory studies and match analysis have well established the

importance of carbohydrate stores for football performance. In addition, the significance of food ingestion according to training and performance individual's needs have been recognised in the literature. Most studies point-out the important role of CHO before, during and post-exercise (Baldwin, 2003). In step, the appropriate monitoring scientific methods must be carefully selected in order to receive valid and reliable results, which will be of assistance to scientists for all parameter accurate estimations.

5.7 Limitations of the current research

The following section outlines limitations associated with the research process that was undertaken in relation to the studies which make up this thesis and recommends focuses for future research.

Initial research design included four (4) teams, two from UK and two from Cyprus. For every team, coach or fitness coach had been contacted for permission letter and their co-operation was requested. Even though permission letters had been received from all participating teams, the UK-recreational (UKrec) team did not act in accordance with what was agreed e.g. absence of coach at pre-scheduled meetings or minimum number of training sessions per week. Consequently, that team was excluded from the research.

Imperative limitations of resources to conduct a battery of physiological tests as part of the research design appeared during both pilot test and main experimental study. The scheduled running tests should have been assessed following the same protocols and due to the participation of footballers from two different countries, the use of laboratories in UK and Cyprus was needed. Therefore, Hertfordshire University Laboratory and Nicosia University laboratory offered the permission to use their facilities for the required tests. Unfortunately, despite the prior reassurance that Cyprus based Nicosia University Laboratory would have availability for the requested dates, on arrival to the laboratory to assess the tests, several required equipment was out of usage or even missing. Additionally, consumables were out of stock. This was one reason why the laboratory physiological testing had to be removed from the research protocol.

A second pilot test was decided in order to assess the validity and reliability of a field VO_{2max} intermittent running test to exhaustion. However, the design of this running test protocol appeared not to be suitable for all fitness level footballers and therefore was eventually removed from the research protocol. Furthermore, as was noted in methodology chapter, the whole physiological testing protocol had to be eventually excluded from the research due to the unwillingness of CYsem participating team's coach to allow the footballers to perform any kind of physiological test.

With respect to the football season calendar, the research design included three different testing time blocks. Thus, all participants should have remained recruited for the prolonged period of ten months. Respectively each group started out with an average of 20 participants and in total the research recruited 80 participants, but only 45 participants completed successfully all throughout the experimental study. A required sample size of six at each group was estimated via a priori analysis to detect significant differences in performance data. The small participant population represents a significant limitation in the analysis of performance. Also due to the different geographical locations of the teams involved in the research, it was only logistically possible to timetable following weeks in the same order during each time testing block. Consequently if a player missed any of the monitored block of training sessions due to injury, absence from training or even been transferred to a different team, the opportunity to collect data from the individual footballer in question was lost.

Nutritional intake was reported through 4-day recall diary and analysed for macronutrient and micronutrient intake. However, the results rely upon accurate reporting of ingested foods by the participants. In an ideal study participants would be weighing all the consumed food and drinks in order to export more accurate results. Unfortunately, the indirect control on the footballers groups along with the large subject group made this method not practicable.

Statistical analysis was a parameter that delayed progression, due to the difficulty of finding supportive training from University of Nicosia. Through the lack of cooperation and communication between departments and teaching staff at the University of Nicosia, the statistical analysis of data was completed owing to tutorial statistical sessions via several Skype conferences with the principal supervisor.

5.8 Implications for future research

Future studies should aim to recruit larger participant numbers in order to provide a sufficient number of athletes to detect statistical significance in small differences, particularly with reference to saliva stress markers. Future research using a group of athletes that report to a standardised training facility daily such as professional footballers would enable tighter control of such variables. The current study compared saliva stress markers from teams of different geographical locations. Logistical time restrictions between collection and transport of samples for analysis to Ipro Interactive Ltd Laboratory required extra financial cost for express delivery.

Finally, monitoring of all the parameters above was only assessed at three time blocks of one week each. In future research it would be more beneficial to record on a daily basis to create a more detailed profile of nutritional intake, training load and saliva stress markers. Furthermore, more investigation is needed for the Mediterranean diet and how is associated with the traditional football nutritional energy intake. Most probably, the ethnic and local attributes of what is considered as Mediterranean diet at each country must be reflected. In step, any positive results to training impact from such rich in antioxidants diet need to be investigated in depth. The current study was not able to do this because of limitations to time, controlled by clubs access to participants, plus travel and other project costs.

CHAPTER 6

CONCLUSION

6.0 Conclusion

The results demonstrated that there were significant differences between teams from different countries and ranking. The periodization principle seemed to differ between teams, most probably due to different season targets and followed strategies. In parallel, despite the fact that findings showed inadequate energy intake for all teams, an adjustment of nutritional energy intake according to training energy cost is observable. Finally, it was demonstrated that physiological salivary stress markers established useful indications regarding training and performance impact to footballers and therefore can be applied in real-life football by sport scientists.

Accordingly, monitoring of both training energy cost and nutritional energy expenditure could be of assistance to avoid insufficient recovery, exhaustion and weak immune system. The athlete's needs are not static, but rather move between categories according to changes in the daily, weekly or seasonal goals and exercise commitments in a periodized training programme.

CHAPTER 7

REFERENCE LIST

7.0 Reference List

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CHAPTER 8

APPENDICES

8.0 Appendices

8.1 Ethics

UNIVERSITY OF HERTFORDSHIRE
HEALTH AND HUMAN SCIENCES

M E M O R A N D U M

TO **Konstantinos Rostantis**

CC **Dr Justin Roberts**

FROM Dr Richard Southern, Health and Human Sciences

DATE **26/04/13**

Protocol number: LMS/PG/UH/00023

Title of study: An investigative comparison between UK and Cyprus based male football athletes in terms of habitual intake, markers of physiological stress and training load

Your application for ethical approval has been accepted and approved by the ECDA for your school.

