THE EFFECTS OF TRAINING ORGANISATION ON THE PHYSIOLOGICAL, METABOLIC AND MOLECULAR RESPONSES TO A SOCCER-SPECIFIED LABORATORY BASED TRAINING SIMULATION

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Figure 2.6 page 12

Figure 2.2 page 18

Figure 2.3 page 20

Figure 2.4 page 23

Figure 34 page 34

Figure 37 page 37

Appendix 4

Appendix 5

Contact details in appendix 1

Contact details in appendix 2

CONTENTS

CHAPTER 3

SEASONAL VARIATIONS IN THE PHYSIOLOGICAL LOADS AND ACTIVITY PROFILES OF PROFESSIONAL SOCCER PLAYERS

CHAPTER 4

THE DEVELOPMENT OF A LABORATORY-BASED SOCCER-SPECIFIC TRAINING SIMULATION

CHAPTER 5

ACUTE MOLECULAR RESPONSES ASSOCIATED WITH MITOCHONDRIAL BIOGENESIS TO A SINGLE BOUT OF SOCCER-SPECIFIC INTERMITTENT EXERCISE

CHAPTER 6

THE INFLUENCE OF A PRIOR BOUT OF EXERCISE ON THE ACUTE MOLECULAR RESPONSES TO SOCCER-SPECIFIC INTERMITTENT EXERCISE

CHAPTER 7
SYNTHESIS OF FINDINGS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVATIONS

% of $HR_{max,}$	Percent of maximal heart rate
ACC	Acetyl CoA-carboxylase
ADP	Adenosine di -phosphate
AICAR	5-aminoimidazole-4-carboxamide ribonucleoside
AMP	Adenosine mono-phosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
ATP	Adenosine-tri-phosphate
AU	Arbitrary unit
BCAA	Branched-chain amino acids
CaMK	Calcium/calmodulin-dependent protein kinase
CD	Cool down
cDNA	Complementary DNA
CHO	Carbohydrate
CI	Confidence interval
COXIV	CyclooxygenaseIV

CP	Creatine phosphate
CREB	Cyclic AMP response element-binding
CS	Citrate synthase
DNA	Deoxyribonucleic acid
Dw	Dry weight
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
ETC	Electron transport chain
FFA	Free fatty acid
FIFA	Fédération Internationale de Football Association
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GAS	General adaptation syndrome
GPS	Global position system
GSK-3β	Glycogen synthase kinase-3β
HAD	3-hydroxyacyl co-enzyme A dehydrogenase
HCl	Hydrogen chloride
HR	Heart rate
KCl	Potassium chloride
KOH	Potassium hydroxide
LPS	Local position system
LSD	Least Significant Difference
LSSTS	Laboratory-based soccer-specific training simulation
MAPK	Mitogen-activated protein kinase
MEF2	Myocyte-specific enhancer factor 2
MJ	Mega joule
mRNA	Messenger RNA
mtDNA	Mitochondrial DNA
mTOR	Mammalian target of rapamycin
NaCl	Sodium chloride
NEFA	Non-estrified free fatty acid
NRF	Nuclear respiratory factor
PDK	Pyruvate dehydrogenase kinase
PGC-1α	Peroxisome proliferator receptor-γ co-activator-1α
PKB	Protein kinase B
pH	Potential of hydrogen
PRC	PGC-1-related cofactor
PT	Physical training
PT/TT	Physical training and technical/tactical training
qPCR	Quantitative PCR
RBP	RNA-binding proteins
RNA	Ribonucleic acid
ROS	Reactive oxygen species
ROX	6-Carboxyl-X-Rhodamine
RPE	Rating of perceived exertion
RT-PCR	Reverse transcription-polymerase chain reaction
Ser172	Serine172
SDH	Succinate dehydrogenase
TBST	Tris-Buffered Saline Tween-20
TF	Transcription factor
TFAM	The mitochondrial transcription factor
TG	Triacylglycerol

Thr180	Threonine180
TIM	Translocases of the inner membrane
TL	Training load
TOM	Translocases of the outer membrane
TSC2	Tuberous sclerosis protein 2
TT	Technical/tactical training
Tyr182	Tyrosine182
UK	United Kingdom
USA	United States of America
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen consumption
WU	Warm-up

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DECLARATION

I declare that the work contained in this thesis is entirely my own.

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ABSTRACT

Player's performance in competitive matches is partly determined by the systematic training programmes that they complete. The differences in the organisation of training may result in differences in the physiological stress placed on players. This study aimed to investigate the influence of training organisation on the physiological, metabolic and molecular responses to soccer-specific intermittent exercise in skeletal muscles.

In Chapter 3, the physical demands of professional soccer training were examined by quantifying the physiological loads and work-rate profiles of elite players throughout the programmed pre-season and in-season training for a one week period. The physiological loads in pre-season were significantly higher than those in the in-season period ($p < 0.05$). Similar activity profiles were, however, observed during each training period irrespective of the time of the year. These findings demonstrate that pre-season training was more intensive than in-season training though these differences were not linked to changes in the activity patterns during sessions. Technical/tactical training seems to be an important component in increasing the physiological strain observed in pre-season training. This study also indicates that soccer training seems to elicit different demands to those associated with match-play.

In Chapter 4, a laboratory-based soccer-specific training simulation (LSSTS) was devised on a motorized treadmill. Attempts were made to re-create both similar overall exercise intensities and patterns of discrete activity observed in training. The validity of this protocol was evaluated by comparing the physiological responses of professional players with those of healthy subjects who completed the LSSTS. Physiological measurements such as mean HR and % of HR_{max} associated with the simulation were similar to those obtained in the actual training session. These data suggest that the protocol is suitable in re-creating a soccer-specific training session in the laboratory. This protocol is, therefore, sufficient to use in investigations to study the physiological responses and the molecular adaptations of skeletal muscle to soccer-specific intermittent exercise.

In Chapter 5, the effect of a single bout of soccer-specific intermittent exercise on metabolic stress and acute molecular responses associated with mitochondrial biogenesis was investigated in human skeletal muscle. The LSSTS was utilised as the sports-specific exercise protocol. The levels of blood metabolites and muscle glycogen were significantly altered during and after exercise ($p < 0.05$). Simulated soccer-specific training also acutely activated the expression of *PGC-1α* mRNA in human skeletal muscle ($p < 0.05$). There was, however, no significant change in the phosphorylation of *AMPK* and *p38MAPK*. This would suggest that the global effect of soccer-specific intermittent exercise on aerobic performance may be partly mediated by adaptations associated with mitochondrial biogenesis in skeletal muscle.

In Chapter 6, the effect of prior soccer-specific training on the physiological, metabolic and molecular responses to a subsequent bout of soccer-specific intermittent exercise was evaluated. Two experimental trials (*BETWEEN DAY trial*, one bout of soccer-specific intermittent exercise in a day Vs *WITHIN DAY trial,* two consecutive bouts of soccer-specific intermittent exercise performed in a day) were completed on two separate occasions. There were significant increases in physiological responses during the second bout of exercise in the WITHIN DAY trial, compared to those obtained in the BETWEEN DAY trial ($p < 0.05$). A more pronounced increase in NEFA and glycerol was observed in the WITHIN DAY trial compared with the BETWEEN DAY trial post-exercise following the second bout of exercise ($p < 0.05$). The expression of *PGC-1α mRNA* significantly increased following exercise compared to pre- and post-exercise values. There was, however, no difference in phosphorylation of *AMPK and p38MAPK* and the expression of *PGC-1α mRNA* between either trial. Based on these findings, it would seem that different approaches to training organisation may be more important for the acute physiological responses to soccer-specific intermittent exercise than the molecular changes underpinning chronic adaptations.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Soccer can be classified as high-intensity intermittent exercise which includes in excess of 1000 acyclic activities during a game (Bangsbo, 1994d). Players cover in the region of 9~14km during a match at a number of exercise intensities ranging from walking to maximal sprinting (Reilly, 1997). The diverse nature of the demands of soccer necessitates players completing systematic training. Soccer training must not only incorporate technical and tactical elements but also develop the physical capacity of players (Bangsbo, Mohr & Krustrup, 2006; Reilly, 2007c). Important aims include developing the aerobic and anaerobic energy systems and developing strength through specific muscle training (Bangsbo et al., 2006; Reilly, 2007c).

The necessity to prepare for the competitive fixtures and the complexity of the training prescription frequently leads to conflicts between the available training time and the amount of training that needs to be completed. For example, training in pre-season usually focuses on the *rebuilding* of fitness levels in players following the off-season. This in turn leads to the requirement to complete multiple training sessions within a day (Bangsbo et al., 2006). This contrasts with the aim of 'in-season' training, where the aim is frequently on the *maintenance* of the specific capacities developed during 'pre-season' (Bangsbo, 1994b; Reilly, 2007f). Professional teams typically carry out between 4~6 training sessions per week during the competitive season (Bangsbo et al., 2006). Such differences in the training organisation may result in differences in the physiological load across similar time periods (Stich et al., 2000; Ronsen, Haug, Pedersen & Bahr, 2001; Goto, Ishii, Mizuno & Takamatsu, 2007). However, few studies have systematically attempted to quantify physiological loading completed by elite professional players over a short-term period of training at different phases of a competitive annual cycle.

The different organisation of soccer training may impact upon the physiological adaptations that occur as a consequence of the completion of such work. The daily training frequency, the length of rest between exercise bouts and the order of exercise can change the hormonal and metabolic adaptations observed following a period of training (Hansen et al., 2005; Goto,

Ishii, Kurokawa & Takamatsu, 2007; Goto, Ishii, Mizuno et al., 2007; Goto, Ishii, Sugihara, Yoshioka & Takamatsu, 2007). For example, the completion of prior exercise significantly alters the hormonal response to a second bout of exercise completed on the same day (Goto et al., 2007c). Training twice every second day enhanced the glycogen restoration and oxidative enzyme activities in skeletal muscle more than training once a daily (Hansen et al., 2005). Also, when short rest intervals were given between two consecutive bouts of exercise, the concentration of hormone such as growth hormone was attenuated (Goto et al., 2007a). These findings clearly illustrate the impacts of the organisation of training on the adaptations in the metabolic and endocrine system (Hansen et al., 2005, Goto et al., 2007a).

Changes in the musculoskeletal system following training are partly related to the molecular adaptations within specific muscle groups (Coffey & Hawley, 2007). These molecular adaptations in skeletal muscles are the cumulative result of a number of acute responses to individual training session within a programme of repeated exercises (Widegren, Ryder & Zierath, 2001a). The systemic responses including hormonal and metabolic changes may partly determine the molecular adaptations in skeletal muscle as such adaptations are highly specific to the mode, intensity and duration of the stimulus (Booth & Thomason, 1991; Coffey & Hawley, 2007). These findings would suggest that a coherent understanding of the interaction between training organisation and the acute physiological and metabolic responses to a specific exercise stimulus would be of benefit to both the exercise scientist and applied practitioner.

A clear understanding of the complex interactions between the training stimuli and acute adaptations that seems vital for the design of effective training programmes is not also currently available. This is especially the case for research that is focused on the specificities of training organisation that has relevance to soccer. It is, therefore, unclear what the physiological consequences are for players when soccer-specific training sessions are performed more than once within a given day or across days especially when the type of training completed may be very different.

1.2 Aims and Objectives

The *overall aim* of this thesis is

To investigate the influence of training organisation on the physiological, metabolic and molecular responses to soccer-specific intermittent exercise in skeletal muscles.

This aim has been achieved through the completion of four studies.

The objective in the **first study** is

1) *To characterise the physical demands of professional soccer training by quantifying and comparing the physiological training loads and activity profiles of professional players during the pre-season and in-season periods and*
2) *To quantify and compare the physiological loading and activity profiles of specific training types (e.g. physical, tactical, technical etc.) or sub-components (e.g. warm up, cool down) during the 'pre-season' and 'in-season' training periods.*

These data will also be used to provide a template for the development of a laboratory-based soccer-specific training simulation.

The objective in the **second study** is

1) *To develop a laboratory based treadmill simulation that replicates the physiological requirements and activity profiles of professional soccer training and*
2) *To examine validity of the protocol.*

Such a simulation developed in this study will permit investigation into the physiological responses and the molecular adaptations of skeletal muscle to a soccer-training specific training session in the later chapters of this thesis.

The objective in the **third study** is

1) *To test the effect of a single bout of soccer-specific intermittent exercise on physiological, metabolic responses in blood and acute signaling responses associated with mitochondrial biogenesis in skeletal muscle of healthy men.*

The objective of the **fourth study** is

1) *To investigate the effect of performing soccer-specific intermittent exercise once versus twice daily on physiological, metabolic responses in blood and acute signaling responses associated with mitochondrial biogenesis in skeletal muscle of healthy men.*

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Soccer is categorised as an acyclic intermittent sport with repeated bouts of high-intensity activity (Bangsbo, 1994d; Reilly, 2005). In order to prepare players for competition, soccer training must cover the various components required to be successful in a game (Reilly, 2005). The fulfilment of these requirements can be achieved by developing systematic training programmes which include sport-specific technical/tactical exercises and specific fitness work (Reilly, 2007c).

Training programmes for sports are frequently periodised into a competitive phase, a transitional off-season phase and a preparatory phase (Bompa, 1999). Athletes in each phase perform specific types of training with variable frequency, duration and intensity. This model is also applied in soccer with the specific organisation of soccer training depending on the particular purpose of a phase of the annual plan, the number of fixtures in the season and/or the experience of the coach (Bangsbo, 2003; Impellizzeri, Rampinini & Marcora, 2005; Bangsbo et al., 2006; Impellizzeri et al., 2006). It is, however, not clear how the physiological loads of soccer training differ at specific parts of the annual cycle. Understanding the extent of these differences in the physiological stimuli may permit the development of training models for improving performance and fitness levels in players.

The specific organisation of training in soccer may stimulate different training adaptations. This is a consequence of the adaptive response in skeletal muscle being specifically altered by types or modes of exercise (Coffey & Hawley, 2006; Coffey & Hawley, 2007). The metabolic and physiological responses to different types of training are relatively well understood (Bangsbo, 1994d; Drust, Reilly & Cable, 2002; Hawley, Tipton & Millard-Stafford, 2006). This is, however, not the case for the cellular and molecular responses to soccer-specific intermittent exercise. Investigations of the molecular responses induced by soccer-specific exercise may, therefore, provide useful evidence that helps identify the cellular regulatory systems that are associated with the development of fitness levels in soccer.

The aim of the present review is to outline the physical demands of soccer match-play and to

present some background information on the metabolic responses to soccer. Strategies for the preparation of players for competition will also be reviewed using the available literature. This will be done in relation to both soccer-specific training and the monitoring of training loads. Finally, the exercise-induced molecular adaptations in human skeletal muscle will be outlined, using information that is relevant to adaptations to soccer-specific training.

2.2. Demands of Soccer Match-play

Individual performance in competitive soccer matches is determined by the tactical and technical ability of players, their psychological make-up and their physical capabilities (Bangsbo, 1994d). It is difficult to separate the importance of each discrete element in order to clearly differentiate the effect of each variable, in isolation, on a player's performance (Hoff, 2005). For example, a well-developed tactical knowledge and a high technical standard in a player could lead to a team's success (Bangsbo, 1994d). Alternatively, a certain fitness level may be an important attribute for a player and his/her ability to influence a game. In reality, it is likely that a player's performance is a consequence of a delicate balance of a player's technical and tactical ability and his/her ability to maintain these skills throughout a match (Bangsbo, 1994b; Bangsbo, 1994d; Mohr, Krustrup & Bangsbo, 2003).

2.2.1 Physiological Demands of Match-play

Understanding the physiological demands of soccer enables us to appreciate the metabolic requirements of the activity. The assessment of the physiological demands of match-play requires observations on specific responses that may represent an individual players' involvement in the match (Drust, Atkinson & Reilly, 2007). The nature of the game, and its' rules, may prevent the use of invasive methodologies as they interfere in the performance of players. Non-invasive and indirect measurement techniques such as HR monitoring, the assessment of the distance covered and the profiles of activities completed during games have, therefore, been commonly used to provide an indication of the overall exercise intensity of match-play (Reilly, 2005; Drust et al., 2007). The activity profiles observed in match-play have been evaluated by several different research groups with several different

methodologies using a range of representations of the data. Methods such as the coded commentary on audio-tapes (Thomas & Reilly, 1976; Yamanaka, Haga & Shindo, 1988), and the filming and analysis of video-tapes (Ohashi, Togari, Isokawa & Suzuki, 1988; Bangsbo, Norregaard & Thorso, 1991; Bangsbo, 1994d; Rienzi, Drust, Reilly, Carter & Martin, 2000; Mohr et al., 2003) were originally used to evaluate the distance covered and/or the durations of separate activities. Modern technology now allows the distance covered and time spent in each activity to be determined more accurately and extensively through the use of complex computerized multi-camera tracking systems (Rampinini, Cottus, Castagna, Sassi & Impellizzeri, 2007; Rampinini et al., 2008; Bradley et al., 2009; Di Salvo, Gregson, Atkinson, Tordoff & Drust, 2009). These modern methods provide good indications of the activity profiles and energy requirements of soccer, thereby enhancing our understanding of the game.

2.2.1.1 Profiles of activities during match-play

Top-class players who were playing in the South American international tournament and/or the English Premier League covered 8-13km during match-play (Rienzi et al., 2000; Stolen, Chamari, Castagna & Wisloff, 2005; Bradley et al., 2009; Di Salvo et al., 2009). For example, the greatest percentage of this activity was covered in low-intensity activities such as walking (36~42%) and jogging (32~40%) in terms of distance covered (Rienzi et al., 2000; Bradley et al., 2009) **(Figure 2.1)**. This would indicate that soccer predominantly taxes the aerobic energy system. Top-level players, who were playing for the Italian professional teams that were competing in the European Champions League, would complete around 1350 discrete bouts of activities during a game (Mohr et al., 2003). These activities will include around 220 runs at high speed (>15 $km \cdot h^{-1}$) (Mohr et al., 2003). Such high-intensity running accounts for only 11~25% of the total distance in a game (Rienzi et al., 2000; Bradley et al., 2009). Di Salvo et al. (2009) reported that the actual distance associated with such high intensity running was around 885~919m with sprinting averaging 222~234m. These high intensity activities are important to the game as they can determine the result of matches (Reilly, 2005; Bradley et al., 2009; Di Salvo et al., 2009). The energy for such intense efforts will be partly derived from the anaerobic energy system (Drust et al., 2007).

There are other types of game-related and energy-demanding activities in a match in addition to the locomotion pattern. These include discrete actions such as passes, interceptions, shots, dribbles, jumping, tackling, kicking, changing direction and getting up from ground. These types of activities are related directly to the players involvement in play (Bangsbo, 1994d; Reilly, 2005; Stolen et al., 2005; Reilly, 2007d). Specific studies that demonstrate the distance covered in possession of the ball report around 2% of the overall distance covered (Bangsbo, 1994d; Reilly, 2007d). These activities, quantitatively small, do require a greater energy-cost than normal locomotion (Bangsbo, 1994d; Reilly, 2005; Stolen et al., 2005; Reilly, 2007d). This suggests that such specific match activities could elevate the physiological stress during match-play and hence the overall physiological load on the players.

The physiological demands of match-play can be altered as a result of factors such as the specific positional roles fulfilled by players, the environmental conditions, and/or the style of play and level of play (Bangsbo, 1994d; Rienzi et al., 2000; Mohr et al., 2003; Reilly, 2005; Di Salvo et al., 2009). For example, Rienzi et al. (2000) have illustrated that midfielders cover a 13~27% greater distance than that of forwards and defenders. This study also demonstrated the variations in work-rate that can be associated with the level of competition. Rienzi and colleagues (2000) suggest that international matches appear to emphasize retaining possession and producing quick decisive passing movements compared to club matches in the Premier League which seem to require high work-rates throughout the game. These specific tactical differences in match-play can reduce the total distance covered by 17% (Rienzi et al., 2000). Other evidence from Mohr and colleagues (2003) also investigated changes in activity profile with level of play; *higher level players* who were playing for an elite European team, competing in the Italian league and in the European Champions League Vs *moderate-level players* who were playing for their respective national teams, which were ranked 1~10 on the official FIFA list. They reported that higher level players covered 5%, 28% and 58% more total distance, high-intensity running and sprinting, respectively than moderate-level players.

2.2.1.2 Physiological responses to match-play

The intensity of match-play has been evaluated by measuring and analyzing HR responses in games. Heart rate is a useful indicator of the overall physiological strain associated with exercise (Billlows, Reilly & George, 2005; Drust et al., 2007) and has been the most commonly used strategy to quantify the internal training loads of players in soccer (Hoff, Wisloff, Engen, Kemi & Helgerud, 2002; Impellizzeri et al., 2005; Little & Williams, 2007). This is a consequence of contemporary short-range radio-telemetric systems that make it possible to measure the HR of all players in both competitive and friendly matches with little restriction to the players involved (Drust et al., 2007). Evaluating the available data from a number of studies (Rhode & Espersen, 1988; Bangsbo, 1994d; Bangsbo, 1994c; Stolen et al., 2005; Krustrup et al., 2006) indicates that the intensity of match-play is observed to be on average 80-93% of HR_{max} or 156- 175 beat·min^{-1}. These data imply that the overall exercise intensity in match-play is high thereby suggesting that players were placed under significant levels of physiological strain.

Additional information on the physiological strain of players during match-play can be provided using estimates of energy expenditure from the HR-$\dot{V}O_2$ regression line determined under laboratory conditions. By this method, the intensity of match-play corresponds to around 70~75% of $\dot{V}O_{2max}$ (Bangsbo, 1994d; Bangsbo, 1994c; Reilly, 1997). There are, however, some suggestions that HR can be influenced by factors other than the physiological requirements of exercise such as the thermal load, emotion and the type of exercise completed (Drust et al., 2007). This may affect the accuracy of the energy expenditure estimates obtained, thereby limiting the usefulness of the data. Previous studies have, however, provided evidence that there are suitable linear relationships between HR and $\dot{V}O_2$ during intermittent exercise that includes dynamic activities such as vertical jumps (Bot & Hollander, 2000). Bangsbo (1994) has also confirmed that the indirect HR-$\dot{V}O_2$ relationship is applicable for estimating energy expenditure in soccer. These findings suggest that the estimation of $\dot{V}O_2$ by measuring HR can be a useful addition in determining the physiological responses to soccer-specific exercise (Bangsbo, 1994d; Hoff et al., 2002). This data would also seem to indicate that the physiological stress associated with games is substantial.

2.2.2 Metabolism in soccer

The wide variations in the intensities of activities in soccer-specific intermittent exercise result in a need to generate *adenosine-tri-phosphate (ATP)* from both the aerobic and anaerobic metabolic systems (Bangsbo, 1994d; Reilly & Bangsbo, 1998). This complex pattern of energy provision makes it difficult to evaluate the dominant energy system used during soccer match-play. Understanding the available information on the energy systems that could be used within soccer-specific intermittent exercise will help to plan and organise optimal training programmes for players.

2.2.2.1 Anaerobic energy production

The multiple sprints within soccer result in periods of high energy demand (Balsom, Seger, Sjodin & Ekblom, 1992; Balsom, Wood, Olsson & Ekblom, 1999). The outcome of matches can be decided by such explosive actions as sprints, shots, jumps, headers and tackles (Bangsbo, 1994d; Rienzi et al., 2000; Stolen et al., 2005). The evidence that exists would suggest that such brief activities are predominantly supported by the anaerobic energy production system (Bangsbo, 1994d; Stolen et al., 2005; Bangsbo et al., 2006).

The breakdown of ATP stored in the muscle and the degradation of *creatine phosphate (CP)* can provide energy for short intense bouts of exercise (Bangsbo, 1994d; Stolen et al., 2005; Bangsbo et al., 2006; Krustrup et al., 2006; Reilly, 2007f). Krustrup et al (2006) reported that the muscle ATP content was lowered to around 15% of the resting level immediately after an intense period in the match, whereas the level of muscle ATP observed after a match decreased around 13% of the pre-match value. These findings suggests the total muscle concentration of ATP is largely preserved during a match (Bangsbo, 1994d) despite periods of time in which this energy source is depleted. The recovery processes that replenish ATP after intense exercise, however, seem to be slow (Krustrup et al., 2006). This may result in a significant contribution of CP to the energy provision during intense bouts of soccer-specific exercise as degradation of CP provides a considerable amount of energy (Bangsbo, 1994d). This would suggest that CP can be a powerful energy buffer during soccer-specific intermittent exercise (Spencer, Bishop, Dawson & Goodman, 2005). Such ideas are supported by previous studies that observed the continuous fluctuation of muscle CP concentrations as a result of the high-intensity exercise bouts (Gaitanos, Williams,

Boobis & Brooks, 1993; Bangsbo, 1994d). The available data implies that the repeated pattern of high-intensity activities may not always allow the replenishment of CP in muscle when the recovery periods are short (Spencer et al., 2005). These findings would indicate the length of the recovery bout is important in the re-synthesis of CP during the subsequent period of low-intensity activities (Bangsbo, 1994d).

When players repeat exhaustive exercise with only short recovery periods or perform very high-intensity sustained exercise (e.g. a long sprint from a defensive to attacking position) muscle glycogen is also utilised by the anaerobic energy system (Bangsbo, 1994d; Stolen et al., 2005; Bangsbo et al., 2006; Reilly, 2007f). Lactate is produced by glycolysis and can be accumulated in the muscle and blood if the exercise bout results in its production exceeding its' clearance rate (Bangsbo et al., 2006; Reilly, 2007f). The blood lactate levels during a game are on average 2.1~10.3 mmol·L^{-1} and 1.6~8.6 mmol· L^{-1} in the first and second halves, respectively (Bangsbo et al., 1991; Bangsbo, 1994d, 1994a; Krustrup et al., 2006). These high blood lactate levels are, however, influenced by the types of exercise completed just before sampling (Bangsbo et al., 1991; Krustrup et al., 2006). This would indicate that blood lactate concentration reflects an accumulated response to a number of high-intensity activities but not the lactate production of muscle to a single action during match-play (Bangsbo et al., 2006; Krustrup et al., 2006). Nevertheless, these results suggest that the rate of glycolysis is high during a match and subsequently it is important to develop specific training programmes for energy provision that utilise the glycolytic energy pathways.

2.2.2.2 Aerobic energy production

The energy contribution during strenuous exercise is not entirely derived from the anaerobic energy system (Bangsbo et al., 1990; Parolin et al., 1999). Bangsbo and colleagues (1990) have reported that anaerobic energy contribution during the first 30-seconds of an intense exercise bout accounted for 80% of the total energy turnover. This figure was subsequently decreased to 30% during the last phase of the exercise. This data demonstrates that the aerobic energy system is also important for intense exercise that is prolonged in nature. These findings have been supported by other studies where ATP turnover rates from anaerobic systems were progressively reduced as a consequence of increases in oxidative phosphorylation during maximal bouts of

exercise (Parolin et al., 1999). This information would suggest that the aerobic energy system can be highly taxed during intense periods of match-play as well as during maximal activities in games (Rienzi et al., 2000). The aerobic energy system regenerates ATP by the oxidation of substrates such as carbohydrate and fat stored within the muscle or delivered from the liver or adipose tissue via blood to the muscle (Reilly & Bangsbo, 1998).

One of the major substrates for aerobic energy provision would be glycogen that is stored within the exercising muscles (Bangsbo, 1994d; Krustrup et al., 2006; Bangsbo, Marcello Iaia & Krustrup, 2007) **(Figure 2.2)**. Bangsbo (1994c) reported that glycogen stored in the active muscles is mobilized faster than those located in hepatic tissue during exercise. The recent research by Krustrup et al. (2006) illustrated the use of glycogen during intermittent exercise by demonstrating the gradual decrease in the glycogen concentration in muscle during a match. Forty-two percent of the muscle glycogen initially stored in muscle was utilised during match-play. Analysis of individual muscle fibres after the match indicated that 11 and 36% of the total individual muscle fibres were 'completely empty' and 'almost empty' following the game (Krustrup et al., 2006). These findings would indicate that muscle glycogen is a major energy source for soccer. Krustrup et al. (2006) suggested that the reduction of glycogen in some fibres may limit maximal efforts, thereby could lead to a decrease in sprint performance at the end of the match (Krustrup et al., 2006). This may suggest that muscle glycogen depletion is related to fatigue during match-play (Krustrup et al., 2006; Bangsbo et al., 2007). Carbohydrate could also be utilised during a match from the breakdown of hepatic glycogen. The levels of blood glucose during a match are higher than at rest and are well maintained over 4 $mmol \cdot l^{-1}$ throughout the 90 min of play (Ekblom, 1986; Bangsbo, 1994d; Krustrup et al., 2006). These findings suggest that the availability of endogenous glucose is high enough to compensate for the use of blood glucose throughout a game (Bangsbo et al., 2007).

Aerobic energy can be also provided by fat oxidation **(Figure 2.2)**. Such substrates may help to compensate for the lower glycogen level observed in muscle during a competitive match though this change in substrate utilisation may have consequences for work-rate (Bangsbo, 1994d; Reilly, 2007f). The free fatty acid (FFA) level in the blood progressively increases during match-play (Bangsbo, 1994d; Bangsbo et al., 2006; Krustrup et al., 2006). These responses may be

explained by the changes in concentrations of hormones, such as the insulin and the catecholamines, over the 90 min of play. The available research would suggest that the catecholamine levels are progressively elevated towards the end of the game (Bangsbo, 1994d), whereas the insulin concentrations are lowered throughout both of the halves during match-play (Bangsbo, 1994d; Krustrup et al., 2006). The elevated catecholamine and lowered insulin concentrations will, thus, stimulate lipolysis from the adipose tissue and enhance the release of FFA into the circulation (Bangsbo, 1994d; Bangsbo et al., 2006). Other possible explanation for the increase in the concentration of FFA as the game progresses are the more frequent rest and low-intensity exercise periods observed in the second half which may allow for a significant increase in blood flow to adipose tissue, subsequently promoting the release of FFA (Bangsbo, 1994d; Krustrup et al., 2006). A high rate of lipolysis during a game is also supported by an observed increase in the glycerol concentration in the circulation (Bangsbo, 1994d; Krustrup et al., 2006). The available data shows that the glycerol level in the early intense periods of the first and second halves is 1.5~3-fold higher than at rest (Krustrup et al., 2006). These findings suggest that fat is a source of fuel in a soccer game and one which would be more predominant during the low-intensity exercise periods.

The uptake of FFA and the amount of fat oxidation cannot, however, be determined simply from the blood FFA and glycerol concentrations (Bangsbo, 1994d). The other major contributor to fat oxidation is *triacylglycerol* (TG) stored within the muscles. Helge et al (2006) has reported that muscle TG is significantly utilised during prolonged cycling exercise. This study suggests that the intra-muscular TG could contribute to the energy requirements during soccer by compensating for the progressive lowering of muscle glycogen during the match. That is, a higher utilisation of muscle TG may help to maintain high glucose availability during exercise (Bangsbo et al., 2006; Helge, Biba, Galbo, Gaster & Donsmark, 2006; Krustrup et al., 2006).

Figure 2.2 Estimated relative aerobic and anaerobic energy distribution and corresponding substrate utilisation (Bangsbo, 1994d)

Protein metabolism could also be important for exercise periods that are sustained for 90 min at average work-rates equivalent to that observed in match-play (Lemon, 1994). During prolonged exercise, *branched-chain amino acids* (BCAA) degraded from skeletal muscle are converted to *alanine*. This alanine formed in skeletal muscle is transferred through the blood to the liver and subsequently used to make glucose by *gluconeogenesis* (Huston, 1995). This glucose can then leave the liver and can contribute, via oxidation, to the energy requirements of the exercise (MacLaren, 2003). The contribution of protein is, however, likely to be only 1-5% of the total energy metabolism during exercise (Tarnopolsky, 2004; Reilly, 2007f) **(Figure 2.2)** as proteins are maintained in a constant state of metabolic flux with both simultaneous synthesis and degradation (Tarnopolsky, 2004). Few researches, however, have attempted to evaluate the importance of protein metabolism for soccer. As a consequence the exact role that protein plays in supporting the energy requirements is unclear.

2.2.3 Fatigue in soccer

It is clear from the previous sections that the energy requirements during high level soccer are high. These requirements may lead to some players, in some circumstances, been unable to fulfil the physical requirements of the game. This may lead to reductions in the high-intensity activity that is completed. A comprehensive understanding of such declines in activity will provide a useful framework for developing physical conditioning for players (Bangsbo et al., 2006). Fatigue can be defined as the inability to sustain work-rate (Reilly, Drust & Clarke, 2008). This can result from a decline in the capability of the muscle to generate the required level of force for a prolonged period of time (Reilly et al., 2008). Such impairments may not just be demonstrated by a decrease in a player's activity profile (e.g. the amount of distance covered) as impairments may also occur in technical and tactical performance.

2.2.3.1 Fatigue towards the end of a game

Many studies have demonstrated significant declines in the total distance covered (Rienzi et al., 2000; Mohr et al., 2003; Rampinini, Cottus et al., 2007; Rampinini, Impellizzeri, Castagna, Coutts & Wisløf, 2009), the total distance completed in high-intensity running and total sprint distance (Mohr et al., 2003; Rampinini, Cottus et al., 2007; Bradley et al., 2009; Di Salvo et al., 2009; Rampinini et al., 2009) during the second half compared with those of the first half. Impairments in a physical (Mohr, Krustrup, Nybo, Nielsen & Bangsbo, 2004; Krustrup et al., 2006) and technical performance (Rampinini et al., 2008; Rampinini et al., 2009) have been reported **(Figure 2.3)**. Other studies that have attempted to recreate soccer-specific exercise in the laboratory have also illustrated decrements in muscle strength and muscular activation in the lower limbs both during and following intermittent exercise protocols on the treadmill (Rahnama, Reilly, Lees & Graham-Smith, 2003; Rahnama, Lees & Reilly, 2006) indicating that the muscles cannot sustain force generating activities throughout 90 min of exercise. These findings suggest that fatigue is frequently experienced by players towards the end of the game. This may suggest that they would, therefore, benefit from additional physical training in order to minimize the impairment of technical performance (Rampinini et al., 2008)

Figure 2.3

It is unclear what the main mechanisms involved in the development of fatigue in soccer may be. One potential mechanism is the amount of glycogen stored in the muscles prior to the match (Reilly, 2007d). Several researchers have demonstrated that the depletion of glycogen stores is linked to decrements in performance in soccer (Bangsbo, 1994d; Reilly, 1997; Krustrup et al., 2006). For example, the players with low muscle glycogen levels before the match completed 25% less distance during the game than those of a control group according to Saltin (Saltin, 1973). In the only other study that has used muscle biopsies to evaluate muscle glycogen concentration during match-play (Krustrup et al., 2006), it was observed that over 40% of glycogen stored in muscle prior to the game is depleted after the match. Such depletion of glycogen in individual muscle fibres seems to be related to the impairment of both single and repeated sprint performance during match-play (Krustrup et al., 2006). This relationship between muscle glycogen levels and fatigue necessitates specific training sessions and nutritional preparation for enhancing glycogen storage in muscle before competition.

2.2.3.2 Temporary fatigue during a game

Changes in performance may also be observed on a more transient basis during a game (Reilly, 2007d). Mohr (2003) and Rampinini et al. (2008) have suggested that such transient changes in performance can be defined as temporary fatigue. The available data has illustrated that the total distance completed in high-intensity running is reduced by around 50% in the 5 min period after the most intense 5 min-section recorded during the game (Mohr et al., 2003; Rampinini et al., 2008). Other studies have shown that sprint performance is also reduced after the most intense period of exercise during the first half, whereas there is no change in such performance at the end of the first half when compared to that observed before the game (Krustrup et al., 2006). Such transient reductions in sprint performance during a game might be caused by metabolic factors such as low potential of hydrogen *(pH)*, high lactate, low CP, low ATP or high extracellular potassium in the muscle (Bangsbo et al., 2007). A complex interplay between those factors may lead to the manifestation of the temporary impairments of performance observed (Bangsbo et al., 2006; Bangsbo et al., 2007) though tactical or psychological factors may also be associated with the transient decrease in performance (Bangsbo & Krustrup, 2009). Bangsbo and Krustrup (2009) have suggested that such reductions in performance after intense exercise may be a result of natural variations in the intensity of a game as opposed to any physiological decrement in

individual players. Further study would, therefore, seem warranted to understand the mechanisms of the transient decreases in performance during match-play.

2.3. Preparation for competition

2.3.1 Multiple components of soccer training

Training in soccer has to be planned and prepared in order to include all the requirements needed for players to cope with the various demands of the game (Bangsbo, 1994b). Soccer training can be divided into four main areas, namely technical, tactical, psychological/social, and fitness training (Bangsbo, 1994b; Bangsbo, 1994d) **(Figure 2.4)**. Psychological preparation during training may enable players to exhibit their best performances as it may help to keep them motivated and manage game-related stress (Eubank & Gilbourne, 2003). Developing the tactical knowledge and ensuring a high technical standard may also lead to an increased opportunity for success (Bangsbo, 1994d). The match-related physical performance and technical abilities of top-level professional players are closely related to the physical capacities of individuals (Bangsbo, 1994d; Rampinini, Bishop et al., 2007; Rampinini, Cottus et al., 2007; Rampinini et al., 2009). This would suggest that developing a squad's overall fitness level may also lead to successful performance in competitive games (Rampinini et al., 2008). A major component of the training of a top-class players should, therefore, focus on improving fitness levels to enable players to perform explosive actions, to repeat high-intensity exercise and to recover following intense periods of match-play (Bangsbo et al., 2006). This can be done by completing systematic conditioning programmes that include the stimulus of match-play on a regular basis (Bangsbo, 2003).