This approval is valid:

From: 01/05/13

To: 30/09/13

Please note:

Approval applies specifically to the research study/methodology and timings as detailed in your Form EC1. Should you amend any aspect of your research, or wish to apply for an extension to your study, you will need your supervisor's approval and must complete and submit form EC2. In cases where the amendments to the original study are deemed to be substantial, a new Form EC1 may need to be completed prior to the study being undertaken.

8.2 Risk assessment

UNIVERSITY OF HERTFORDSHIRE

ETHICS COMMITTEE FOR STUDIES INVOLVING THE USE OF HUMAN PARTICIPANTS ('ETHICS COMMITTEE')

FORM EC5: STANDARD RISK ASSESSMENT FORM

FORM EC5 – STANDARD RISK ASSESSMENT FORM

NAME:

DATE:

Activity Description					
1. IDENTIFY HAZARDS	2. WHO COULD BE HARMED & HOW?		3. EVALUATE THE RISKS		4. ACTION NEEDED
Activities/tasks and associated hazards Describe the activities involved in the study and any associate hazards. Check sector specific/UH specific Guidance. (NB. Describe any equipment to be used and read manufacturer's instructions and note any hazards that arise, particularly from incorrect use.)	Who is at risk? e.g. participants, researchers, other people at the location, the owner / manager / workers at the location etc.	How could they be harmed? What sort of accident could occur, e.g. trips, slips, falls, lifting equipment etc., handling chemical substances, use of invasive procedures and correct disposal of equipment etc. What type of injury is likely?	Are there any precautions currently in place to prevent the hazard? Are there standard operating procedures or rules for the premises? Have there been agreed levels of supervision of the study? Will trained medical staff be present? Etc.	Are there any risks that are not controlled or not adequately controlled?	List the action that needs to be taken to reduce/manage the risks arising from your study?
Anthropometric measurements	Participants	Trips/ falls (very low possibility). Adverse emotional disturbance - denial about their fat % (low possibility) Infection on feet from body analyser bare foot contact (low possibility).	All lab and equipment will be cleared prior to any assessment for possible trip hazards. All participants are training 3-9 sessions / week Disinfectant spray for bacteria and viruses will be used on all the equipment prior to testing.	No	Researcher is first aid qualified and will ensure area is clear from trip hazards prior to use. Researcher is adequately trained and experienced in providing feedback to subjects. Protocol of safe working 1.11 Measurements of body composition and Protocol of safe working 1.2. The recording of bio-electrical potentials will be adhered to.
Completion of 7-day estimated record of food, training load, mood and illness episodes, with a verbal feedback session	Participants	Food miss representation (Low/ medium probability) Miscalculation of training load Negative emotional impact (Low probability)	Advices will be provided through examples in the diaries pages, so as to assist the participants in acourate feedback estimations about foods' weight and training load quantification.	No	Researcher is adequately trained and experienced in providing feedback to subjects. Protocol of safe working 2.2 Interviews, focus groups and surveys will be adhered to.

Physiological tests: *Maximal oxygen uptake incremental running test;* *Acceleration test, 0-20m;* *Counter Jump;* *Counter Movement Jump.*	Participants	Fatigue, tiredness, soreness (moderate/ high) Collapse (moderate, rare) Musculo-skeletal injury (low/moderate, rare) Negative emotional impact (low/ moderate) Cardio-vascular accident (high, rare)	Cardiovascular and respiratory monitoring to be undertaken during test. Warm-up (5mins at 100W) to be undertaken as standard practice (also 5mins at 100W cool down)	No	Health screen to be completed and assessed prior to exercise (incl. consent form) Pre-exercise guidelines provided to subjects ie: no strenuous exercise for 48 hours prior to test. Professionals and recreationally active subjects taking part Two testers per subject to be present, at least one with current first aid qualifications Protocol follows guidelines outlined by the British Association of Sport and Exercise Sciences, as assessed during laboratory accreditation. Protocol of safe working 1.5 'Exercise Testing' adhered to. BASES accredited staff present.
Saliva sampling – oral fluid collector *And* *Capillary blood sampling from the finger tip*	Participants	Cross contamination and infection of pathogenic organisms (high, rare) Swab material to cause gums, tongue or inside cheek irritation (low, rare)	Disposable gloves will be worn by the investigator during collection and handling of saliva. Fluid collector consists of a single use synthetic polymer-based swab material.	No	All the requirements of 'Protocol of Safe working 1.4 'Code of Practice for the collection of blood and the handling of blood-contaminated body materials and equipment' will be followed.

Signed by applicant:	Signed by Off Campus Location Representative: (NB. This needs to be the person who has given permission to use the location)

8.3 Subject Briefing

UNIVERSITY OF HERTFORDSHIRE

ETHICS COMMITTEE FOR STUDIES INVOLVING THE USE OF HUMAN PARTICIPANTS

('ETHICS COMMITTEE')

FORM EC6: PARTICIPANT INFORMATION SHEET

Title of Research

An investigative comparison between UK and Cyprus based male football athletesin terms of habitual dietary intake, markers of physiological stress and training load.

Introduction

You are being invited to take part in a research study. Before you decide whether to do so, it is important that you understand the research being conducted and what your involvement will include. Please take the time to read the following information carefully and discuss it with others if you wish. Please do not hesitate to ask for further information or to clarify any details in orderto help you make your decision. Please do take your time to decide whether or not you wish to take part. Thank you for reading this.

With an increasing scientific interest in the beneficial effects of Mediterranean, and in particular, polyphenol and antioxidant rich diets on aspects of cardiovascular health, insulin resistance and visceral obesity, less is known about the potential influence of cultural diets on aspects of athletic training and performance.

This study therefore aims to compare the habitual diets of both recreational and trained football athletes in contrasting geographical locations to assess whether specific dietary trends, food choices, macro- micro- and antioxidant nutrient intake may relate to management of training load and markers of physiological stress during the pre-season, mid-season and end of season.

The study population will comprise two main groups (one UK based, one Cyprus based), with each group being made up of two cohorts of male football athletes (recreational and professional); hence four cohorts in total.

All participant athletes will be assessed on habitual food intake, physiological screening and immunological markers according to the purpose of this study.

What is the purpose of this study?

The purpose of this study is:

To investigate habitual dietary intake between selected Cyprus based football cohorts and UK based football cohorts, especially in terms of antioxidant nutrition and cultural trends, between above cohorts over selected points of a training season;

To assess and compare training load and field based markers of physiological stress between above cohorts at selected points of a training season;

To assess relationships between habitual dietary intake and markers of physiological stress between above cohorts at selected testing periods of a training season.

Do I have to take part?

It is completely up to you whether or not you decide to take part in this study. If you do decide to take part you will be given this information sheet to keep and be asked to sign aninformed consent form. Agreeing to join the study does not mean that you have to complete it. You are free to withdraw at any stage without giving a reason. If you agree, then you may at some time in the future be contacted again in connection with this or another study.

What will happen to me if I take part?

If you decide to take part in this study, you will be asked to undertake the following over a typical training week (this will be undertaken during the pre-season phase, the mid-season and end of season):

Complete a daily food recall diary, a daily training diary and a weekly illness episode diary – this will involve simple completion of a diary to quantify all foods/fluids consumed each day for a period of 7 days; additionally you will be asked to record all training undertaken in the same period. The illness episode diary will be a simple quantification of any bouts of listed symptoms that may occur over the testing week.

Be assessed during your training sessions for total training load – this will be undertaken in line with your coach

Undertake a standard assessment of fitness markers including: body composition, counter movement jumps, and a field based assessment of maximal fitness

Provide three saliva samples for the analysis for training stress markers

The first thing to happen will be:

Before you begin the study, you will be asked to complete a standard consent form and a health screen questionnaire. You will also be given clear details on how to complete the diaries. All testing will take place at your club within normal training sessions in conjunction with your team coach/sports scientist. The actual burden to you is very low, and this will not impede or interfere with your normal training. At the beginning of each testing week, you will be provided with the diaries which will be collected at the end of the week. The researcher will be present daily over each testing week to support or help you with providing accurate information in your diaries and to collect saliva samples and undertake training load assessment.

What are the possible disadvantages, risks or side effects of taking part?

Exercise testing - As with any exercise that is undertaken to maximal conditions it is possible that you may experience a degree of exertion exhaustion, dizziness, musculo-skeletal soreness or injury, nausea or collapse. However, considering the training background and participants' age, the risk is minimised.

Blood& Saliva collection – with any form of saliva or blood collection there is the risk of cross-contamination or infection. Such risks are very rare, and full precautions are taken during all testing to minimise such risks. Only qualified staff will be involved in taking samples, under standard laboratory conditions, using single kit equipment for each test. All testers are first aid qualified and adhere to strict protocols for collection and handling of blood and saliva samples.

What are the possible benefits of taking part?

The latest scientific methods will be assessed fortesting, monitoring and evaluating your results. This is an opportunity to use these results as individuals to influence subsequent training behavior and establish your own training profile.

How will my taking part in this study be kept confidential?

Data will be recorded using a code number for each participant. Names will not appear in any published materials and will be deleted once data collection has been completed. Personal data will be recorded so as not to be individually identifiable. Personal data will involve the following: name, age, sex, email address/telephone number (for contact purposes only).

All data will be stored in a secure file, on the lead researchers password protected laptop. All data will be kept confidential during the research and only accessible to the research team. By giving consent you also acknowledge the right of the investigator to publish personal data recorded in the experiment, with exception to your name, which will not appear in published research.

What will happen to the results of the research study?

Data will be used for the researcher's study using code numbers and will be destroyed once the examination process is completed.

Who has reviewed this study?

This research has been reviewed by Dr A Mitchell, based at the University of Hertfordshire, School of Life and Medical Sciences.