The multiple requirements of soccer training programmes lead to complex considerations in the planning and organising of training programmes for the sport. Generally, the programmes in soccer must try and include strategies that integrate both the physical training and technical/tactical preparation (Reilly, 2005). If one of those components is neglected, then the overall results in the game may be adversely affected (Reilly, 2007f). Such programmes also necessitate a consideration of the specific requirements of individual players both in terms of their

physical status and playing-position (Bangsbo, 1994d). The implication here is that training programmes in soccer need to be planned on an individual basis and be associated with the demands of individual players rather than those associated with everyone in the team (Alexiou & Coutts, 2008). This set of requirements is very complicated and necessitates a detailed approach to planning.

2.3.2 Theoretical approaches planning and organising training

Understanding the theoretical mechanisms associated with physiological adaptation may provide useful background information for the appropriate planning and organisation of training programmes. The application of such knowledge may help individuals maximise the adaptations associated with a given training stimulus, thereby subsequently improving the physical performance of players in the most efficient way.

The processes of the body's responses to such physiological disturbances have been linked to the

General adaptation syndrome (GAS) theory proposed by Selye (1974). The model of *GAS* (Selye, 1974) has been accepted by many trainers and coaches for several decades and translated into their training methodology in an attempt to appropriately manipulate physical stress and regeneration. This relationship between exercise and physical responses has been termed as *the super-compensation theory* in the context of training theory (Bompa, 1999; Whyte, 2006). The super-compensation cycle consists of 4 phases: *training, fatigue, recovery* and *adaptations (super-compensation)* (Whyte, 2006). When the body is stressed by *training*, the utilisation of energy and the accumulation of by-products lead to *fatigue*. This fatigue is characterized by temporary decrements of performance. The body during the *recovery* phase compensates for this fatigue by the restoration of homeostasis by regenerating energy and removing the by-products of exercise metabolism. The last part of the super-compensation cycle is the subsequent phase of *super-compensation* or *physiological adaptation*. At this time the body achieves a new, higher level of homeostasis (Whyte, 2006) that may then be associated with an increased level of physiological function and performance.

2.3.2.1 Periodisation

The application of super-compensation theory in the real world necessitates periodic training programmes which enable soccer players to achieve a careful balance between training and recovery. Depending on the number of fixtures, professional teams carry out between 4~6 training sessions in a week during the competitive season (Bangsbo et al., 2006). This fixture congestion may result in players completing fewer training sessions, thereby leading to a subsequent deterioration in performance (Reilly et al., 2008). The training load can also be increased to 1 to 2 training sessions a day over a similar time period during pre-season (Impellizzeri et al., 2006; Svensson, 2007). For example, English professional players completed 2 training sessions a day in an attempt to emphasize the development of specific fitness levels during the mid pre-season period (Unpublished data, Svensson, 2007). These fluctuations in training load are frequently associated with very specific phases of a competitive year (Bangsbo, 2003) and are a consequence of individual coaches planning strategies to maximize physiological adaptations and the performance level of players at key times (Bangsbo & Krustrup, 2009).

The typical training year in soccer is periodised into a competitive phase (e.g. in-season), a

transitional off-season phase and a preparatory phase (between the former two periods) (Bompa, 1999; Bangsbo, 2003). Such purposeful variations of the training programme over time has been termed *periodisation (Bompa, 1999; Whyte, 2006)*. The training programmes in the off-season are based around the need of players to recover from the stresses of a competitive season. This often includes providing an opportunity for the gradual transition between individual training programmes completed while away from the squad and training as a team. Such training attempts to help players to maintain elements of their fitness as well as relax mentally from the stresses of competition (Bangsbo, 1994b; Bangsbo, 2003). The pre-season training periods should emphasize high-intensity training so that players can attain a high fitness level at the beginning of the season (Bangsbo, 1994d). During the in-season period, the quality of training should be emphasized to consolidate and maintain the fitness level of players (Bangsbo, 2003; Reilly, 2007f). This is important as the games programme forced on teams provides little opportunity for multiple training sessions and hence the development of fitness. This variability in the training requirements across different times of the annual phase helps ensure that players achieve their peak level of preparation.

2.3.2.2 General principles of training

Effective planning necessitates coaches and conditioners to be able to utilise a number of general principles associated with the delivery of specific sessions, to deliver the physical loads and recovery required by the general framework of the training plan. The systematic repetition of exercise as a physiological stressor results in functional improvements to the anatomical, physiological and biochemical systems (Viru, 1984). In order to achieve such adaptations, the training completed must not only stress the body's physiological systems but be specific to both the muscle groups and energy system that are required to complete the performance (Reilly, 2007c). That is to say, the gains acquired by training tend to be limited to the tissues and systems that are stimulated during training. This has been termed the *principle of specificity*. Soccer training should, therefore, consider the specific demands of the game and be designed for players to train in a soccer-specific way. This may require the inclusion of drills with a ball as a means of delivering a training stress (Bangsbo, 1994d; Reilly, 2005, 2007f). The physiological stress placed on the athletes should also be greater than that which players are normally used to. This is defined as *the principle of overload* (Reilly, 2007c). If this overload is too great, the players may

become overstressed during team-based training sessions. Such sessions, if frequently repeated, may lead to *overreaching and/or overtraining syndrome* (Impellizzeri et al., 2005). This can cause decrements in performance and increases in risk of injury. If, however, the loading is appropriate in magnitude, training sessions are likely to improve performance of players.

Another principle is *the principle of individuality*. In football, the training needs of a player should reflect their specific requirements (Bangsbo, 1994b; Bangsbo, 1998). For example, the fitness level of the player should be an important determinant of the work that they should complete. It was recently reported that the players with the highest fitness level had the lowest physiological response to a small-sided-game aimed at developing endurance performance (Hoff et al., 2002). This would indicate that the structure of this activity was not suitable for some of the players in question. The delivery of an appropriate load will enable players to consolidate their strengths and improve their weaker abilities (Impellizzeri et al., 2005). The adaptations achieved in training will also be progressively lost if the training session are not maintained or its stimulus is not delivered above the threshold that is required for adaptation (Reilly, 2007f). This *principle of reversibility* relates to such deterioration in training adaptations. For example, the tapering of volume and intensity of exercise during the off-season can lead to reductions in the fitness levels of players (Bangsbo, 1994d).

2.3.2.3 Monitoring physiological load in soccer training

These theoretical approaches to training adaptation and periodization can be optimised by implementing procedures that monitor the physiological stress during training sessions. This enables a more systematic control of the training load to be achieved, thereby ensuring a better adherence to key principles of training. Evaluation of the individual responses to training ensures that all players are provided with an adequate training stimulus during training sessions (Impellizzeri, Rampinini, Coutts, Sassi & Marcora, 2004; Little & Williams, 2007; Alexiou & Coutts, 2008). Such accurate quantification of the players' physiological responses will clearly lead to players attaining peak condition for competition as well as avoiding *under-training* or *over-training* (Achten & Jeukendrup, 2003; Impellizzeri et al., 2005; Little & Williams, 2007).

The relative physiological stress imposed on the players defined as the *internal training load (TL)*

(Impellizzeri et al., 2004; Alexiou & Coutts, 2008) requires the quantification of the intensity and duration of the physiological stress during training for a particular player (Foster et al., 1995; Impellizzeri et al., 2004). The complexities in the evaluation of the internal TL seem to be associated with the balance between the *validity* of the parameter that is chosen as an indicator of TL and the *practicality* of using that parameter for load assessment during exercise (Achten & Jeukendrup, 2003). Drust et al. (2007) suggested a framework to evaluate the physiological responses associated with soccer training and match-play. They suggested that any observation on the internal responses to any exercise load must not interfere with the performance of players and be non-invasive. Secondly, methods should have the same validity to the dynamically changing exercise intensities that comprise soccer-specific activities in training as to steady state exercise conditions. Many studies have complied with this framework and used HR-based and RPE-based monitoring methods for quantifying the internal TL of soccer players (Rhode & Espersen, 1988; Impellizzeri et al., 2004; Impellizzeri et al., 2005; Little & Williams, 2007). These studies, however, have focused on developing methodologies for determining training intensity of soccer players rather than direct comparison of physiological stress associated with the different seasonal periods. Few studies have, therefore, systematically attempted to quantify the TL completed by elite professional players over a short-term period of training at different time points. Further studies comparing the stress associated with different training periods would, therefore, provide a better understanding of training optimisation for soccer.

2.3.3 Specific types of training for soccer

The theoretical models and general principles of training are useful guides for developing conditioning programmes for all sports. These general principles should be supported by theoretical and practical information on the demands of the sport to ensure the appropriateness of the prescription (Stone & Kilding, 2009). Such systematic training programmes for soccer should clearly include provision for both the aerobic and anaerobic energy systems and specific musculature. This multifactorial approach to training will help attain peak fitness levels in players ahead of competition (Bangsbo, 1994d; Hoff, 2005; Stolen et al., 2005). The remaining sections of this aspect of the literature review will, therefore, briefly cover the potential impact of soccer-specific training on the aerobic and anaerobic energy systems as well as facets of specific muscle function such as strength. Understanding these physiological adaptations and changes in

performance following such training programmes will provide the scientific bases for planning and organising practical training sessions in soccer. A brief overview of approaches to the types of training practices that have been commonly used in the literature for these aspects of fitness is also provided to provide a frame of reference for the future experimental investigations in this thesis.

2.3.3.1 Aerobic training

Aerobic performance in soccer is related to the ability to perform prolonged high-intensity intermittent exercise periods (Bangsbo, 1994d; Reilly, 2007a). A greater endurance capability can enable players to repeat high-intensity activities and to recover more quickly after any given period of high-intensity exercise during a game (Reilly, 2007a). This capability would be a fundamental fitness requirement for players and/or the team both for physical performance and the maintenance of technical and tactical performance towards the end of a game.

This aspect of fitness can be improved by training sessions designed to improve the oxygen transport system that is able to extract and utilise oxygen. This type of training will also increase fat availability from muscle by enhancing responses of hormones such as catecholamines. This will help to spare the muscle glycogen levels for use in any high-intensity periods towards the end of game (Bangsbo, 1994b; Bangsbo, 2003; Reilly, 2007a). Enhancing oxygen transport is a consequence of changes in both *central* and *peripheral components* (Reilly, 2007a). *The central components* include increases in pulmonary and cardiac capacities (e.g. heart size, cardiac output, blood volume etc.). *Peripheral factors* improve the ability to utilise the delivered oxygen in the skeletal muscle. This could be mediated by means of capillary proliferation and increases in mitochondrial enzymes (Ross & Leveritt, 2001). Improvement in aerobic capabilities can be reflected in increases in match-related performance such as distance covered, number of sprints and number of involvements with the ball (Helgerud, Engen, Wisloff & Hoff, 2001), thereby leading to the potential for better team success (Hoff & Helgerud, 2004; Hoff, 2005).

Aerobic training can be classified into various categories according to the intensity of exercise that is used in the training session (Bangsbo, 2003; Reilly, 2007a). Using a classification related to the intensity of exercise, the types of training include *low-intensity (recovery) training* (50~80% of

HR_{max}, 20~70% of $\dot{V}O_{2max}$), *moderate-intensity training* (70~90% of HR_{max}, 60~85% of $\dot{V}O_{2max}$) *and high-intensity training* (80~100% of HR_{max}, 70~100% of $\dot{V}O_{2max}$). Low-intensity (Recovery) training would be normally completed after a match and/or a hard training session to facilitate regeneration of body and to avoid over-reaching or over-training syndrome. Players perform light activities such as jogging and/or alternative types of training (e.g. football tennis, swimming etc.) in this type of session. Moderate-intensity training attempts to improve aerobic fitness by increasing the peripheral factors that enhance the oxidative potential and utilisation of specific substrates in the active muscles. High-intensity training sessions are thought to focus more on the central factors, thereby enabling players to repeat high-intensity bouts of exercise for prolonged periods during match-play.

In order to develop the aerobic capacities of players, training should be performed with interval type exercises (Reilly et al., 2008). Although this type of exercise can be delivered with or without a ball, interval drills with a ball can provide a more soccer-specific training stimulus. For example, high-intensity interval dribbling around a soccer-specific track improves the maximal oxygen uptake of soccer players (McMillan, Helgerud, Macdonald & Hoff, 2005). Small-sided games can also be utilised for a soccer-specific interval training as this type of exercise not only motivates players through increased involvement with the ball (Reilly, 2007a) but developing a similar physiological stress to that associated with match-play. If small-sided games are completed with a diverse range of intensities (*via* the control of pitch-size, the number of players and setting limits to touch a ball), they are equally as effective as the other types of aerobic interval training in improving both physiological fitness levels and match-related performances (Reilly, 2005; Reilly & White, 2005; Sassi, Reilly & Impellizzeri, 2005; Impellizzeri et al., 2006; Reilly, 2007a).

2.3.3.2 Anaerobic Training

A players' anaerobic performance is determined by the capabilities to produce and/or maintain power and energy rapidly during intense exercise (Bangsbo, 1994b). These abilities translate in a match to situations that require the rapid development of force such as sprinting, quickly changing direction and the maintenance of these all-out efforts over a short period of time (Bangsbo, 1994d, 2003). Anaerobic performance can, therefore, be categorized into two aspects; those requiring short duration and highly explosive activities (e.g., sprint performance) and those that are

performed at a high-intensity for relatively longer periods of time (e.g., speed-endurance) (Reilly, 2007b).

Anaerobic performances are partly influenced by metabolic factors. The activation of a number of key enzymes associated with the ATP-CP system and anaerobic glycolysis, such as creatine kinase, phosphofructokinase, myokinase and glycogen phosphorylase, seems to be related to increases in the ability to perform high-intensity efforts (MacDougall et al., 1998; Reilly & Bangsbo, 1998; Ross & Leveritt, 2001; Bangsbo, 2003; Mohr et al., 2007). Oxidative enzyme activities such as citrate synthase, succinate dehydrogenase and malate dehydrogenase may also be involved in the improvements in the performance of all-out efforts following anaerobic training (Dawson et al., 1998; MacDougall et al., 1998). This type of training can also lead to elevations in glycogen levels in muscles (Reilly & Bangsbo, 1998; Ross & Leveritt, 2001). This would seem important to help maximise muscle glycogen concentration prior to match-play although aerobic training induces similar adaptations. Anaerobic training can also reduce the inhibitory effects of hydrogen ions (H^+) within the muscle cell by increasing the muscle membrane transport proteins involved in pH regulation and enhancing muscle buffering capacity (Juel et al., 2004; Iaia, Rampinini & Bangsbo, 2009) (Mohr et al., 2007; Iaia et al., 2008; Bangsbo, Gunnarsson, Wendell, Nybo & Thomassen, 2009). The alterations in the ion transport system can preserve the cell excitability and force development, thereby reducing the contraction-induced net loss of K^+ from the working muscles. Increases in the proportion of type II muscle fibres or their cross-sectional area after training are also possibly related to enhancements in anaerobic performances (Dawson et al., 1998; Ross, Leveritt & Riek, 2001). These adaptations are, however, specific to the type of anaerobic training that is completed by players. For example, Mohr and colleagues (2007) reported that speed training (6-s runs at ~95% maximal running speed with 1-min recovery) promoted metabolic adaptations that enhanced peak sprint performance, whereas indicators of recovery from high-intensity exercise were only improved following speed endurance training (30-s runs at ~130% maximal running speed separated by 1.5-min recovery). These findings indicate that it is essential to conduct anaerobic training with very specific prescriptions in order to induce general improvements in high-intensity exercise performance.

Speed training for sprint performance mainly focuses on developing technical elements such as

stride length and stride rate (cadence) (Stein, 1998; Reilly, 2007b). These technical aspects may be supported by increasing functional performance in parameters such as muscle strength and power (Wisloff, Castagna, Helgerud & Hoff, 2004) and coordination and agility (Venturelli, Bishop & Pettene, 2008). Training for these factors can maximise the initial acceleration and the top speed that players can obtain (Ross et al., 2001; Reilly, 2007b; Venturelli et al., 2008). Combining different training elements may be more effective than training related physical parameters in isolation. Kotzamanidis and colleagues (2005) have reported that sprint speed is significantly increased in a group that combines high-intensity strength and speed training rather than a group that completes strength training only (Kotzamanidis, Chatzopoulos, Michailidis, Papaiakovou & Patikas, 2005). This synchronization of muscular hypertrophy and neural adaptation, referred to *complex training* (Harris, Stone, O'Bryant, Proulx & Johnson, 2000), seems to influence the level of the strength gain or neural factors, thereby facilitating sprint performance (Kotzamanidis et al., 2005). Speed training may be more effective when the drills incorporated into practice replicate the rapid decision-making and quick movements in combination with a ball as completed during the game (Bangsbo, 1994b). This indicates that speed training should ideally be performed under football-specific conditions and include the ball (Bangsbo, 1994b).

The capacity in *speed-endurance* is improved by the repetition of maximal or near maximal intensity bouts of exercise (Bangsbo, 1994b; Bangsbo, 2003; Mohr et al., 2007; Reilly, 2007b). Speed endurance training can be divided into "production training" and "maintenance training" (Reilly & Bangsbo, 1998). The ability to repeat maximal efforts could be improved by "production training", whereas "maintenance training" could enhance the capacity to sustain high-intensity activity (Iaia & Bangsbo, 2010). Previous studies show that sprint velocity and average power output in a single exhaustive effort were increased through training that included repeated sprinting at near maximal intensities (Dawson et al., 1998; MacDougall et al., 1998; Mohr et al., 2007; Ferrari Bravo et al., 2008). The ability to sustain exercise at a high intensity and to maintain speed during a sequence of maximal efforts were also improved by similar types of exercise (Dawson et al., 1998; MacDougall et al., 1998; Mohr et al., 2007). These findings, therefore, indicate that such speed-endurance training enables players to perform intense activities repeatedly and to recover faster after the intense periods during a game, thereby coping with the number of high-intensity phase of the match.

2.3.3.3 Specific muscle training in soccer

Strength and power are important factors in soccer performance (Hoff, 2005). Explosive actions such as sprinting, jumping, turning, shooting and tackling are elements of performance that are dependent on these specific attributes (Bangsbo, 1994b). These explosive game-related activities that are critical to match-play can be improved by increasing the forces generated in muscular contractions in appropriate muscle groups (Bangsbo, Norregaard & Thorso, 1991). Such increase in muscular strength may not only lead to the success of the team (Wisloff, Helgerud & Hoff, 1998) but also prevent injuries (Bangsbo, 1994b; Reilly, 2007d).