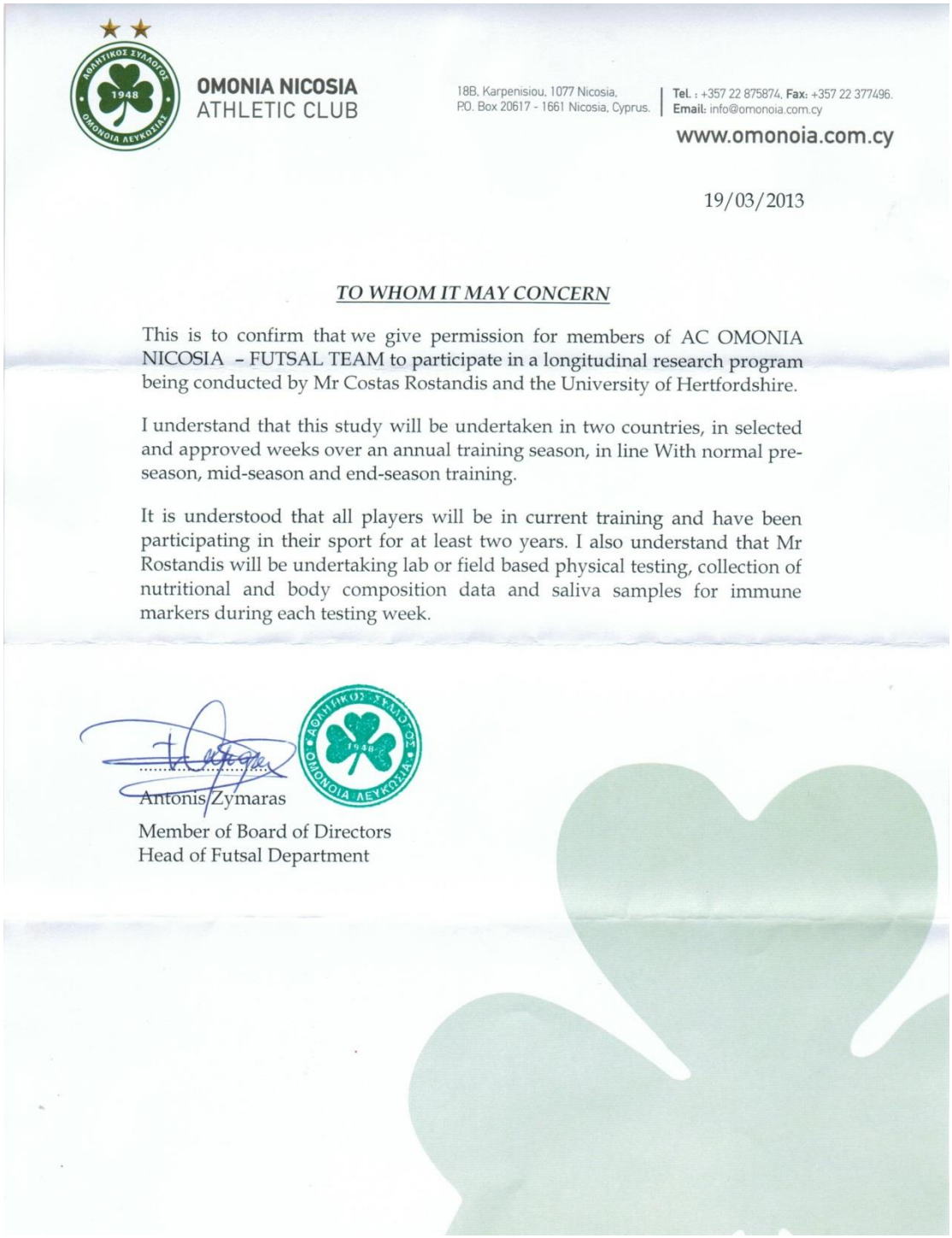
Who can I contact if I have any questions?

If you would like further information or would like to discuss any details personally, please contact me, in writing, by phone 0035799680708 or by email rostandicos@yahoo.com

Although we hope it is not the case, if you have any complaints or concerns about any aspect of the way you have been approached or treated during the course of this study, please write to the University Secretary and Registrar.

Thank you very much for reading this information and giving consideration to taking part in this study.

8.4 Consent letter from Clubs



OMONIA NICOSIA
ATHLETIC CLUB

18B, Karpenisiou, 1077 Nicosia,
P.O. Box 20617 - 1661 Nicosia, Cyprus.
Tel. : +357 22 875874, Fax: +357 22 377496.
Email: info@omonoia.com.cy
www.omonoia.com.cy

19/03/2013

TO WHOM IT MAY CONCERN

This is to confirm that we give permission for members of AC OMONIA NICOSIA – FUTSAL TEAM to participate in a longitudinal research program being conducted by Mr Costas Rostandis and the University of Hertfordshire.

I understand that this study will be undertaken in two countries, in selected and approved weeks over an annual training season, in line With normal pre-season, mid-season and end-season training.

It is understood that all players will be in current training and have been participating in their sport for at least two years. I also understand that Mr Rostandis will be undertaking lab or field based physical testing, collection of nutritional and body composition data and saliva samples for immune markers during each testing week.

Antonis Zymaras
Member of Board of Directors
Head of Futsal Department

Brentford FC
Griffin Park
Braemar Road, Brentford
Middlesex TW8 0NT

Telephone: 020 8380 9933
Fax: 020 8568 9940
Mob: 07983636541
Email: chaslam@brentfordfc.co.uk

www.brentfordfc.co.uk

28/03/2013

Dear Sirs

This is to confirm that we give permission for members of Brentford FC to participate in a longitudinal research program being conducted by Mr Costas Rostandis and the University of Hertfordshire. I understand that this study will be undertaken in two countries, in selected and approved weeks over an annual training season, in line with normal pre-season, mid-season, and end-season training.

It is understood that all players will be in current training and have been participating in their sport for at least two years. I also understand that Mr Rostandis will be undertaking lab or field based physical testing, collection of nutritional and body composition data and saliva for immune markers during each testing week.

Chris Haslam
Head of Sport Science and Conditioning
Brentford Football Club

Tel: 07817523637
Email: chaslam@brentfordfc.co.uk

8.5 Consent form for participants

University of Hertfordshire

CONSENT FORM FOR STUDIES INVOLVING HUMAN PARTICIPANTS

I, the undersigned *[please give your name here*, *in BLOCK CAPITALS]*

……
…….

of *[please give contact details here, sufficient to enable the investigator to get in touch with you, such as a postal or email address]*

…..……
…… ..

Hereby freely agree to take part in the study entitled;

An investigative comparison between UK and Cyprus based male football athletes in terms of habitual dietary intake, markers of physiological stress and training load.

1 I confirm that I have been given a Participant Information Sheet (a copy of which is attached to this form) giving particulars of the study, including its aim(s), methods and design, the names and contact details of key people and, as appropriate, the risks and potential benefits, and any plans for follow-up studies that might involve further approaches to participants. I have been given details of my involvement in the study. I have been told that in the event of any significant change to the aim(s) or design of the study I will be informed, and asked to renew my consent to participate in it.

2 I have been assured that I may withdraw from the study at any time without disadvantage or having to give a reason.

3 I have been given information about the risks of my suffering harm or adverse effects. I have been told about the aftercare and support that will be offered to me in the event of this happening, and I have been assured that all such aftercare or support would be provided at no cost to me.

4 I have been told how information relating to me (data obtained in the course of the study, and data provided by me about myself) will be handled: how it will be kept secure, who will have access to it, and how it will or may be used.

5 I have been told what will be done if the study reveals that I have a medical condition which may have existed prior to the study, which I may or may not have been aware of, and which could affect the present or future health of myself or others. If this happens, I will be told about the condition in an appropriate manner and advised on follow-up action I should take. Information about the condition will be passed to my GP, and I may no longer be allowed to take part in the study.

6 I have been told that I may at some time in the future be contacted again in connection with this or another study.

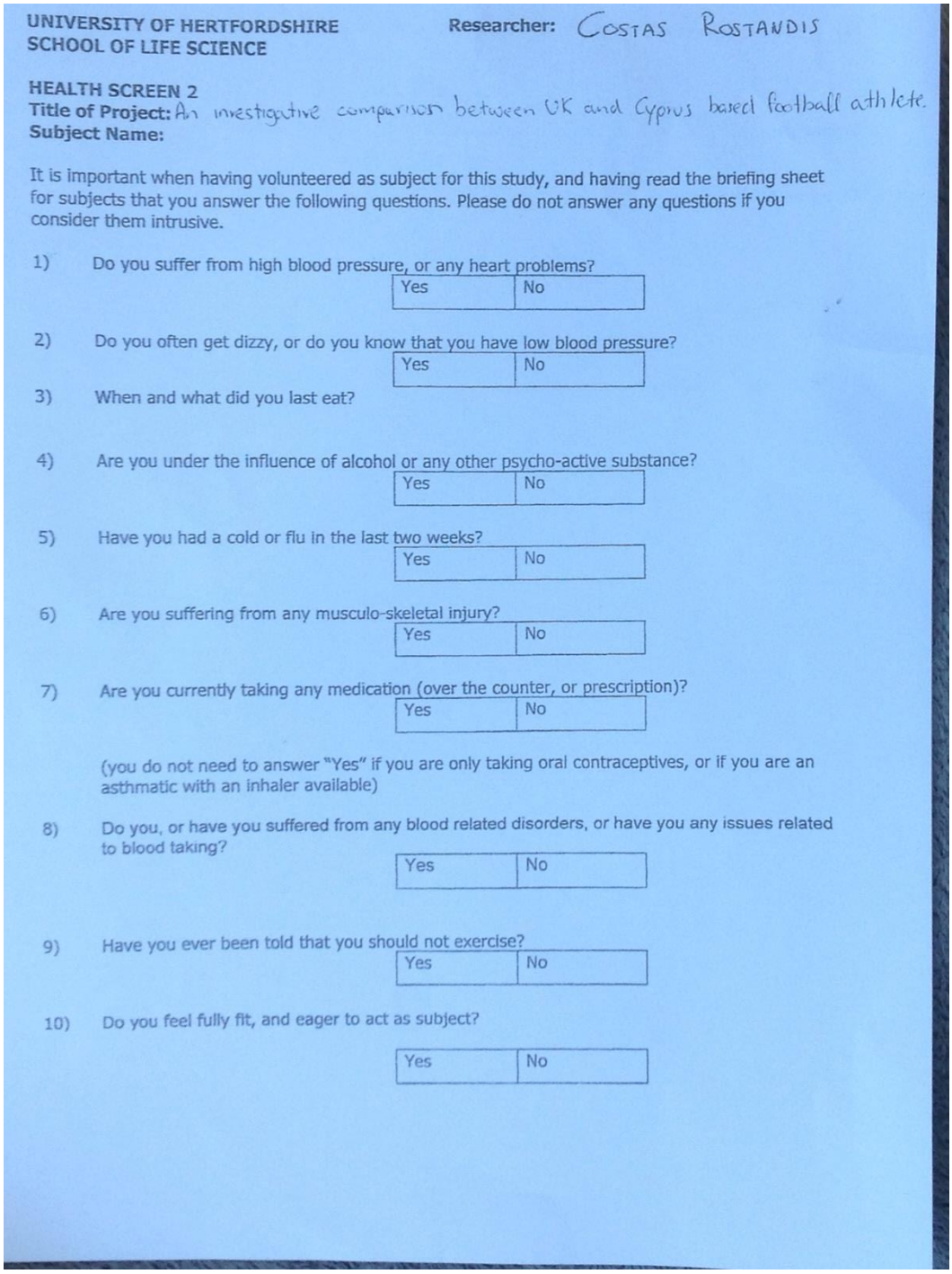
Signature of participant…… Date……..….

Signature of (principal) investigator……
Date……

Name of (principal) investigator

KONSTANTINOS ROSTANTIS

8.6 Health Screen



UNIVERSITY OF HERTFORDSHIRE **Researcher:** COSTAS ROSTANDIS
SCHOOL OF LIFE SCIENCE

HEALTH SCREEN 2
Title of Project: An investigative comparison between UK and Cyprus based football athlete.
Subject Name:

It is important when having volunteered as subject for this study, and having read the briefing sheet for subjects that you answer the following questions. Please do not answer any questions if you consider them intrusive.

1) Do you suffer from high blood pressure, or any heart problems?

Yes	No

2) Do you often get dizzy, or do you know that you have low blood pressure?

Yes	No

3) When and what did you last eat?

4) Are you under the influence of alcohol or any other psycho-active substance?

Yes	No

5) Have you had a cold or flu in the last two weeks?