Muscle strength can be developed by working against resistance (Reilly, 2007e). Two different mechanisms of adaptations to strength training have been described; *muscular hypertrophy* and *neural adaptation* (Ingham, 2006). *Hypertrophy* (increases in size of the muscle) of muscle is produced by altering the balance between protein synthesis and degradation. Increases in the cross-sectional area of muscle lead to improvements in force development as force is related to muscle sixe (Ingham, 2006). Adaptive changes in the nervous system, such as selective activation of motor units, synchronization, antagonist co-contraction and increases in the motor unit recruitment can also contribute to improved muscle strength (Behm, 1995). Such neural adaptations may change the activation of fast twitch fibre recruitment *via* increases in motoneuron excitability, thereby subsequently contributing to superior sprint performance (Ross et al., 2001). These findings indicate that specific muscle training in football would simultaneously emphasise improvements in both muscle hypertrophy and neural adaptation in order to maximise performance associated with such explosive actions.

2.4. Cellular Adaptations in Skeletal Muscle to Training Patterns Relevant to Soccer

Chronic adaptations in skeletal muscle include morphological and metabolic alterations that are derived from the cumulative effect of acute sub-cellular responses (Hawley, 2002; Flueck, 2009). The training completed by soccer players supplies the acute biological stimulus that is required to initiate these adaptive processes in muscle. The initiation signals for such adaptations are thought to include factors such as mechanical stretch, calcium flux and redox and phosphorylation

changes in cellular homeostasis during and after exercise. These factors subsequently influence changes in the sub-cellular regulatory cascades (Coffey & Hawley, 2006; Coffey & Hawley, 2007). The accumulation of acute molecular changes following repeated bouts of physical activity over time leads to increases in the number of proteins (Flueck, 2009). The increased protein content augments the mass of sub-cellular structures in skeletal muscle (e.g. mitochondria, enzymes, receptors, and myofibril), and subsequently enables a player to improve exercise capacity and function (Viru, 1984; Booth & Thomason, 1991; Flueck, 2009). An example of this concept can be illustrated by the schematic model for mitochondrial adaptations presented in **Figure2.5.**

The molecular consequences of cellular adaptations are highly specific to the volume, intensity and duration of the training (Booth & Thomason, 1991; Coffey & Hawley, 2007). The type of stimulus, i.e. the mode of exercise, also affects the training adaptations (Coffey & Hawley, 2007). For example, Atherton and colleagues (2005) have indicated that the selective activation of specific signaling pathways in the animal experimental model results in specific adaptive responses to both endurance and resistance training. These data indicated that a pattern of electrical stimulation representative of endurance training activated a specific signaling pathway that is associated with mitochondrial adaptations (*e.g., AMPK-PGC1α cascade*) whereas electrical stimulation that was resistance training-specific selectively activated the *PKB-TSC2-mTOR cascade,* a pathway that is related to the upregulation of protein synthesis and muscle growth (Atherton et al., 2005). This specificity of regulatory pathways to different contractile activities has also been observed in trained human skeletal muscles (Coffey, Shield et al., 2006; Coffey, Zhong et al., 2006; Leger et al., 2006; Mayhew, Kim, Cross, Ferrando & Bamman, 2009). Human studies have demonstrated that a single endurance exercise bout will activate the specific genes associated with mitochondrial synthesis, carbohydrate and lipid metabolism and angiogenesis (Coffey, Shield et al., 2006; Coffey, Zhong et al., 2006) while resistance training increases phosphorylation of anabolic genes such as Akt, glycogen synthase kinase-3β (GSK-3β) and mTOR (Leger et al., 2006; Mayhew et al., 2009).

2.4.1 Training responses and adaptations to soccer training

The endurance capacity of a soccer player is fundamentally important to enable the repeated performance of high-intensity activities during match-play (Stone & Kilding, 2009). One major aim of training in soccer should, therefore, be to enhance the endurance capacity (aerobic fitness) of players. This can be achieved through morphological and metabolic adaptations in the skeletal muscles and the associated increases in the rate of energy production from oxygen-dependent pathways (Hawley et al., 2006).

The available evidence would indicate that soccer training can induce long-term alterations in mitochondrial enzyme activities in skeletal muscle that are similar to those observed following

endurance training (Bangsbo & Miznuno, 1988; Bangsbo, Nielsen et al., 2009; Krustrup et al., 2009). Bangsbo and Miznuno (1988) observed that the level of *3-hydroxyachl co-enzyme A dehydrogenase (HAD)* activities was significantly increased after 4 weeks of soccer-specific training. Other studies have examined skeletal muscle adaptations following longer periods of small-sided soccer-specific exercise in untrained men (Krustrup et al., 2009) and women (Bangsbo, Nielsen et al., 2009). Such extended periods of soccer training increase *citrate synthase (CS)* and *HAD* activity by 9~14% (Bangsbo, Nielsen et al., 2009; Krustrup et al., 2009) and 8% (Bangsbo, Nielsen et al., 2009) respectively. These findings seem to be related to the observed functional improvements in endurance performance following soccer-specific training, in variables such as maximal aerobic capacity (McMillan et al., 2005; Reilly & White, 2005; Sassi et al., 2005; Impellizzeri et al., 2006). This indicates that the global effects of soccer training may be partly mediated by increases in mitochondrial function and the associated changes in substrates oxidation.

The available data, while linking mitochondrial adaptations to changes in performance does not, however, explain the potential regulatory mechanisms that may lead to increases in oxidative enzymes (e.g., CS and HAD) in response to soccer training. It is, therefore, still unclear what the impact of soccer-specific training has on the complex signaling networks that may underpin the molecular changes associated with such chronic adaptations in skeletal muscle. Obtaining such information to address these limitations in the available data in the field is very difficult. This makes it important to utilise other experimental models in an attempt to elucidate the mechanisms associated with mitochondrial adaptations to soccer training. Such models may include the use of soccer-specific intermittent exercise protocols completed in the laboratory. The findings from such potential research projects would improve our understanding of the cellular and molecular adaptations to both general and soccer-specific intermittent exercise programmes. This may support the development of novel training approaches in the field. The following sections will, therefore, review the acute molecular responses that lead to mitochondrial adaptations in human skeletal muscle following endurance training. The information in this section will help provide a rationale for elements of the future experimental studies included in this thesis.

2.4.2 Molecular responses underpinning mitochondrial biogenesis

Mitochondria are the main sub-cellular organelle in skeletal muscle that determines the oxidative capacity and fatigue resistance of athletes to prolonged contractile activity (Hoppeler & Fluck, 2003). Mitochondrial synthesis, which is frequently termed *mitochondrial biogenesis,* leads to increases in the enzyme activities associated with carbohydrate and fat oxidation in skeletal muscle (Adhihetty, Irrcher, Joseph, Ljubicic & Hood, 2003). Such alterations allow the muscle to more efficiently utilise substrates for ATP production and subsequently lead to improvements in endurance performance (Coffey & Hawley, 2007; Rockl, Witczak & Goodyear, 2008). The regulation of mitochondrial biogenesis appears to be controlled by a complex molecular process that require numerous transcriptional factors and transcriptional co-activators (Coffey & Hawley, 2007). **Figure 2.6** illustrates the signaling pathways involved in mitochondrial biogenesis. These pathways include: 1) the activation of signaling *kinases* to initiate biogenesis, 2) the induction of co-activator proteins such as *peroxisome proliferator receptor-γ co-activator-1α (PGC-1α) and NRF* transcription factor proteins and their trans-activation of target genes, 3) the import of these precursor proteins into mitochondria, and 4) the co-ordinated incorporation of both mitochondrial and nuclear gene products into an expanding organelle reticulum. All of these steps play a key role in regulating cellular homeostasis and in the adaptations to endurance exercise (Ljubicic et al., 2010).

Figure 2.6 Contractile activity-induced mitochondrial biogenesis (Ljubicic et al., 2010). (1) A single bout of contractile activity induces the activation of kinases through alterations in cellular *milieu*. (2) Multiple signaling cascades activated by kinases induce the binding of transcription factor (TF) to the promoter region of *PGC-1α* and elicit the transcription of *PGC-1α*. (3) *PGC-1α* binds to the promoter regions of target genes such as NRF-1/2 and stimulates their expressions. (4) NRF-1 activates nuclear genes encoding mitochondrial-destined proteins such as TFAM. (5) mRNAs of mitochondrial-destined proteins are exported into the cytoplasm where mRNA transcripts can be stabilized or destabilized by the binding of RNA-binding proteins (RBP). (6) Following translation in cytoplasm, mitochondrial proteins are imported by the protein import complex such as the translocases of the outer membrane (TOM) and translocases of the inner membrane (TIM). (7) Such mitochondrial proteins (e.g. TFAM) that are translocated into mitochondria can induce the expression of mtDNA-encoded proteins which consists of part of the electron transport chain (ETC).

2.4.2.1 Activation of protein kinases

A protein kinase is an enzyme altering the function of other proteins via the chemical addition of phosphate groups; a process which is referred to as *phosphorylation* (Hardie, 2004). Such phosphorylation of signaling kinases regulates the majority of cellular pathways including signal transduction in response to external stimuli such as exercise. Acute contractile activities of skeletal muscle following a single exercise bout elicit rapid changes in cytoplasmic calcium (Ca^{2+}), energy reserves, oxygen consumption and reactive oxygen species (ROS) (Ljubicic et al., 2010). These alterations in the cellular *milieu* lead to the activation of relevant protein kinases that are associated with mitochondrial biogenesis. Those protein kinases include *calcium/calmodulin-dependent protein kinase (CaMK)*, *AMP-activated protein kinase (AMPK)* and *p38 mitogen-activated protein kinase (MAPK)* (Ljubicic & Hood, 2008). These three exercise-activated kinases seem to initiate mitochondrial biogenesis. Understanding the specific responses of such kinases in accordance with the mode, duration and intensity of exercise will provide information on molecular mechanisms leading to improvement in oxidative capacity following training.

The activation of *CaMKs* are primarily associated with the acute elevation of cytosolic Ca^{2+} levels by contractile stress (Pilegaard, Saltin & Neufer, 2003; Hawley et al., 2006; Wright, Geiger, Han, Jones & Holloszy, 2007). A study by Wright and colleagues (2004) demonstrated that raising the cytosolic Ca^{2+} level (by caffeine administration which causes the release of Ca^{2+} from the sarcoplasmic reticulum (SR)) enhanced the phosphorylation and activation of *CaMKII* in vitro (Wright, Hucker, Holloszy & Han, 2004). Alterations in amplitude and duration of Ca^{2+} flux, which may also be important for adaptation, seem to be dependent on the type of the contractile stimulus. For example, endurance exercise results in sustained elevations of intracellular Ca^{2+} levels, whereas resistance exercise may increase intracellular Ca^{2+} levels with short cycles (Baar & Esser, 1999). This finding suggests that the rate of Ca^{2+} flux between the cytosol and SR may play a role in the regulation of the specificity of exercise-induced phohsphorylation of CaMKs (Chin, 2005; Coffey & Hawley, 2007). The phosphorylation of *CaMKII* is also related to exercise intensity as greater increases are observed after high-intensity exercise bouts than low-intensity bouts of exercise in humans (Rose, Kiens & Richter, 2006; Egan et al., 2010). It is, however, currently not known whether these phenomenon will differentially affect the regulation of downstream signaling cascades associated with mitochondrial biogenesis. For example, the rate of cytosolic Ca^{2+} flux

and the subsequent activation of CaMKs following intermittent or interval running programmes maybe different to those associated with more continuous exercise patterns. This would indicate that it is important to complete investigations using specific exercise patterns such as soccer-specific intermittent exercise patterns in order to fully understand the impact of such exercise protocols on adaptations. As a consequence, little information is available on influence of CaMKs signaling and training that is representative of real world football practice.

The activation of *p38MAPK* signal transduction cascades could be stimulated by exercise-induced factors such as growth factors, cytokines, hypoxia, alterations in intracellular Ca^{2+} levels, mechanical stress and reactive oxygen species (ROS) (Widegren, Ryder & Zierath, 2001b; Wright, Geiger et al., 2007). For example, contraction-induced Ca^{2+} release may be one of the other possible mechanisms leading to the activation of *p38MAPK* cascades (Widegren et al., 1998; Wright, Han et al., 2007) as the available data have demonstrated that increases in cytosolic Ca^{2+} levels can upregulate the phosphorylation of *p38MAPK* (Wright, Geiger et al., 2007). This Ca^{2+}-mediated phosphorylation of *p38MAPK* may coincide with activation associated with mechanical perturbations (Egan et al., 2010). Yamazaki (1995) and Komuro et al. (1996) first reported that mechanical stretch activated the *MAPK* family in cardiac myocytes. These mechanical stresses, such as are associated with myofibrillar contractions may then also directly modulate *p38MAPK* signaling pathways in human muscle (Yamazaki et al., 1995; Komuro et al., 1996; Coffey & Hawley, 2007). The *p38MAPK*-related regulatory pathways may then convert the mechanical and/or biochemical stimuli that are elicited by muscle contraction into appropriate intracellular responses associated with mitochondrial biogenesis and changes in substrate oxidation (Widegren et al., 2001b). The activation of *p38MAPK* has been also observed in human skeletal muscles following exercise (Widegren et al., 1998; Egan et al., 2010; Little, Safdar, Cermak, Tarnopolsky & Gibala, 2010). In particular, Egan and colleagues (2010) reported the activation of *p38MAPK* following a bout of cycling exercise irrespective of the intensity. The few available studies in this area indicate that the specific responses on *p38MAPK* following both different types and intensities of exercise are not yet fully defined. For example, no information is available on soccer-specific intermittent type exercise protocols that may induce different mechanical perturbations compared to steady-state exercise. This would indicate that investigating the effects of soccer-specific patterns of contractile activity on activation of *p38MAPK* is critical in order to

understand the role of *p38MAPK* in regulating downstream cascades during soccer training.

The *AMPK* activation in exercise seems to be mainly induced by cellular alterations in energy turnover; predominantly ATP depletions either by an acceleration of ATP consumption or through the inhibition of ATP production (e.g. elevation of free AMP levels and increase in AMP/ATP ratio) (Winder & Hardie, 1999; Hardie & Hawley, 2001). This may imply that *AMPK* acts as a "fuel gauge" or "cellular energy sensor" (Hardie & Hawley, 2001). *AMPK* may be especially stimulated by cellular energy reserves such as glycogen (McBride, Ghilagaber, Nikolaev & Hardie, 2009). The β-subunit of *AMPK* contains a glycogen-binding domain that binds *AMPK* to glycogen (Polekhina et al., 2003). Such specific sites on the *AMPK* molecule may suggest that *AMPK* may have a role as a "glycogen sensor" (Hardie, 2004; McBride et al., 2009) thereby supporting the idea that the depletion of energy stores, specifically muscle glycogen may play an important role in activating *AMPK* (McBride et al., 2009; Richter & Ruderman, 2009). Acute cycling increases *AMPK* activation in an intensity-dependent manner (Wojtaszewski, Nielsen, Hansen, Richter & Kiens, 2000; Chen et al., 2003; Wojtaszewski et al., 2003; Lee-Young, Koufogiannis, Canny & McConell, 2008) above a threshold intensity of around 60% of $\dot{V}O_2$max. *AMPK* activation has been also increased following exercise at a maximal intensity performed with brief exercise durations (4 repetitions of 30 s all out exercise bout) (Gibala et al., 2009) and at lower intensities when periods of activity are prolonged (around 40% $\dot{V}O_2$max for 3.5h) (Wojtaszewski, Mourtzakis, Hillig, Saltin & Pilegaard, 2002). These findings suggest that the *AMPK* activation may be related to the intensity, duration, types and mode of exercise. It is, however, currently unknown whether such variations, especially in one acute bout of exercise can acutely influence the exercise-induced regulation of mitochondrial synthesis and oxidative metabolism.

2.4.2.2 *PGC-1α* regulatory pathways

PGC-1α has been established as a regulator of a variety of metabolic processes such as gluconeogenesis in hepatocytes, brown fat thermogenesis in skeletal muscle and more significantly mitochondrial biogenesis (Lin, Handschin & Spiegelman, 2005; Yan, 2009). *PGC-1α* mRNA and protein are highly expressed in slow-oxidative fibres, compared with fast-glycolytic fibres muscle, as a principal factor regulating muscle fibre-type determination (Lin et al., 2002; Wu et al., 2002). *PGC-1α* is a transcriptional co-factor that binds and further activates transcription

factors leading to increases in the expression of their target genes. The expression of *PGC-1α* following contractile activities is also a downstream signaling pathway regulated by protein kinases such as *AMPK, CaMKs* and *p38MAPK* (Ljubicic et al., 2010). Such key protein kinases have been suggested to increase the promoter activity of *PGC-1α* through an up-regulation of its transcriptional activity (Wu et al., 2002; Hardie, 2004; Wright, Geiger et al., 2007; De Filippis et al., 2008; Yan, 2009; Egan et al., 2010; Ljubicic et al., 2010). For example, an exercise-induced activation of *PGC-1α* has been observed following a single bout of exercise in human skeletal muscle (Mortensen et al., 2007; De Filippis et al., 2008; Mathai, Bonen, Benton, Robinson & Graham, 2008; Wang, Psilander, Tonkonogi, Ding & Sahlin, 2009; Egan et al., 2010; Perry et al., 2010). These data suggest that *PGC-1α* seems to be a key factor in mediating and coordinating the adaptive responses to regular endurance exercise (Pilegaard & Richter, 2008). An acute upregulation of *PGC-1α* following exercise in human skeletal muscles may be responsible for initiating the mitochondrial biogenesis observed following exercise.

The potential link between *AMPK* activation and increases in *PGC-1α* expression has been demonstrated by several studies in animals (Terada et al., 2002; Taylor et al., 2008) and in humans (De Filippis et al., 2008; Gibala et al., 2009; Little et al., 2010). Terada (2002) and Taylor et al. (2008) have demonstrated that treatment with *5-aminoimidazole-4-carboxamide ribonucleoside* (*AICAR,* an *AMPK*-activator) directly induced an increase in *PGC-1α* expression and mitochondrial biogenesis in rodent muscle. The mechanism of *AMPK*-mediated *PGC-1α* activation by acute exercise in humans have also been indirectly explained by the robust phosphorylation of *acetyl CoA-carboxylase (ACC*) that is a direct downstream target of *AMPK* (Gibala et al., 2009; Little et al., 2010). De Filippis et al (2008) have also suggested that decreased *AMPK* phosphorylation may lead to a diminished *PGC-1α* activation in response to high-intensity interval cycling exercise. These findings indicate that *PGC-1α* would be regulated by activation of *AMPK* as its downstream signaling pathway following a bout of exercise.

Alternative signaling transduction pathways could also activate *PGC-1α* during exercise in human skeletal muscle. Alterations in mechanical stress and intracellular Ca^{2+} level would regulate *PGC-1α* transcriptional activities. Puigserver and colleagues (2001) have suggested that *p38MAPK* can directly phosphorylate *PGC-1α* in *vitro* and *vivo*. They demonstrated that the protein contents of

PGC-1α were increased in both a bacterial recombinant protein expression system *(in vitro)* and cell culture *(in vivo)* that are activated by *p38MAPK* (Puigserver et al., 2001). *CaMKs* may also enhance the *PGC-1α* expression through the regulation of *p38MAPK* (Wright, 2007; Wright, Han et al., 2007). Wright and colleagues (2007) have provided evidence *in vivo* that raising the cytosolic Ca^{2+} level can increase the phosphorylation of *p38MAPK* and activate *PGC-1α*. Such increases in *p38MAPK* and *PGC-1α* have been also blocked by both *CaMK* and *p38 MAPK* inhibitors. *CaMK* can also activate *PGC-1α through activating several transcription factors, such as cyclic adenosine monophosphate (CREB) and myocyte-specific enhancer factor 2(MEF2)* (Wu et al., 2002). These findings indicate that protein kinases such as *CaMK* and *p38MAPK* could also regulate the expression of *PGC-1α* gene transcription.

The activation of *AMPK, CaMKs and p38MAPK* signaling cascades seems to be well observed as upstream modulators of *PGC-1α* expression in skeletal muscle. Nevertheless, further study is required in order to determine how the activity of these protein kinases are exactly linked to the transcription factors involved in *PGC-1α* expression following exercise in human skeletal muscle (Yan, 2009). For example, there are few available controlled studies that demonstrate that higher energetic stress is directly associated with a greater activation of *AMPK* and subsequently an enhanced up-regulation of *PGC-1α* in human skeletal muscle (Wang et al., 2009). As relatively little is also known about the extent of phosphorylation of each kinase in response to soccer-specific intermittent exercise, it is clearly uncertain as to the role of these kinases in controlling *PGC-1α* expression in this form of exercise. Such investigations may help to identify important characteristics of the training stimulus (e.g. Ca^{2+} flux, energy stores, mechanical stress) for improvements in mitochondrial synthesis and oxidative metabolism following soccer training.

2.4.2.3 Response of *PGC-1α* signaling cascades to a single bout of exercise

The acute activation of mechanical and metabolic signaling following contractile activities increases the transient transcription of mRNA (Ljubicic et al., 2010). Accumulation of such transcriptional activation would lead to the effects of training *via* subsequent translation of the nuclear-encoded information into amino acid sequence and protein assembly of intracellular organelle (Flueck, 2009). Alterations in protein contents may not always consistently coincide with activation of its transcriptional activities (Watt, Southgate, Holmes & Febbraio, 2004; Mathai et al.,

2008). For example, Watt and colleagues (2004) demonstrated a 12-fold increase in *PGC-1α* mRNA content induced following prolonged cycling exercise at ~60% $\dot{V}O_{2max}$, whilst expression of *PGC-1α* protein was not enhanced. These findings implicate that *PGC-1α* protein content may not represent the acute cellular responses to a bout of exercise. This indicates that measuring *PGC-1α* mRNA would provide better information on initial cellular responses associated with mitochondrial biogenesis following contractile activities than assessing protein content. Recent studies consistently reported increases in the acute expression of *PGC-1α* mRNA in response to a variety of exercise such as knee extension exercise (Pilegaard et al., 2003; Mortensen et al., 2007), cycling (Sriwijitkamol et al., 2007; De Filippis et al., 2008; Mathai et al., 2008; Wang et al., 2009; Egan et al., 2010) and running (Harber et al., 2009).

Such increases in *PGC-1α* mRNA seems to be mediated via AMPK activation that is associated with the mode, duration and intensity of exercise (Booth & Thomason, 1991; Nader & Esser, 2001; Hildebrandt, Pilegaard & Neufer, 2003; Pilegaard et al., 2003; Egan et al., 2010). Wang and colleagues (2009), however, suggested that the type of exercise, such as continuous or intermittent may not influence the transcription of genes associated with mitochondrial biogenesis. This study demonstrated that continuous (67% $\dot{V}O_{2max}$) and interval cycling exercise (12 s at 120% and 18 s at 20% of $\dot{V}O_{2max}$) for 90-min with the same total amount of work resulted in a similar increase in the levels of mRNA encoding *PGC-1α* and *PDK* (Wang et al., 2009). The available evidence does not therefore seem to provide a clear indication of the importance of the exercise protocol for the increase in *PGC-1α*. It is then unclear whether a high-intensity intermittent exercise such as soccer would induce a similar effect on *PGC-1α* expression to that of steady state or interval endurance exercise. Understanding the extent of a *PGC-1α* regulation following a single bout of soccer-specific intermittent exercise may provide important preliminary molecular data on the mechanisms that lead to improvements in the fitness level of soccer players.

2.4.3 Molecular responses to the repeated training sessions

The training programmes of soccer teams combine multiple training sessions in a day. These repeated training sessions are used in order to integrate the large volume of training required to ensure high-standard levels of performance in players. Recently it has been suggested that such training programmes may amplify physiological adaptations and subsequently enhance the fitness

levels of players compared to training days that include a single session (Ronsen et al., 2001; Baar & McGee, 2008; Yeo et al., 2008). The training effects of multiple daily sessions when compared to single daily sessions are, however, currently unknown especially with regard to the molecular responses to training sessions that are representative of soccer. Understanding the acute molecular responses to repeated bouts of exercise may provide practical insights into the optimal training organisation in the sport.

2.4.3.1 Acute responses to the repeated bouts of exercise

Multiple bouts of exercise on the same day may provide additionally stress on the physiological systems of the body compared to a single session of exercise. These repeated training sessions may promote more effective training adaptations through a greater perturbation of homeostasis than is associated with a single session (Yeo et al., 2008). Recent findings from a number of research studies provide evidence that multiple exercise bouts can affect the physiological and metabolic responses to a subsequent exercise bout (Stich et al., 2000; Ronsen et al., 2001; Goto, Ishii, Mizuno et al., 2007) and hence may alter the potential cellular signals for muscle adaptation (Yeo et al., 2008). For example, a prior bout of prolonged cycling exercise significantly augments fat availability for a second bout of exercise at an equivalent exercise intensity (~ 60% of $\dot{V}O_{2max}$) (Stich et al., 2000; Goto, Ishii, Mizuno et al., 2007). Repeated cycling exercise at a high intensity (75% of $\dot{V}O_{2max}$) demonstrated more prominent neuroendocrine responses to the following bout of exercise on the same day compared to when a single bout of exercise was completed (Ronsen et al., 2001). These increases are most likely associated with more pronounced epinephrine and norepinephrine levels in the later sessions (Stich et al., 2000; Ronsen et al., 2001; Goto, Ishii, Mizuno et al., 2007). The levels of these hormones may also be elevated due to the incomplete resynthesis of muscle glycogen between bouts of exercise on the same day. This results in the stimulation of hepatic glycogenolysis and gluconeogenesis (Ronsen et al., 2001). Such changes in plasma glucose levels may implicate increases in the energy requirements associated with the second bout of exercise (Ronsen et al., 2001). The available data suggests that repeated training sessions are associated with a greater exercise-induced change in metabolism than is observed following a single bout of exercise (Stich et al., 2000). The potential magnitude of the change in these responses following a change in the organisation on the exercise stimulus is likely mediated by variables such as the frequency, recovery time and order of exercise incorporated in

the approach to the repeated training sessions (Hansen et al., 2005; Goto, Ishii, Kurokawa et al., 2007; Goto, Ishii, Mizuno et al., 2007; Goto, Ishii, Sugihara et al., 2007; Coffey, Pilegaard, Garnham, O'Brien & Hawley, 2009)

It has been suggested that the pre-exercise muscle glycogen level is important in mediating the acute molecular responses to multiple bouts of exercise (Hawley et al., 2006; Baar & McGee, 2008). Glycogen may directly regulate the activity, localization, structure and function of proteins that contain glycogen-binding domains (Baar & McGee, 2008). Those proteins are associated with diverse cellular functions such as substrate oxidation and gene transcription (Hargreaves, 2004). Several studies have manipulated pre-exercise muscle glycogen state and documented a greater signaling response in the lower glycogen compared to the higher glycogen state (Wojtaszewski et al., 2003; Chan, McGee, Watt, Hargreaves & Febbraio, 2004; Pilegaard et al., 2005; Steinberg et al., 2006; Mathai et al., 2008; Cochran, Little, Tarnopolsky & Gibala, 2010). Such greater signaling responses induced by lower pre-exercise muscle glycogen levels could subsequently lead to altered training adaptations (Baar & McGee, 2008). This, therefore, implies that the prior training session could lower the muscle glycogen store and subsequently affect the glycogen-binding domains.

Several studies have investigated the effects of low muscle glycogen levels on specific signaling proteins (e.g. *AMPK, p38MAPK, PGC-1α*) associated with acute mitochondrial adaptations to endurance training (Wojtaszewski et al., 2003; Chan et al., 2004; Pilegaard et al., 2005; Steinberg et al., 2006; Mathai et al., 2008; Cochran et al., 2010). Glycogen-depletion in the exercising muscles has been induced in these investigations by exercise and/or manipulating exogenous carbohydrate intake. Low glycogen concentrations in exercising muscles seem to greatly activate *AMPK*. McBride and colleagues (2009) have suggested that glycogen binding to glycogen-binding domains on the *AMPK β* subunit allosterically inhibits *AMPK* activity in vitro. This implies that depleted glycogen levels would possibly help activate this molecule. Recent studies have also reported that the level of *AMPK* activity measured using immunoprecipitates is higher by 34~60% in glycogen-depleted muscles than that of glycogen-loaded muscles (Wojtaszewski et al., 2003) and muscles with normal glycogen (Steinberg et al., 2006) in humans. Compared with normal glycogen availability, *AMPK* phosphorylation has also been increased by around ~35 % when

exercise is performed with low muscle glycogen levels (Yeo et al., 2009). These findings would seem to suggest that glycogen is an important mediator in *AMPK* activity during exercise (Polekhina et al., 2003; McBride et al., 2009).

Muscle glycogen availability also appears to influence *p38MAPK* expression following a bout of exercise (Chan et al., 2004). Chan and colleagues have demonstrated that glycogen-depletion greatly increases the nuclear abundance of phosphorylated *p38MAPK* after exercise compared with normal muscle glycogen level. This study, however, could not confirm whether *p38MAPK* was directly activated by the lowered muscle glycogen level as these changes could have been a consequence of *AMPK* activity (Chan et al., 2004). Recent studies (Yeo et al., 2009; Cochran et al., 2010) have alternatively suggested that *p38MAPK* activation is not mediated by lowered muscle glycogen levels. For example, Yeo and colleagues (2009) illustrated that *p38MAPK* phosphorylation following acute exercise was similar between groups when the glycogen concentration was both low and normal. Cochran and colleagues (2010) also demonstrated that two groups with similar muscle glycogen concentration showed significantly different *p38MAPK* phosphorylation levels in response to an acute bout of exercise. These findings would suggest that it is still unclear whether *p38MAPK* is directly affected by the glycogen status in the muscle.

It has been suggested that the induction of *PGC-1α* mRNA in human skeletal muscle is also influenced under different metabolic states (Norrbom et al., 2004; Pilegaard et al., 2005). When circulating substrate utilisation is reduced by restricting the blood flow to exercising muscles, the increase in *PGC-1α* mRNA content is 3-fold higher than that observed in the non-restricted condition (Norrbom et al., 2004). The muscle glycogen depleted by the prior bout of exercise seems not to be re-synthesized if the recovery period between two consecutive training sessions is short (Yeo et al., 2008; Cochran et al., 2010) or if carbohydrate ingestion is restricted post-exercise (Mathai et al., 2008). This implies that the rest period after the bout of exercise likely provides the temporal and energetic resources for the acute activation of the molecular regulatory system (Mathai et al., 2008; Ljubicic et al., 2010). Such phenomenon possibly activates glycogen-binding domains of AMPK subunits aforementioned and may lead to enhancing transcriptional activities of *PGC-1α* mRNA. Indeed, recent studies (Pilegaard et al., 2005; Cochran et al., 2010) suggested that the regulation of *PGC-1α* was sensitive to muscle glycogen levels. Pilegaard and

colleagues (2005) reported that *PGC-1α* mRNA content following exercise was elevated for a longer time in muscles with low-glycogen than those with normal levels of glycogen during the recovery period. Such ideas are also supported by the data of Cochran et al. (2010). This study demonstrated that *PGC-1α* mRNA contents were significantly enhanced following the second bout of exercise that reduced pre-exercise muscle glycogen levels. These findings provide indirect evidence that the *PGC-1α* mRNA expression would be greatly influenced by the depleted muscle glycogen level.

It is, however, debatable whether such different training programmes (i.e., twice or once daily training) can induce different adaptations on *PGC-1α* mRNA expression. The study by Mortensen et al. (2007) demonstrated that increases in mRNA expression of *PGC-1α* were similar 2-hr after an acute bout of exercise irrespective of the organisation of the training programme into twice or once daily regimens. These data could not confirm the effect of muscle glycogen levels on the acute expression of *PGC-1α* mRNA. Available data in this study did not show whether training commenced with lower muscle glycogen levels following the second training session in training twice daily model than those observed in training once model (Mortensen et al., 2007). It is, therefore, still necessary to investigate the acute effect of pre-exercise muscle glycogen contents, which is induced by repeated training sessions, on exercise-induced responses of *PGC-1α* mRNA. This investigation could also provide information on the effects of other factors such as energy reserves and recovery periods.

2.4.3.2 Chronic adaptations to the repeated training sessions

The repeated impact of acute exercise converts the transient signaling responses to the more permanent changes in the structure and function of organelles observed in a trained state (Flueck, 2009). In order to examine the chronic effects of such repeated training, recent studies have employed experimental models that manipulate training organisation (e.g. training twice daily every second day as opposed to training just once each day) (Hansen et al., 2005; Yeo et al., 2008; Morton et al., 2009). These experimental models can reduce pre-exercise glycogen levels in skeletal muscles. For example, the group training twice every second day commence every second bout of training with low muscle glycogen reserves in all studies irrespective of the mode of exercise utilised (one-legged knee extension protocol, Hansen et al., 2005; cycling, Yeo et al.,

2008; interval running, Morton et al., 2009). These studies have demonstrated that training under conditions of reduced carbohydrate availability can induce significantly greater increases in resting muscle glycogen content (Hansen et al., 2005; Yeo et al., 2008) and mitochondrial enzyme activities including CS (Hansen et al., 2005; Yeo et al., 2008), β-HAD (Yeo et al., 2008) and SDH (Morton et al., 2009). These findings seem to be relatively consistent irrespective of differences in the mode, duration and intensity of exercise (Helge, Stallknecht, Richter, Galbo & Kiens, 2007). This data would seem to confirm that commencing training with low muscle glycogen levels can potentiate the adaptations in oxidative enzyme activities (Morton et al., 2009).

Repeating training sessions on the same day may not, however, lead to superior training adaptations in *PGC-1α* in skeletal muscle compared to those protocols that utilise training once a day models. Mortensen et al (2007) compared the regulation of the PGC-1 family (*PGC-1α/-1β*, PGC-1-related cofactor (*PRC*)) following different training programmes (e.g., training once daily vs. training twice daily every second day) for 10 weeks. The mRNA contents of *PGC-1α and PRC* were similarly increased following a bout of exercise after completing both training programmes for 10 weeks. The phenomenon observed in the study by Mortensen et al. (2007) could be explained by two possible mechanisms. First, there may be no difference in the muscle glycogen levels between both training models and this subsequently may lead to the similar responses to training. Second, a significant difference may not be detected due to the late enhancement in the expression of *PGC-1α* mRNA; indeed, the expression of *PGC-1α* mRNA in human muscles has been observed to peak 5-hr after an acute bout of exercise (Pilegaard et al., 2005). Third, it is possible for *PGC-1α* mRNA levels to be attenuated as muscle adapted to the exercise challenge in the long term (Perry et al., 2010). These findings are also in agreement with the work of Morton et al. (2009) in which signaling proteins associated with mitochondrial biogenesis have been examined. They reported that *PGC-1α* and *COXIV* protein contents are similarly promoted irrespective of the training frequency used across the programme. In order to clarify the specific effect of pre-exercise muscle glycogen levels on chronic mitochondrial adaptations, it is, therefore, essential to develop a training model that clearly depletes muscle glycogen and that obtains muscle tissue at optimal times for the determination of key parameters. Such a controlled model would provide more useful information on optimising training programmes in sport than the currently available data.

Discrepancies between the responses of oxidative enzymes and *PGC-1α* to chronic training may be explained by a mechanism proposed in recent studies (Wright, Han et al., 2007; Perry et al., 2010). The rapid increase in exercise-induced enzymatic activities could be activated by the pre-existing *PGC-1α* and upstream signaling cascades rather than an increase in absolute *PGC-1α* protein contents following training programmes (Wright, Han et al., 2007; Perry et al., 2010). This mechanism may lead to more rapid increases in oxidative enzyme activities than *PGC-1α* protein content in the early phase of exercise training. We are, however, currently unsure of the role and mechanism of the depleted muscle glycogen level on the adaptations of oxidative enzymes and *PGC-1α* expression in response to the chronic endurance training. This clearly necessitates further controlled studies to investigate the impact of repeated training sessions with low muscle glycogen levels on *PGC-1α* expressions and oxidative enzyme activities.

In summary, the information reviewed above has demonstrated that acute bouts of exercise enhance exercise-induced signaling pathways associated with mitochondrial biogenesis and substrate oxidation. These changes may be potentially mediated by energy reserves, calcium flux and mechanical stretches in exercising muscles. Training programmes largely changing cellular homeostasis may, therefore, provide greater metabolic stimuli and facilitate training adaptations that are superior to those following training with minimally altering intracellular milieu. There are, however, limited studies that evaluate the effect of consecutive bouts of running exercise, especially soccer-specific intermittent exercise on protein kinases and *PGC-1α*. Further, no available study has attempted to determine the specific metabolic stress following an acute bout of soccer-specific intermittent exercise and the associated molecular responses especially when multiple sessions are programmed within a day. These investigations may provide data on the optimal organisation of soccer-specific training programmes.

CHAPTER 3
SEASONAL VARIATIONS IN THE PHYSIOLOGICAL LOADS AND ACTIVITY PROFILES OF PROFESSIONAL SOCCER PLAYERS

3.1 Introduction

Performance in competitive matches is determined by the tactical and technical ability of players, their psychological make up and their physical capabilities (Bangsbo, 1994d). The complex demands of soccer necessitate players completing systematic training programmes that include a range of activities designed for specific training effects. These programmes must not only incorporate technical and tactical elements but also develop the physical capacity of players in a number of fitness areas. Important aims include developing the aerobic and anaerobic energy systems and developing strength through specific muscle training (Bangsbo et al., 2006; Reilly, 2007c)

The weekly training programmes of soccer players vary according to the phase of the annual plan, the number of fixtures during the season and/or the experience of the coach (Impellizzeri et al., 2005; Bangsbo et al., 2006; Impellizzeri et al., 2006). Training in 'pre-season' usually focuses on the *rebuilding* of fitness levels in players following the off-season. This contrasts with the aim of 'in-season' training, where the aim is frequently on the *maintenance* of the specific capacities developed during 'pre-season' (Bangsbo, 1994b; Reilly, 2007f). Professional teams typically carry out between 4~6 training sessions per week during the competitive season (Bangsbo et al., 2006) though this training load can be increased to 1 to 2 daily training sessions for five days over a similar time period during 'pre-season' (Impellizzeri et al., 2006). Such changes in training pattern therefore dramatically increase the training demands placed on players and may result in differences in the physiological load across similar time periods (Stich et al., 2000; Ronsen et al., 2001; Goto, Ishii, Mizuno et al., 2007).