Yes	No

6) Are you suffering from any musculo-skeletal injury?

Yes	No

7) Are you currently taking any medication (over the counter, or prescription)?

Yes	No

(you do not need to answer "Yes" if you are only taking oral contraceptives, or if you are an asthmatic with an inhaler available)

8) Do you, or have you suffered from any blood related disorders, or have you any issues related to blood taking?

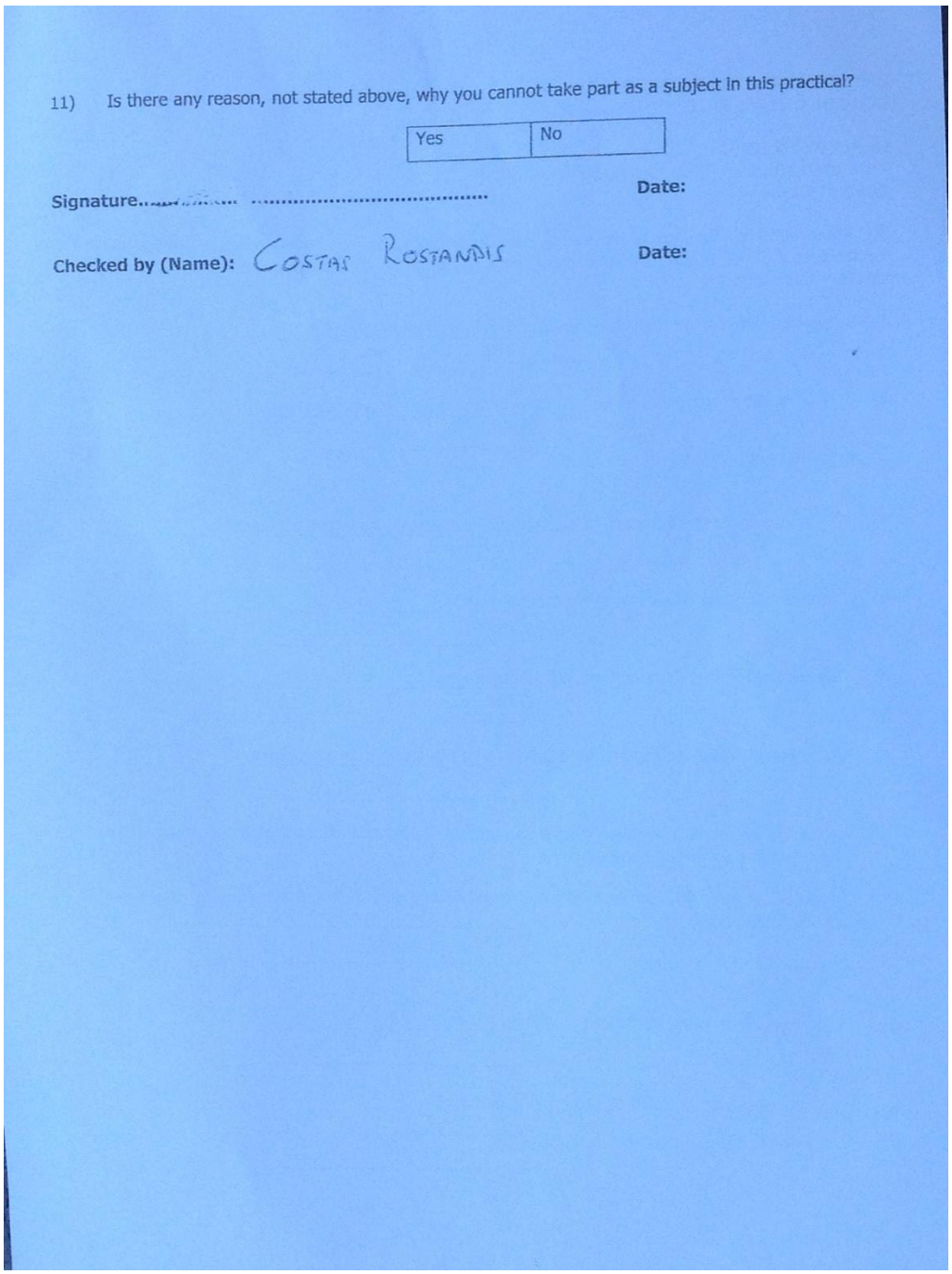
Yes	No

9) Have you ever been told that you should not exercise?

Yes	No

10) Do you feel fully fit, and eager to act as subject?

Yes	No



11) Is there any reason, not stated above, why you cannot take part as a subject in this practical?

Yes	No

Signature..... Date:

Checked by (Name): COSTAS ROSTANDIS Date:

8.7 Raw Data

Nutritional data

UK and Cyprus based football athletes Research

NUTRITION & SLEEP DIARY EXAMPLE

Week: 1

Day: Monday 10/05/13 Time	Profession: Banker Hours of work: 8	Food Consumed Incl. Brands where applicant	Amount(g) & portion size Provide as much info as you can; cooking method, additional sides.	Drinks Consumed Type & Amount (ml) For alcohol drinks indicate the alc.% if possible	Supplements & Medicines Incl. Brands and Dosage	Notes Comments on each meal/ Cooking methods
07:30	Wake up					
.. : ..	Training 1					
08:00	Breakfast	Wheat biscuits (McVities Digestive)	130gr (per 100g/ 481kcal)*	1 Espresso single, ½ sugar 1 coffee with milk 2%, ½ sugar		
10:30	Mid Breakfast	1 Espresso single, ½ sugar 1 banana,			1 liquid amino ampoule 25ml	200ml water with 13,8ml/37kcal minerals
	Training					
13:30	Lunch	Lamb leg Potatoes Baby bean Bread Tahini	400g/ on the grill Roasted 2 small Small portion 1 pc, toast size 3 spoons	Beer 500ml(4.6%) Fred Espresso, 1 sugar	2 Strepsils pastilles	Baby beans steamed with tomato and carrot
14:00	Sleep					
	Training					
15:30	Mid Afternoon	Mandarin Crisps salt&vinegar(Bugles)	Medium 40g/ 208kcal/ fat 13g			
21:30	Dinner	Lamb leg and Broccoli (Lemon and olive oil) Cucumber	280gr/ grill 2 pcs/ grill, Lemon 2 pcs	Squash lemonade 300ml		
10:15	Pre Bed	Hazelnuts Chocolate (Galaxy)	140g Salted 46g/ 250kcal/ Fat 14.9g			
23:45	Sleep					
Comments	Sleep quality 1-10: 9	Mood: relaxed	Energy: good all day	Digestion: normal		