Little, if any, research has systematically attempted to both quantify and compare the training loads completed by elite professional players over a short-term period of training during 'in-season' and the 'pre-season' training. Such omissions from the literature are probably a consequence of the focus on developing methodologies for monitoring the training intensity of professional players [e.g. heart rate (Rhode & Espersen, 1988); session-RPE (Impellizzeri et al., 2005)] rather than directly comparing the stress associated with different training sessions. Nevertheless, understanding the extent of differences in the physiological stimuli which are provided during different training periods may permit the development of a model of the weekly

training loads that could be used to attain optimal performance and fitness levels at different times of the season (Impellizzeri et al., 2004). Such research may also be beneficial in providing information on avoiding overreaching or overtraining syndrome (Foster, 1998; Impellizzeri et al., 2004).

In addition to quantifying training load through traditional measures such as average heart rate and session RPE, it is also important to consider the specific sub-components of each training session in terms of activity profile and training goals. It is possible that subtle changes in the physiological stress associated with specific types of training or in discrete sub-components of a training session may explain the differences that have been anecdotally observed in training at specific phases of the annual plan. However, no researchers to date have attempted to evaluate the specific sub-components of soccer training in order to characterise specifically the physiological loading that is associated with each distinct type of activity. These differences in physiological stress may be a direct function of the movements that players complete in the session (Bangsbo, 1994d; Reilly, 2003) though this has also not been directly examined in the literature.

The aim of the present investigation was to therefore evaluate the physical demands of professional soccer training by quantifying and comparing the physiological training loads and activity profiles of professional players during the 'pre-season' and 'in-season' periods. This study also aimed to quantify and compare the physiological loading and activity profiles of specific training types (e.g. physical, tactical, technical etc.) or sub-components (e.g. warm up, cool down) during the 'pre-season' and 'in-season' training periods. Such approaches, therefore, enabled us to hypothesize that: 1) the training loads and activity profiles of professional soccer players would be higher during the 'pre-season' than the 'in-season' training periods; and 2) that the specific sub-components of training session would explain the differences that were observed in the overall training stress. These data will also be used to provide a template for the development of a laboratory-based soccer-specific training simulation. Such a simulation will permit investigation into the physiological responses and the molecular adaptations of skeletal muscle to a soccer-training specific training session in the later chapters of this thesis.

3.2 Methods

3.2.1 Participants

Twenty Korean professional footballers were monitored throughout the programmed 'pre-season' and 'in-season' training completed by a professional team participating in the highest division of the Korean league. These players were observed for an entire week of training during both the 'pre-season' and 'in-season' training periods. No data was available on the physiological load associated with the games completed during these observation periods as a consequence of restrictions placed on the project by the coaches at the club in question and the rules of the tournament. The middle week of the 'pre-season' training period was chosen for analysis as it was envisaged that this would provide the most representative micro-cycle to evaluate during this training phase. The 'in-season' training analysis was carried out 12 weeks into the season when players were in a regular pattern of games and training. After excluding players who did not participate in all sessions, data from twelve players (mean ± SD age: 24 ± 3 years, body mass: 73 ± 4 kg, height: 1.78 ± 0.06 m, professional career: 3 ± 2 years; 3 forwards, 6 mid-fielders, 3 defenders) in the 'pre-season' and ten players (mean ± SD age: 25 ± 3 years, body mass: 75 ± 5 kg, height: 1.82 ± 0.05 m, professional career: 4 ± 3 years; 3 forwards, 3 mid-fielders, 4 defenders) in the 'in-season' training periods were included for analysis. Five of these players participated in both 'pre-season' and 'in-season' training sessions. The team finished in seventh place of the fourteen teams that competed in the league during this year.

3.2.2 Determining the physiological load

The physiological loads of all training sessions were evaluated by monitoring heart rate (HR) throughout each session. Heart rate was recorded every 5 s using a short-range telemetry system (Polar Team System®, Kempele, Finland). The physiological intensities of all training sessions were indicated by both absolute (beat/min) and relative values (*i.e.* the corresponding percentage of maximal HR; % of HR_{max}) of HR. The time spent within specific HR zones (90~100% of HR_{max}, 80~90 % of HR_{max}, 70~80 % of HR_{max}, 60~70% of HR_{max}, 50~60 % of HR_{max} and < 50 % of HR_{max}) was also measured. The maximal heart rate of each individual player was assessed by using the multistage bleep test (Ramsbottom, Brewer & Williams, 1988). The rating of perceived exertion

(RPE) was also evaluated after the completion of each training session using a modified 10-point rating Borg scale (Borg, Hassmen & Lagerstrom, 1987; Foster et al., 1995). Training load (TL) in arbitrary units (AU) was calculated by multiplying RPE score with the duration of each session (in minutes) to provide an index of the total physiological loads completed during the session (Foster et al., 1995). Daily total TL was calculated from the sum of all TL for specific training sessions performed in a day (Foster, 1998; Impellizzeri et al., 2004). The validity of these approaches in the assessment of TL in football has been well established in previous publications (Impellizzeri et al., 2004; Alexiou & Coutts, 2008).

3.2.3 Categorizing training sessions into sub-components according to specific training goals

In order to evaluate the weekly organisation of training sessions and to provide physiological data on a certain aspect of training, specific sub-components of each session were categorized according to the specific focus of training. This categorization was made following discussion with the coaches of the team. Physical training (PT) was defined as a programmed session that was devised to enable players to cope with the physical demands of match-play. When a session was planned for the player's tactical understanding and/or their technical ability, it was defined as technical/tactical training (TT) (Bangsbo, 1994b). When the session included both technical/tactical activities immediately followed by physical training, the session was defined as physical and technical/tactical training (PT/TT). Warm-up and cool downs were also specifically defined for each training session irrespective of the training type. The duration of all training sessions was recorded using a stopwatch (HS-3V-1, Casio, Japan).

3.2.4 Evaluation of activity profiles during training

Players' movements during a training session were analyzed using a video-based notation system. This analysis was included in the investigation as the activities completed by players in each specific component of training may increase the potential for meaningful interpretations of differences in the physiological loading between each phase of training. Six subjects (2 defenders, 2 mid-fielders, 2 forwards) were filmed in all sessions during 'pre-season' and 'in-season' training. One video camera (GR-DVL520KR, JVC Ltd, Japan) per player was used for filming. The players

were filmed for the entire duration of each of the training sessions.

The activities completed during training were classified into specific movement categories based on the intensity of action including sprinting (maximal running) (Sp), high speed running (HSR), jogging (J), walking (W) and static pause (St) (Bangsbo, 1994d; Rienzi et al., 2000). The categorisation of movement was based around the stride counts observed per second in each activity. The stride counts associated with sprinting, high speed running, and jogging were above 4 strides·s^{-1}, between 3 strides·s^{-1} and 4 strides·s^{-1} and below 3 strides·s^{-1}, respectively. Jogging and walking categories included forwards (Fw), backwards (Bw) and sideways (Sw) movements (Rienzi et al., 2000). Movement categories were determined on a second by second basis using the computerized coding system developed (Sportscode Gamebreaker® software, Sportec Ltd, Australia). After completing the coding of activities for each session, the total time associated with each movement category was summated thereby permitting the calculation of the work-rate for an entire training session to be determined.

Technical elements reflecting the skills of the game were also analysed. These elements included touch of the ball, jumping, heading, throwing, dribbling, tackling, shooting or kicking, and getting up from ground (Bangsbo, 1994d; Bangsbo, 1994b). The specific definitions of each activity are presented in **Table 3.1**.

Table 3.1 Definitions of the technical elements

Skill elements	Definitions
Ball touch	A player contacts the ball with any part of body except their head.
Jumping	A player moves quickly off the ground by pushing himself with his legs and feet.
Heading	A player contacts the ball using their head.
Throwing	A player throws the ball over their head.
Dribbling	A player runs with the ball at their feet.
Tackling	A player acts to prevent an opponent's possession of the ball or to block a pass.
Kick	A player sends the ball to a target or team-mate over long distance.
Getting up from ground	A player stands up from ground after contacts with the floor.

3.2.5 Evaluating the reliability of the methodology for the analysis of activity profiles

The reliability of the movement analysis system was analysed prior to the analysis of the training sessions. The agreement between pairs of observations performed at different times was assessed in order to evaluate the intra- and inter-repeatability of the notational analysis systems of the movement. Intra-rater agreement, which is the comparison of two measurements completed by the same observer, was initially evaluated. Intra-rater agreement was assessed by comparing one analysis of a designated training session with another subsequent analysis of the same session. To avoid the variability between repeated trials within a day (Atkinson & Nevill, 1998), the second analysis was completed 3 days after the first observation. Subsequent re-analyses were also completed for this tape following the analysis of every 15 tapes. This procedure was performed to evaluate any learning effects associated with the notational analysis method. As a consequence of the agreement between categorical assessments being specific to the ability of different observers (Altman, 1991), inter-rater agreement was also evaluated for the system. Inter-rater agreement was measured by comparing the results of the analysis of one training session by two different individuals at different times. Each observer completed one hour long familiarization session to learn how to operate the software and analyze movement.

The exact number of agreements during activities was calculated based on a second-by-second breakdown of the data. The agreement between different observations was then calculated by evaluating the exact number of agreements between each specific assessment. The ratio of the exact agreement was evaluated by the calculation of kappa (k). Kappa statistics enabled the calculation of the numbers of agreement that are in excess of those agreements that would be expected by chance. A 95 % confidence interval (CI) for the population value of k was also obtained by calculating the approximate standard error of k. All kappa values of both intra-rater and inter-rater agreement tests were observed to be in the "very good category" and were in the range of a 95% CI (Landis & Koch, 1977; Altman, 1991) **(Table 3.2)**.

Table 3. 2 Intra-rater and inter-rater agreements for the work-rate analysis

		kappa (*k*)	95% CI of *k*	Strength of agreement
Intra-rater agreement				
	1st	0.92	0.90-0.94	very good
	2nd	0.90	0.88-0.92	Very good
	3rd	0.91	0.89-0.93	Very good
Inter-rater agreement		0.90	0.88-0.92	very good

CI – Confidence interval

In order to evaluate reliability of the observation system for technical elements, Scott's π coefficient of reliability was calculated (Craig, 1981; Van der Mars, 1989). The agreement between two different observations was estimated by calculating both the observed proportion of agreements and the proportion that would be expected by chance in a similar manner to the calculation of *k*. The reliability coefficient, π , was then computed as the ratio of the difference between obtained and expected proportions of intra-rater or inter-rater repeatability to the difference between perfect and expected agreement. When all π values of both intra-rater and inter-rater agreements were applied to the strength standards of *k* (Landis & Koch, 1977; Craig, 1981; Altman, 1991), the values of π obtained in the current investigation were in the "very good category" (**Table 3.3**).

These findings indicate that the notational analysis system used within this study had an acceptable level of repeatability and objectivity.

Table 3.3 Intra-rater and inter-rater agreements of analysis for technical elements

		pi (π)	Strength of agreement
Intra-rater agreement			
	1st	0.83	very good
	2nd	0.81	Very good
	3rd	0.85	Very good
Inter-rater agreement		0.82	very good

3.2.6 Statistical Analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences software program (version 15.0). After verification of normality which was confirmed by the *Shapiro-Wilk* test and visual inspection of Q-Q plots, Student's t-tests for independent samples were used to compare the mean duration, frequency and physiological loads of an entire training session between the 'pre-season' and 'in-season' periods. The physiological loads of each specific type of training session (*e.g.* PT, TT and PT/TT) were also compared between both training periods with an independent t-test. The RPE-based training loads over each week were analysed by a repeated-measures *ANOVA* (two factors: time x training period) for the comparison of the daily TL across days between the 'pre-season' and 'in-season' training periods. All data are presented as means ± SD with *p values* < 0.05 indicating statistical significance. Analysis of work-rates was illustrated by only descriptive statistics due to the insufficient sample size ('pre-season', n = 3; 'in-season', n = 3).

3.3 Results

3.3.1 Overview of weekly training schedule

An overview of the weekly training schedule and the specific sub-components of each training

session are shown in **Figure 3.1**. The weekly training programme consisted of 34 discrete sub-components of training in 'pre-season' and 18 sub-components of training during the 'in-season' period. The frequency and duration of each subcomponent of training are presented in Table 3.4. The 'pre-season' training programme was performed over 6 days a week with players performing 2 sessions every day except Wednesday. In contrast, during the 'in-season' period players trained 5 days a week and generally only completed one session per day (Tuesday was the only day on which players completed two sessions in a day). The duration of an entire training session did not differ between training periods ('pre-season': 92 ± 17 min; 'in-season': 83 ± 15 min, p = 0.86). Durations of specific sub-components of training sessions were also similar between both training periods **(Table 3.4)**.

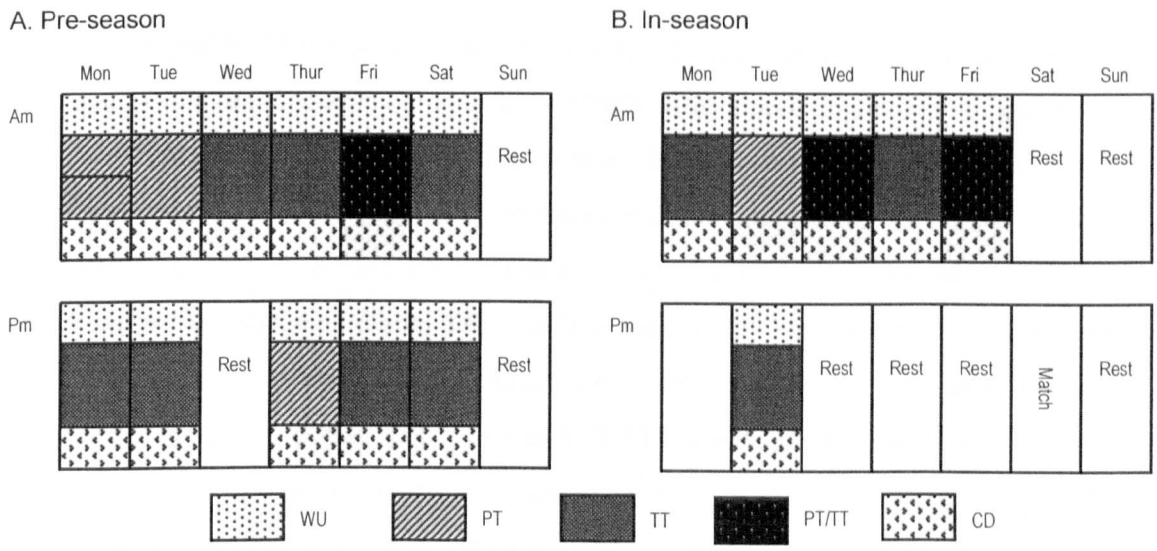


Figure 3.1 Weekly training programmes of the Korean professional team during 'pre-season' (A) and 'in–season' (B). WU: Warm up, PT: Physical training, TT: Technical/tactical, PT/TT: Physical and technical/tactical training, CD: Cool down.

Table 3.4 Frequency and average duration for all training sessions during 'pre-season' and 'in-season'

		Sessions	Sub-components of Sessions				
			WU	PT	TT	PT/TT	CD
Pre-season	Frequency	11	11	4	7	1	11
	Duration (min)	92 ± 17	24 ± 8	43 ± 21	56 ± 18	60	11 ± 4
In-season	Frequency	6	6	1	3	2	6
	Duration (min)	83 ± 15	24 ± 7	50	45 ± 13	54 ± 16	10 ± 5

WU: Warm up, PT: Physical training, TT: Technical /tactical training, PT/TT: Physical and technical/tactical training, CD: Cool down.

3.3.2 Quantification of training loads between 'pre-season' and 'in-season' training

Average HR and % of HR_{max} of an entire session was significantly higher in 'pre-season' compared with those observed in 'in-season' **(Table 3.5)**. These differences appear to be attributable to a significantly higher physiological strain for TT sessions observed in 'pre-season' period than 'in-season' **(Table 3.5)**. During an entire session in 'pre-season', the time distributions in HR zones between 80~90 and 90~100% of HR_{max} were significantly increased compared to those observed in 'in-season' **(Table 3.6)**. The time spent in the highest intensity HR zone for specific PT and TT sessions in the 'pre-season' period was also significantly greater than that observed in 'in-season' **(Table 3.6)**. The sum of RPE-based TL over each week showed significant differences between 'pre-season' and 'in-season' (4343 ± 329 vs. 1703 ± 173 AU, respectively) **(Table 3.7)**. The daily TL of each day in 'pre-season' was significantly higher than that observed in 'in-season' except for a Tuesday **(Figure 3.2)**. In terms of specific training sessions, both TT and PT/TT sessions during 'pre-season' showed significantly higher RPE-based TL than comparable sessions completed in 'in-season' **(Table 3.7)**.

Table 3.5 HR and % HR_{max} for all training sessions during 'pre-season' and 'in-season' training

		All sessions	WU	PT	TT	PT/TT	CD
HR ($b \cdot min^{-1}$)	Pre-season	124 ± 7*	105 ± 7	138 ± 7	137 ± 8*	118 ± 8	113 ± 6*
	In-season	112 ± 7	104 ± 7	137 ± 8	114 ± 9	113 ± 6	99 ± 7
%HRmax (%)	Pre-season	64 ± 3*	54 ± 2	71 ± 3	70 ± 4*	61 ± 4	58 ± 3*
	In-season	58 ± 6	54 ± 3	72 ± 3	60 ± 5	59 ± 3	52 ± 9

* $p < 0.05$, significant difference between the 'pre-season' and 'in-season' periods. HR: Heart rate, HR_{max}: Maximal heart rate, WU: Warm up, PT: Physical training, TT: Technical /tactical training, PT/TT: Physical and technical/tactical training, CD: Cool down.