Training load data

dev.st		1.2	3.3		13	2%	2%	0.5	2	3	2.1%	1.70%	4.5%	4%			peso	Kcal/h/kg			6%	2.4	1.8	2.5%	0.50	9%	
media		11.3	32.3		116	10%	13%	1.7	17.0	142	22%	6.2%	41.7%	27%	1100		67	5.06			45%	20	11	13.9%	4.25	64%	
		w med	Vo2 med	FC med	dist/ min	a int	dec int	CdD /min W>	CdD/ min >30°	dist/min EQ	% Dist Equiv	%vel> 16	%ana er	% w>20 t>=3"	kcal	min reg	dist tot	vel km/ h	T vel> 16	T w> 20	%T vel/w HI	y (lung h)	x (larg h)	% t w>20 /Ttot	n az int/mi n	Tr 0-10w <60"	n rec > 60"
0	8408	5.9	16.9	0	62	5%	6%	1.2	6.6	77	24%	2.7%	40%	33%	792	140.1	8664	3.71	228	608	37%			7.3%	1.80	23%	8
1784	2053	11.5	32.8	0	123	9%	12%	0.4	12.9	150	22%	0.2%	30%	0%	49	4.5	552	7.38	1	31	2%	0.0	0.0	11.4%	3.35	50%	0
2256	3134	7.3	20.9	0	74	8%	11%	1.4	13.6	95	28%	1.9%	34%	12%	102	14.6	1089	4.46	16	66	25%	0.0	0.0	7.6%	2.26	39%	5
3773	6510	12.5	35.8	0	129	11%	11%	3.1	13.2	163	26%	7.6%	49%	38%	546	45.6	5900	7.76	207	503	41%	0.0	0.0	18.5%	4.41	54%	2

8.8 Statistics

2way anova TLDaily/ General Linear Model

Notes	
Output Created	05-Dec-2014 17:08:35

Comments		
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	Split File	<none>
	N of Rows in Working Data File	45
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data for all variables in the model.
Syntax		GLM T1 T2 T3 BY Group /WSFACTOR=time 3 Polynomial /METHOD=SSTYPE(3) /POSTHOC=Group(BONFERRONI T3) /EMMEANS=TABLES(OVERALL) /EMMEANS=TABLES(Group) COMPARE ADJ(LSD) /EMMEANS=TABLES(time) COMPARE ADJ(LSD) /EMMEANS=TABLES(Group*time) /PRINT=DESCRIPTIVE /CRITERIA=ALPHA(.05) /WSDESIGN=time /DESIGN=Group.
Resources	Processor Time	00 00:00:00.188
	Elapsed Time	00 00:00:00.234

[DataSet1] C:\Users\Costas\Documents\FAT kcal all.sav

Within-Subjects Factors

Measure:MEASURE_1

time	Dependent Variable
1	T1
2	T2
3	T3

Between-Subjects Factors

		N
Group	1.00	14
	2.00	16

Between-Subjects Factors

		N
Group	1.00	14
	2.00	16
	3.00	12

Descriptive Statistics

	Group	Mean	Std. Deviation	N
T1	1.00	2583.9286	194.25378	14
	2.00	1991.1875	181.81647	16
	3.00	2150.4167	263.55488	12
	Total	2234.2619	330.99768	42
T2	1.00	2544.6192	228.95963	14
	2.00	2172.9898	214.13363	16
	3.00	2177.1021	203.93371	12
	Total	2298.0412	275.13634	42
T3	1.00	2614.8943	236.91782	14
	2.00	2110.8974	205.73511	16
	3.00	2197.6096	169.82261	12
	Total	2303.6713	303.13187	42

Multivariate Tests[c]

Effect		Value	F	Hypothesis df	Error df	Sig.
time	Pillai's Trace	.198	4.697[a]	2.000	38.000	.015
	Wilks' Lambda	.802	4.697[a]	2.000	38.000	.015
	Hotelling's Trace	.247	4.697[a]	2.000	38.000	.015
	Roy's Largest Root	.247	4.697[a]	2.000	38.000	.015
time * Group	Pillai's Trace	.512	6.711	4.000	78.000	.000
	Wilks' Lambda	.489	8.170[a]	4.000	76.000	.000
	Hotelling's Trace	1.043	9.643	4.000	74.000	.000
	Roy's Largest Root	1.040	20.285[b]	2.000	39.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

c. Design: Intercept + Group

Within Subjects Design: time

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]	
					Greenhouse-Geisser	Huynh-Feldt
time	.653	16.196	2	.000	.742	.80