Table 3.6 Time proportion (%) in HR zones during 'pre-season' and 'in-season' training

		HR zones (% of HR_{max})					
		100-90	90-80	80-70	70-60	60-50	<50
Pre-season	All sessions	4 ± 3*	14 ± 4*	16 ± 3	23 ± 2	25 ± 4	17 ± 7
(n=12)	PT	4 ± 3*	20 ± 6	20 ± 5	25 ± 3	20 ± 6	13 ± 5
	TT	8± 6*	22 ± 6*	21 ± 3	21 ± 2	20 ± 4	9 ± 5
	PT/TT	0.2 ± 1	7 ± 7	18 ± 7	29 ± 5	29 ± 4	18 ± 12
In-season	All sessions	0.3 ± 1	5 ± 2	13 ± 5	26 ± 5	29 ± 4	26 ± 10
(n=10)	PT	0.4 ± 1	22 ± 12	41 ± 8	19 ± 3	15 ± 7	2 ± 2
	TT	0.6 ± 1	9 ± 6	15 ± 6	22 ± 9	30 ± 8	24 ± 15
	PT/TT	0.2 ± 1	3 ± 3	16 ± 10	29 ± 8	29 ± 6	22 ± 4

* $p < 0.05$, significant difference between 'pre-season' and 'in-season'. HR: Heart rate, HR_{max}: Maximal heart rate, WU: Warm up, PT: Physical training, TT: Technical/tactical training, PT/TT: Physical and technical/tactical training, CD: Cool down.

Table 3.7 RPE-Based Training Loads during the 'pre-season' and 'in-season' weekly training programme of the Korean professional soccer team

		WU	PT	TT	PT/TT	CD
RPE (AU)	Pre-season	2 ± 1	6 ± 1	5 ± 1*	5 ± 1*	2 ± 1
	In-season	2 ±1	7 ± 1*	4 ± 1	4 ± 1	2 ± 1
TL (AU)	Pre-season	55 ± 8	242 ± 20	321 ± 23*	240 ± 36*	26 ± 6
	In-season	50 ± 9	365 ± 63*	174 ± 27	187 ± 18	24 ± 3

* $p < 0.05$, significant difference between 'pre-season' and 'in-season'. RPE: Rating of perceived exertion, AU: Arbitrary unit, TL: Training load, WU: Warm up, PT: Physical training, TT: Technical /tactical training, PT/TT: Physical and technical/tactical training, CD: Cool down.

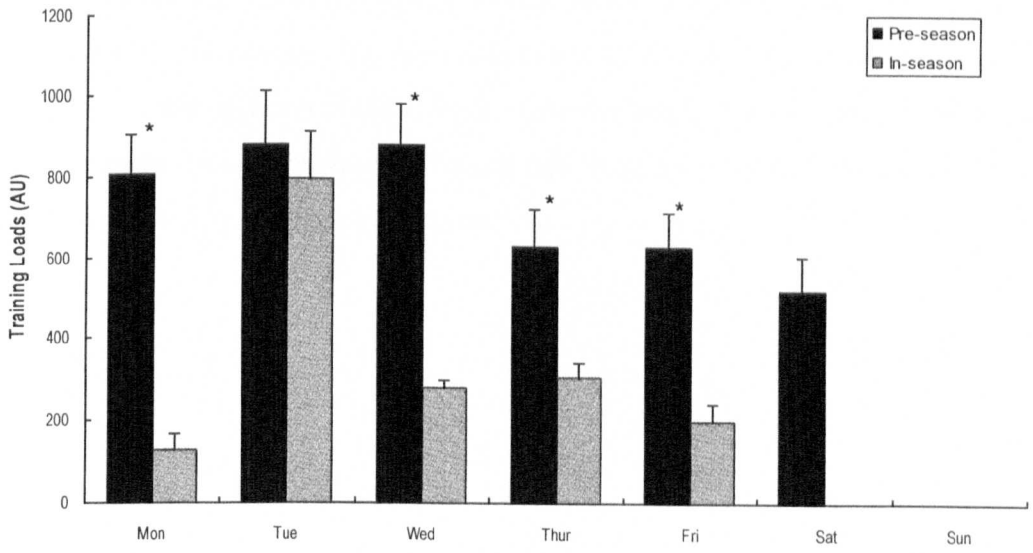
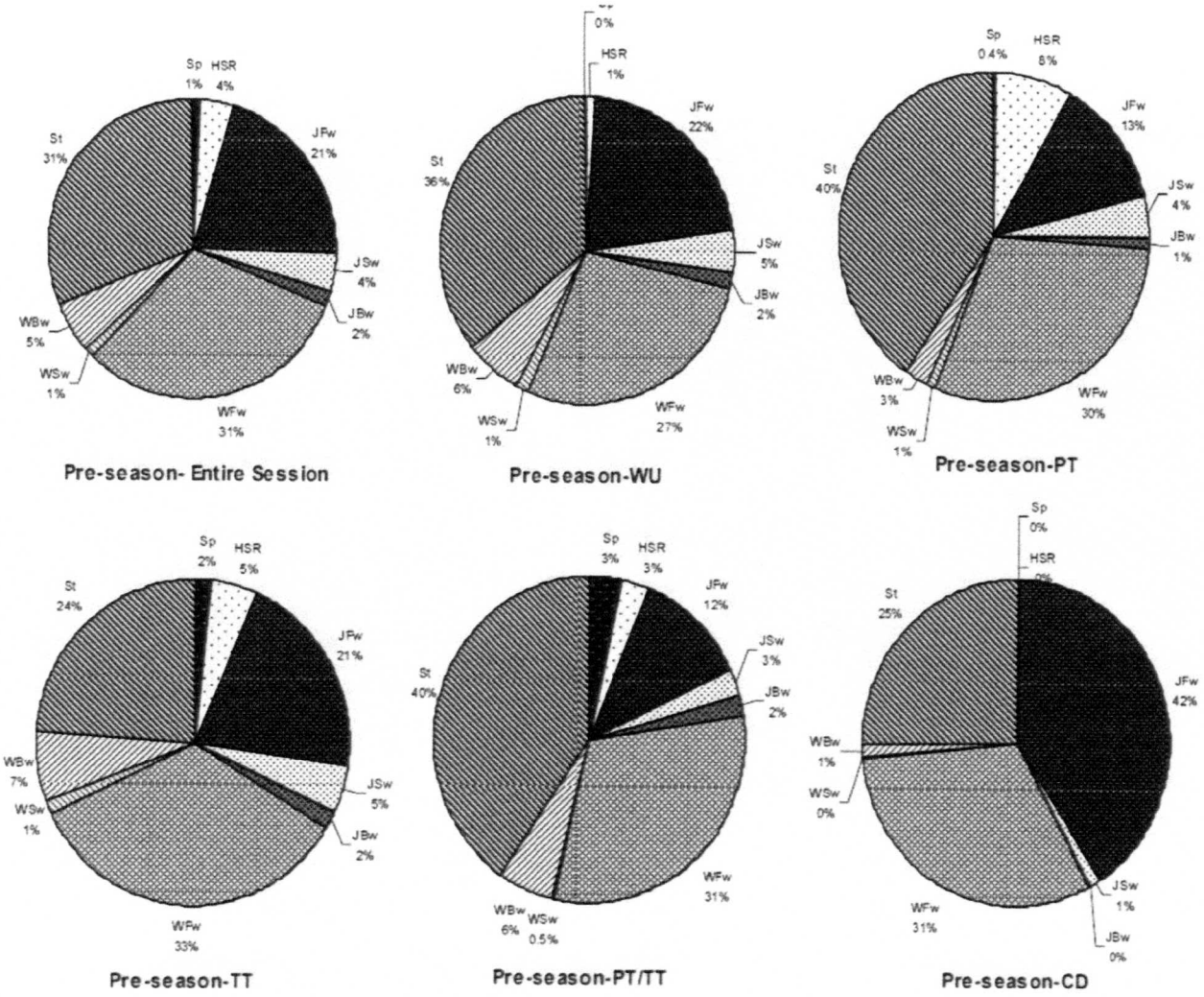


Figure 3.2 Representation of the weekly training loads as calculated using rating of perceived exertion during the 'pre-season' and 'in-season' periods. AU: Arbitrary unit, * $p < 0.05$, significant difference between 'pre-season' and 'in-season' periods.

3.3.3 Comparison of activity profiles between 'pre-season' and 'in-season' training

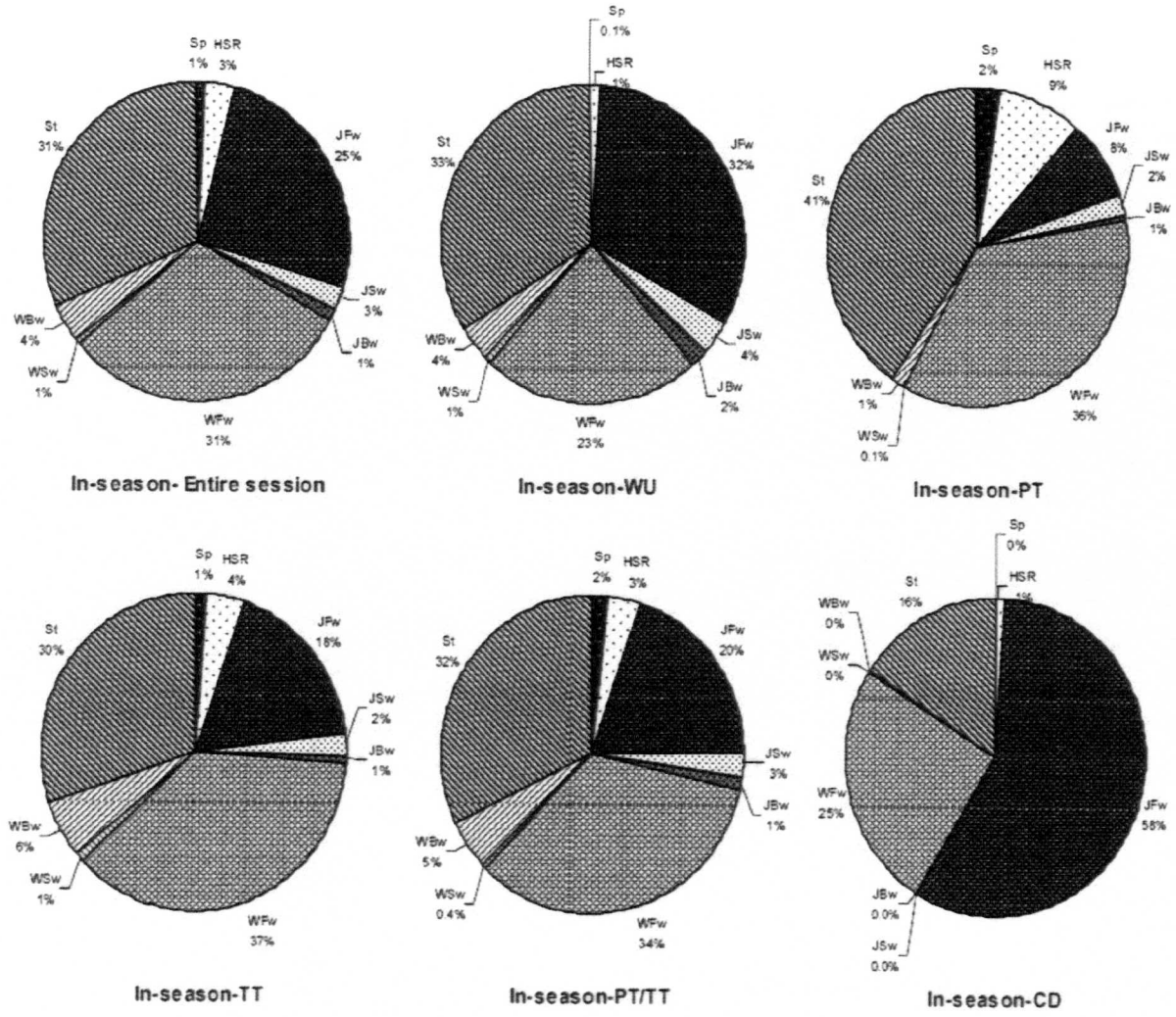
Activity profiles were examined in an attempt to identify possible explanations for the differences in the physiological loads between 'pre-season' and 'in-season' training. However, these data were not analysed using formal statistical procedures though a subjective inspection of the data indicate similar activity profiles during 'pre-season' and 'in-season' training, respectively (St: 31 ± 2 vs. 31 ± 6%; W Fw:31 ± 2 vs. 31 ± 6%; W Sw 1 ± 1 vs. 1 ± 0.3%; W Bw 5 ± 1 vs. 4 ± 2%; J Fw 21 ± 2 vs. 25 ± 3%; J Sw 4 ± 1 vs. 3 ± 1%; J Bw 2 ± 0.4 vs. 1 ± 0.4%; HSR 4 ± 1 vs. 3 ± 1%; Sp 1 ± 0.5 vs. 1 ± 1%). Activity profiles observed in the specific training sessions were also not different between training periods **(Figure 3.3 A and B)**.

The average counts of the technical skills within an entire session were 325 ± 144 and 212 ± 126 during the 'pre-season' and 'in-season' periods, respectively. Ball touch was the most commonly performed technical action in an entire training session completed during both the 'pre-season' and 'in-season' training sessions ('pre-season', 225 ± 107; 'in-season', 130 ± 96). This was numerically followed by jumping ('pre-season', 30 ± 42; 'in-season', 30 ± 27). In 'pre-season' training, the average counts of dribbling, heading, kicking and throwing were 12 ± 9, 18 ± 32, 9 ± 10 and 6 ± 17, respectively. This compared to 9 ± 6, 10 ± 18, 20 ± 17 and 1 ± 3, respectively during 'in-season'. In terms of the activities without a ball, getting up from ground and tackling were completed more in 'pre-season' training than 'in-season' training ('pre-season', 14 ± 24 and 11 ± 6; 'in-season', 6 ± 2 and 5 ± 6) **(Figure 3. 8).**



(A)

Figure 3.3 (A) Activity profiles of training sessions during the 'pre-season' training period. Sp: sprinting, HSP: high speed running, J: jogging, W: walking, St: static pauses, Fw: forward, Sw: sideway, Bw: backward.



(B)

Figure 3.3 (B) Activity profiles of training sessions during the 'in-season' training period. Sp: sprinting, HSP: high speed running, J: jogging, W: walking, St: static pauses, Fw: forward, Sw: sideway, Bw: backward.

Table 3.8 The profiles of game-related technical elements during 'pre-season' and 'in-season' training of Korean professional soccer players

		WU	PT	TT	PT/TT	CD	Total
Ball touch	Pre-season	104±91	170±162	66±35	54	2±8	225±107
	In-season	70±100	39	62±32	68±60	0	130±95
Dribbling	Pre-season	6±7	3±5	6±6	1	0	12±9
	In-season	3±4	16	4±1	2±2	0	9±6
Getting up	Pre-season	4±4	30±45	2±2	3	0±1	14±24
from ground	In-season	3±2	2	2±1	4±1	1±0	6±2
Header	Pre-season	5±6	39±64	4±5	0	0±1	18±32
	In-season	7±18	0	3±1	3±4	0	10±18
Jumping	Pre-season	25±4	69±69	6±5	8	0±1	30±42
	In-season	9±10	26	7±1	40±38	0	30±27
Kicking	Pre-season	5±8	1±1	8±3	3	0	9±10
	In-season	7±11	0	17±2	13±12	0	20±17
Tackling	Pre-season	5±3	10±7	9±3	3	0±1	11±6
	In-season	2±4	7	5±4	0	0	5±6
Throwing	Pre-season	4±13	0±1	2±5	0	0	6±17
	In-season	1±3	0	0	0	0	1±3

WU: Warm up, PT: Physical training, TT: Technical/tactical training, PT/TT: Physical and technical/tactical training, CD: Cool down.

3.4 Discussion

The aim of this study was to quantify the physiological loads and movement profiles of elite professional players during 'pre-season' and 'in-season' training. To our knowledge, this is the first attempt to investigate the demands of programmed soccer training in this way for a professional team at different times of the annual plan. The present data demonstrate that the overall physiological load of the weekly training schedule was significantly greater in 'pre-season' compared with 'in-season' training. This is evident from the significantly higher average heart rates and significantly greater proportion of time spent training in the highest heart rate training zones (*i.e.*, 80-100% of HR_{max}). Interestingly, these differences in training intensity were largely associated with the increased physiological demands induced by the TT specific training sessions and the CD during the 'pre-season' training phase. Such variations in training load are highly likely to be a direct function of the aims and objectives of the coaches during particular training periods

The training sessions observed in our investigation were completed by Korean elite professional players associated with one specific club in the best division in the national league. It is therefore acknowledged that the training carried out by this specific population of players may not be representative of other squads within the same league or across similar leagues in other countries. However, the overall training schedule, training session duration and physiological loading of our participants were similar to those observed in Danish, Italian and English professional soccer teams during both 'in-season' and 'pre-season' periods (Rhode & Espersen, 1988; Bangsbo, 1994d; Impellizzeri et al., 2005; Bangsbo et al., 2006; Svensson, 2007). Such agreements therefore suggest that the data presented here are suitable approximations of the training loads completed across a number of different populations in elite professional soccer. Nevertheless, further research comparing physiological loading of distinct periods of the annual plan for teams based in other countries and/or continents may be warranted. We also acknowledge that our investigation may be limited by the small number of players that were included in our data collection during both 'pre-season' and 'in-season' training periods. This limitation was a direct consequence of the availability of specific players (through injury etc) at the time of our data collection. We were also unable to collect data during competitive matches during the observation periods. This omission may well influence the overall physiological loading associated with these

discrete periods of activity. A more rigorous sampling procedure and the collection of game data in future research may be beneficial to prevent both inter-individual differences in training and under-estimations in the overall physiological load influencing the data set.

The 'pre-season' training period has traditionally been the time when the majority of the physical preparation work is completed by players to enable them to fulfil the physiological requirements of the competitive season (Bangsbo, 1994d). It has therefore been generally accepted that the physiological demands of this phase of training would be greater than at other times in the annual plan. There is, however, no available published scientific data that clearly illustrates the existence of these differences in an objective way. This study clearly demonstrates that the training programmes prescribed to soccer players in the 'pre-season' period are associated with a greater level of physiological stress than similar types of sessions completed 'in-season'. Significant differences were observed in both mean HR, time in the highest intensity HR zones and both weekly and daily RPE-based TL. This increase in physiological stress is probably a consequence of the need for players to complete the high-intensity exercise sessions required to achieve an optimal fitness level for the competitive season (Bangsbo, 1994d).

The 'pre-season' period has traditionally been the time when the majority of the physical preparation work is completed by players (Bangsbo, 1994d). As a consequence one may expect the increased physiological loading observed during 'pre-season' to be attributable to a higher exercise intensity and proportion of time spent in PT specific training sessions. Our data, however, suggest that the increased physiological loading during this period can largely be attributed to the higher intensities and training time spent in TT specific training sessions. Such training sessions were observed to typically consist of a variety of small-sided games (SSG) including practice matches. This form of training is becoming increasingly used as a tool for soccer-specific conditioning (Bangsbo, 1994d; Impellizzeri et al., 2006; Rampinini, Impellizzeri et al., 2007; Kelly & Drust, 2009). The data may reflect this trend as discussion with the coaches indicated that the inclusion of such training practices was a conscious decision. This would seem to indicate that these particular coaches were aware of the potential impact of these drills on their training practices and were prepared to use them to fulfil the objectives of their sessions.