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept + Group

Within Subjects Design: time

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	105243.755	2	52621.878	7.638	.001
	Greenhouse-Geisser	105243.755	1.485	70883.007	7.638	.003
	Huynh-Feldt	105243.755	1.609	65412.028	7.638	.002
	Lower-bound	105243.755	1.000	105243.755	7.638	.009
time * Group	Sphericity Assumed	196601.022	4	49150.255	7.134	.000
	Greenhouse-Geisser	196601.022	2.970	66206.644	7.134	.000
	Huynh-Feldt	196601.022	3.218	61096.602	7.134	.000
	Lower-bound	196601.022	2.000	98300.511	7.134	.002
Error(time)	Sphericity Assumed	537411.757	78	6889.894		
	Greenhouse-Geisser	537411.757	57.905	9280.863		
	Huynh-Feldt	537411.757	62.748	8564.536		
	Lower-bound	537411.757	39.000	13779.789		

Tests of Within-Subjects Contrasts

Measure:MEASURE_1

Source	time	Type III Sum of Squares	df	Mean Square	F	Sig.
time	Linear	90103.113	1	90103.113	9.396	.004
	Quadratic	15140.643	1	15140.643	3.613	.065
time * Group	Linear	33547.934	2	16773.967	1.749	.187
	Quadratic	163053.088	2	81526.544	19.454	.000
Error(time)	Linear	373973.188	39	9589.056		
	Quadratic	163438.570	39	4190.733		

Tests of Between-Subjects Effects

Measure:MEASURE_1

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	6.475E8	1	6.475E8	5348.342	.000
Group	5907402.108	2	2953701.054	24.397	.000
Error	4721669.549	39	121068.450		

Estimated Marginal Means

1. Grand Mean

Measure:MEASURE_1

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
2282.627	31.212	2219.494	2345.760

2. Group

Estimates

Measure:MEASURE_1

Group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	2581.147	53.690	2472.550	2689.745
2.00	2091.692	50.222	1990.108	2193.275
3.00	2175.043	57.991	2057.744	2292.342

Pairwise Comparisons

Measure:MEASURE_1

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1.00	2.00	489.456*	73.518	.000	340.752	638.159
	3.00	406.105*	79.029	.000	246.253	565.956
2.00	1.00	-489.456*	73.518	.000	-638.159	-340.752
	3.00	-83.351	76.716	.284	-238.523	71.821
3.00	1.00	-406.105*	79.029	.000	-565.956	-246.253
	2.00	83.351	76.716	.284	-71.821	238.523

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Univariate Tests

Measure:MEASURE_1

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	1969134.036	2	984567.018	24.397	.000
Error	1573889.850	39	40356.150		

The F tests the effect of Group. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

3. time

Estimates

Measure:MEASURE_1

			95% Confidence Interval	
time	Mean	Std. Error	Lower Bound	Upper Bound
1	2241.844	32.917	2175.264	2308.425
2	2298.237	33.626	2230.221	2366.253
3	2307.800	32.265	2242.539	2373.062

Pairwise Comparisons

Measure:MEASURE_1

					95% Confidence Interval for Difference[a]	
(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	Lower Bound	Upper Bound
1	2	-56.393*	19.855	.007	-96.554	-16.232
	3	-65.956*	21.517	.004	-109.478	-22.435
2	1	56.393*	19.855	.007	16.232	96.554
	3	-9.563	11.864	.425	-33.560	14.433
3	1	65.956*	21.517	.004	22.435	109.478
	2	9.563	11.864	.425	-14.433	33.560

Based on estimated marginal means

Pairwise Comparisons

Measure:MEASURE_1

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-56.393*	19.855	.007	-96.554	-16.232
	3	-65.956*	21.517	.004	-109.478	-22.435
2	1	56.393*	19.855	.007	16.232	96.554
	3	-9.563	11.864	.425	-33.560	14.433
3	1	65.956*	21.517	.004	22.435	109.478
	2	9.563	11.864	.425	-14.433	33.560

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.
Pillai's trace	.198	4.697[a]	2.000	38.000	.015
Wilks' lambda	.802	4.697[a]	2.000	38.000	.015
Hotelling's trace	.247	4.697[a]	2.000	38.000	.015
Roy's largest root	.247	4.697[a]	2.000	38.000	.015

Each F tests the multivariate effect of time. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

4. Group * time

Measure:MEASURE_1

Group	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.00	1	2583.929	56.622	2469.401	2698.457
	2	2544.619	57.842	2427.622	2661.616
	3	2614.894	55.500	2502.634	2727.154
2.00	1	1991.187	52.965	1884.056	2098.319
	2	2172.990	54.107	2063.549	2282.431
	3	2110.897	51.916	2005.888	2215.907
3.00	1	2150.417	61.158	2026.712	2274.121
	2	2177.102	62.477	2050.731	2303.473
	3	2197.610	59.947	2076.355	2318.864

Post Hoc Tests

Group

Multiple Comparisons

Measure:MEASURE_1

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	1.00	2.00	489.4558*	73.51763	.000	305.5399	673.371
		3.00	406.1046*	79.02907	.000	208.4009	603.808
	2.00	1.00	-489.4558*	73.51763	.000	-673.3718	-305.539
		3.00	-83.3512	76.71553	.852	-275.2672	108.564
	3.00	1.00	-406.1046*	79.02907	.000	-603.8083	-208.400
		2.00	83.3512	76.71553	.852	-108.5648	275.267
Dunnett T3	1.00	2.00	489.4558*	73.57884	.000	302.5803	676.331
		3.00	406.1046*	80.96292	.000	198.7470	613.462
	2.00	1.00	-489.4558*	73.57884	.000	-676.3313	-302.580
		3.00	-83.3512	75.83551	.621	-277.8786	111.176
	3.00	1.00	-406.1046*	80.96292	.000	-613.4621	-198.747
		2.00	83.3512	75.83551	.621	-111.1761	277.878

Based on observed means.

The error term is Mean Square(Error) = 40356.150.

*. The mean difference is significant at the .05 level.