Although we observed significant differences in physiological loading between 'pre-season' and 'in-season' periods we did not see any appreciable differences in activity profiles and technical elements between training periods. It is, of course, difficult to make firm conclusions from these data owing to the small sample size that were videoed for this part of the study and the lack of formal statistical analysis for this data. It is also possible that our inability to differentiate between the training periods is also partly a consequence of our approach to determining the activity profiles of players. The usual focus of such methodological approaches is to identify the amount that different classifications of activities contribute to an individual's entire activity profile. The physiological responses that are provoked from this activity are, however, more likely to be a consequence of the specific *pattern* or *combination* of these different activities within a session rather than the total amount observed during a training session. This is because the average physiological and metabolic responses to high-intensity intermittent exercise (e.g. oxygen uptake, blood lactate, blood glucose, free fatty acid, glycerol concentration etc) are highly dependent on the pattern of the exercise completed (Balsom et al., 1992). Further research which attempts to evaluate and co-ordinate a combination of data that includes specific patterns of movements, sports-specific actions (*i.e.*, ball contacts, tackling, jumping etc) and exact session details is therefore warranted to try and clarify this issue.

3.5 Conclusion

In summary, our data provide the first report to quantify and verify that the overall physiological loads associated with 'pre-season' soccer training are higher than those observed in 'in-season'. Such differences in training intensity between training periods are largely attributable to the higher intensities and time spent in TT specific training sessions during the 'pre-season' period. The apparent emphasis on TT sessions may have been a conscious decision by the coaches to achieve the necessary physiological conditioning through sport-specific approaches given the recent trends in professional soccer to support this training activity. Multiple daily sessions during 'pre-season' seem to induce greater global training loads than those induced by a daily single session during 'in-season'. Such differences in soccer training programmes could be expected to influence metabolic responses in the blood and/or molecular adaptations in skeletal muscles. The data on activity profiles of players from the current study will be therefore available to devise the

soccer-training specific intermittent exercise protocol in later study in order to investigate the effects of such differences in training organisation.

CHAPTER 4
THE DEVELOPMENT OF A LABORATORY-BASED SOCCER-SPECIFIC TRAINING SIMULATION

4.1 Introduction

Soccer is separated from other sports by its intermittent activity profile. This results in the physiological demands being more complex than continuous exercise (Drust, Reilly & Cable, 2000). The demands of soccer-specific intermittent exercise have been obtained by measuring physiological and metabolic responses during competitive and practice games (Rhode & Espersen, 1988; Bangsbo, 1994d; Impellizzeri et al., 2005). Such direct monitoring of actual physiological responses to match-play would seem to be the most effective way of determining the physiological cost of soccer-specific intermittent activity (Drust et al., 2007). In reality these procedures are limited by a number of theoretical and practical methodological issues such as accuracy and reliability. This may therefore limit the usefulness of the data in terms of its ability to accurately describe the physiological requirements of the sport. An alternative model to this approach is to attempt to recreate the demands of the sport using laboratory-based simulations. This approach provides the controlled conditions frequently required in experimental investigations and/or the depth and accuracy of understanding associated with laboratory-based analytical procedures (Drust et al., 2000). A small number of researchers have attempted to devise soccer-specific laboratory-based protocols to replicate the exercise patterns observed during match-play using both motorised (Drust et al., 2000; Gregson, 2002) and non-motorised treadmills (Clarke, Drust, MacLaren & Reilly, 2005). Such protocols can elicit broadly similar physiological responses to those observed in games thereby supporting their efficacy as suitable experimental models to apply to the sport.

The physiological demands associated with matches and training sessions are different (Rhode & Espersen, 1988; Impellizzeri et al., 2005). Soccer training incorporates different intensities and durations of activities than those that are observed in soccer matches (Rhode & Espersen, 1988). Our recent observations of training in Korean professional players (**Chapter 3**) has indicated both lower physiological strain and altered movement characteristics compared to previously published data on match-play (Bangsbo, 1994d; Rienzi et al., 2000). These variations may ultimately lead to differences in the external training loads placed on individual players (Impellizzeri et al., 2005) and subsequently differences in the internal training load. This information would suggest that soccer-specific treadmill simulations that are currently published in the literature may not be appropriate

to simulate the physiological loads associated with soccer training.

The aim of this study was to therefore to devise a laboratory-based soccer-specific training simulation using activity profiles observed in professional soccer training (Chapter 3). The development of such a protocol will permit future investigations into the physiological responses, and the molecular adaptations of skeletal muscle, to soccer-training specific training sessions later in this thesis.

4.2 Methods

4.2.1 Development of a laboratory-based soccer specific training simulation (LSSTS)

The development of the LSSTS attempted to recreate the physiological demands imposed on professional players during a soccer specific training session. The simulation for the study was performed on a motorized treadmill (h/p/cosmos Pulsar® 4.0, h/p/cosmos, Germany). This treadmill was controlled by a computer-based programme. The protocol used activity patterns that were observed during the physical training session of Korean professional players observed during the 'in-season' period **(Chapter 3)**. The proportion of time designated for each discrete activity-category was the same as those recorded in the actual training session **(Table 4.1)**. The movements which were employed in the simulation included walking, jogging, high speed running and sprinting. Static pauses were also included. During this period, subjects remained stationary on the treadmill. A small portion of the activities in the training session involved utility activities such as sideways (2.1%) and backward movements (2%). These types of movements were not incorporated within the protocol due to the technical limitations of the equipment used and the safety issues associated with including these activities. As a consequence of these omissions, the sum of the percentage time in these two movements in training was added to the walking and jogging categories included in the protocol. This allowed the calculation of the percentage time that each activity category would contribute to the final protocol. The speeds of each movement on the treadmill were based on previous observations obtained during match play (Mohr et al., 2003). The relevant speeds utilised for walking, jogging, high speed running and sprinting were 6km·h^{-1} 12km·h^{-1}, 19km·h^{-1} and 23km·h^{-1}, respectively.

The protocol consisted of three identical exercise periods. The time period between each block was designed to be utilised as a window for the recording of physiological variables during exercise. Fifteen min of exercise was initially assigned to each block in the simulation. This time was approximated in order to closely replicate the total duration of the actual training session of Korean professional players (*i.e.*, around 50 min). The actual exercise time within a block (*i.e.*, 15 min) was divided by the percentage time for each activity observed during the training session. The total time for each activity category from training was then divided by the number of discrete bouts of each activity included the simulation in order to give a time each discrete bout of activity for each pattern of movement. The duration of each discrete bout of activity in the protocol was therefore closely matched to those observed during the training session **(Table 4.1)**. This permitted the total number of bouts for each discrete activity in the simulation to be calculated. A block of exercise incorporated 93 discrete activities; these included 26 static pauses, 28 walks, 17 jogs, 16 high speed runs and 6 sprints. The LSSTS thus incorporated a total 279 activities within 3-repeated identical blocks. This was compared to a total of 472 discrete activities that were observed in the actual training sessions of professional players. Once the total numbers of bouts in each activity category were established, the order of the presentation of activities was determined. High-intensity activities were separated by low-intensity recovery periods to replicate the acyclical nature of the movement patterns observed in the training session.

The time required to complete all speed changes between the different activity categories was monitored following the development of the exercise protocol for the simulation. This enabled the total time for the speed transitions included in the treadmill protocol to be calculated. The total time for changing speeds between the different categories of activities was 336 s during each block. The final duration of a block was then determined by summing *the total exercise time* that would be performed at constant speeds of each activity and *the total transition time* that was required to change between speeds. As a result a block of exercise lasted 20 minutes 36 seconds **(Figure 4.1)**. This block was then repeated a total of 3 times, thereby resulting in the total protocol time closely resembling the total duration of the training session

Table 4.1 The activity profiles employed in a laboratory-based soccer-specific training simulation and the actual training session observed

	St	Wk	Jg	HSR	Sp
Training					
Total time (%)	41	37	11	9	2
Total number for each movement	104	147	135	63	23
Average duration of each movement (s)	14	12	6	5	3
Simulation					
Total time (%)	41	37	11	9	2
Total number for each movement	78	84	51	48	18
Average duration of each movement (s)	14	12	6	5	3

St; Static pauses, Wk; walking, Jg; Jogging, HSR; High speed running, Sp; Sprinting

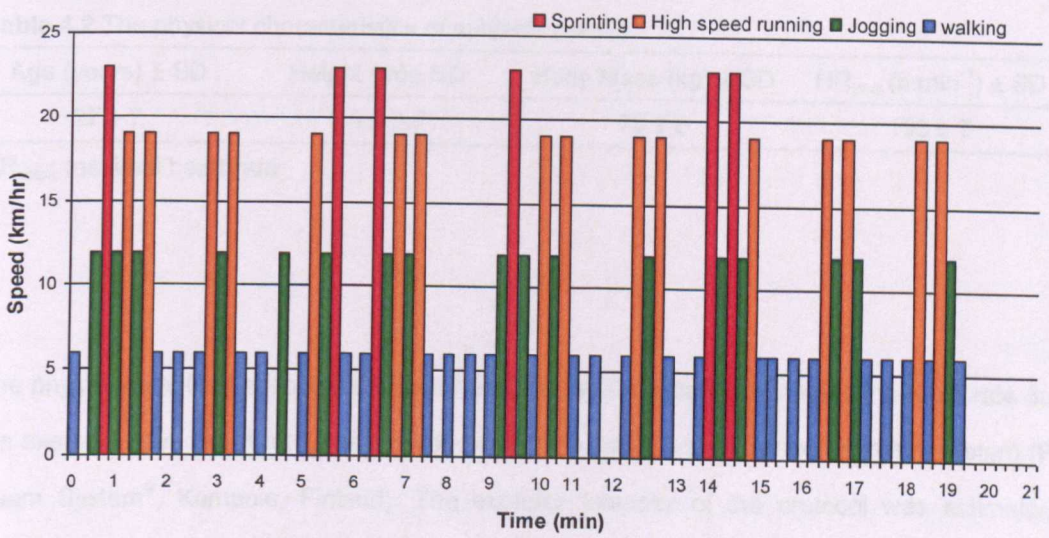


Figure 4.1 The graphical representation of the activity patterns undertaken during one bout (20 min 36 s) of a soccer-training-specific intermittent exercise protocol.

4.2.2 Evaluation of the validity of the simulation

In order to evaluate the appropriateness of the devised simulation as a recreation of actual training activity the physiological responses to the laboratory-based soccer-specific training simulation were compared to those of the professional players (n=10) observed in professional soccer training. Ten healthy active males participated in this evaluation. The mean ± SD physical characteristics of the subjects are represented in **Table 4.2**. All subjects were informed of the experimental procedures and associated risks, and gave their written informed consent to participate. When each participant arrived at the laboratory, a familiarization session including a verbal description of the protocol was conducted. A five minutes warm-up session was then completed on the treadmill before starting the simulation. The warm-up session included two bouts of a walking (30 s) and a bout of a jogging (4 min). The speeds for these activities were the same as those employed in the protocol.

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Table 4.2 The physical characteristics of subjects (n=10)

Age (years) ± SD	Height (m)± SD	Body Mass (kg) ± SD	HR_{max} ($b \cdot min^{-1}$) ± SD
27 ± 7	1.8 ± 0.7	75 ± 3	193 ± 7

HR_{max}; maximal heart rate

The physiological load associated with the protocol was evaluated by measuring heart rate during the exercise bout. Heart rate was recorded every 5 s using a short-range telemetry system (Polar Team System®, Kempele, Finland). The exercise intensity of the protocol was estimated by determining the mean HR and percentage of maximal HR (% of HR_{max}) during the exercise session. The maximal heart rate for each individual participant was estimated using the formula (HR_{max} = 220 –age) of Karvonen et al (Karvonen, Kentala & Mustala, 1957). The time spent within specific HR bands was also calculated. This provided information on the relative distribution of different levels of physiological loading within the protocol. Six different HR zones were used. These were 90~100% of HR_{max}, 80~90 % of HR_{max}, 70~80 % of HR_{max}, 60~70% of HR_{max},

50~60 % of HR_{max} and < 50 % of HR_{max}. Rating of perceived exertion (RPE) was also evaluated after the completion of a bout of the LSSTS using a modified 10-point rating Borg scale (Foster et al., 1995). Training load (TL) in arbitrary unit (AU) was calculated by multiplying RPE score with the duration (in minute) of the LSSTS.

4.2.3 Statistical Analysis

The validity of the laboratory-based soccer specific training simulation was evaluated by comparing the physiological responses of participants (mean HR, % of HR_{max} and TL) associated with the simulation to those of Korean professional players which were obtained in the actual training session. The time distribution in the specific HR zones during the simulation was also compared with that obtained in the training session. Independent T-tests (SPSS 15.0) were used for these analyses. The assumption of normality was confirmed by the Shapiro-Wilk test and visual inspection of Q-Q plots. $P < 0.05$ was considered significant.

4.3 Results

Mean ± SD heart rate during the protocol was 136±10 $b \cdot min^{-1}$. This value was approximately 71± 5 % of HR_{max}. Three ± 3% and 21 ± 15 % of the total time during exercise were spent in the HR zones between 90~100 and 80~90 % of HR_{max}, respectively. Over half of the time (around 59 %) was spent in HR zones between 60~80 % of HR_{max}. About 17% of the time was in the relatively low-intensity HR zones (below 60 % of HR_{max}). The mean RPE-based TL was 365 ± 63 AU (**Table 4.3**).

The mean values of HR, % of HR_{max} and RPE-based TL in the protocol were similar to those observed in the actual training session. Time spent in the HR zones between 80~70% and 60~70% of HR_{max} during the simulation were significantly different than those seen in training ($p < 0.05$) **(Table 4.3)**.

Table 4.3 The physiological responses during the laboratory-based soccer-specific training simulation and the actual training session observed.

	Mean HR ($b \cdot min^{-1}$) ± SD	Mean % of HR_{max} ± SD	% of time spent in the HR zone (% of HR_{max})						TL (AU) ± SD
			100~90	90~80	80~70	70~60	60~50	<50	
Training (n=10)	137±8	72±3	0.4±1	22±12	41±8*	19±3	15 ±7	2±2	365±63
Simulation (n=10)	136±10	71±5	3±3	21±15	33±10	26±7*	13±8	4±6	356±64

* p <0.05, significant difference between both groups. HR-heart rate, HR_{max}-maximal heart rate, TL-training load.

4.4 Discussion

This study aimed to devise a LSSTS using the activity profiles observed in actual training sessions of elite professional players. The LSSTS successfully replicated both the activity profile and the duration of each discrete activity observed in the actual training session. The simulation also stimulated similar heart rate responses and training loads to those obtained in professional soccer training. Such data suggests that the simulations may present a valid model to be used in future laboratory-based investigations on the organisation of training.

No previous attempt has been made to develop a laboratory-based simulation that represents soccer-specific training sessions completed by elite professional players. The development of LSSTS was based upon activity profiles and physiological responses of Korean professional soccer players in physical training session during 'in-season'. All major activity patterns (static pauses, walking, jogging, high speed running and sprinting) observed in the training session were included. The work-rates and the relevant duration of each discrete bout of activity employed in this protocol were designed to similarly re-create those observed during the training session

(Table 4.1). The actual exercise time in the protocol, excluding transition time for attaining relevant treadmill speeds, was also close to the time period associated with PT sessions for the professional training session (simulation, around 45 min vs. training, 50 min).

The LSSTS was composed of 3-repeated identical blocks on a treadmill. This activity included 279 bouts of discrete activities (78 static pauses, 84 walks, 51 jogs, 48 high speed runnings and 18 sprints). The activity profile used in the current simulation incorporated a larger number of activity changes than other simulations performed on a motorised treadmill (Drust et al., 2000; Gregson, 2002). This improvement is a direct consequence of the modern treadmills significantly faster acceleration and deceleration periods (Drust et al., 2007). Such frequencies are, however, still less that those observed in the actual training session of professional players (104 static pauses, 147 walks, 135 jogs, 63 high speed runnings and 23 sprints). These differences in the total number of discrete activity bouts remain a consequence of the limitations of the treadmill used; and the time required to change speeds between different movements. Both utility movements (i.e., sideways and backwards) and soccer-specific technical elements (e.g. ball touch, jumping, heading, tackling, kick, shot, dribbling, passing, getting up from ground etc.) were also excluded due to the technical limitations of the treadmill and safety issues associated with the performance of such actions. Omission of these activity categories, along with the lower frequency of the total number of activities may cause a reduction of the energy cost of the simulation compared to that associated with similar exercise in actual field conditions (Bangsbo, 1994d; Drust et al., 2007). Such reductions in energy cost may, however, be compensated by the extra transition time associated with changing speeds on a treadmill. This may lead to the overall physiological strain closely matching that associated with actual training. The current simulation may, therefore, be accepted as a good representation of the activity profiles and the physiological loads observed in actual training sessions of professional players.

The physiological responses to the LSSTS were also similar to those recorded in the actual training sessions of the elite professional players **(Table 4.3)**. No significant differences between the mean values for HR and RPE-based TL were observed between the two conditions. The time spent in high intensity HR zones (80~100% of HR_{max}) was also equivalent between both groups (training group, 22.4% vs. simulation group, 23%). Such similarities in the physiological responses

between the actual training session and the simulation would seem to be indicative of the suitability of the protocol to act as a recreation of a real world training session. The physiological indicators used in this investigation were limited in their ability to determine the "true" physiological cost of the exercise. The independent groups' experimental design that was employed is also a limitation as the two populations included in the investigation are likely to vary in a number of parameters (e.g. fitness, training history) that may influence the physiological responses to the exercise. Such issues are very difficult to address in studies of this nature. As a consequence the available data may well represent the most realistic and therefore effective evaluation of the simulation that could be performed. Despite of this limitation, such approach could be the most realistic way of evaluating validity of the simulation as the LSSTS will be mostly utilised for laboratory-based investigations to evaluate physiological responses of non-elite participants. These findings, therefore, suggested that the LSSTS was valid enough to re-create the relevant physiological strains during the actual training session of professional players.

4.5 Conclusion

The laboratory-based soccer-specific training simulation devised in this study represents a novel protocol that would seem to replicate the activity profiles of a professional soccer training session during the in-season training period. The protocol leads to similar physiological responses to those observed in the physical training of professional players. This protocol would, therefore, seem to be suitable to use in investigations into the physiological responses and the molecular adaptations of skeletal muscle to soccer-specific intermittent exercise.

CHAPTER 5

ACUTE MOLECULAR RESPONSES ASSOCIATED WITH MITOCHONDRIAL BIOGENESIS TO A SINGLE BOUT OF SOCCER-SPECIFIC INTERMITTENT EXERCISE

5.1 Introduction

The aerobic fitness of soccer players is a fundamental component of an individual's ability to repeatedly perform high-intensity activities during match-play (Stone & Kilding, 2009). One major aim of training in soccer should, therefore, be to enhance the aerobic fitness of players. Increases in aerobic performance following soccer-specific training are associated with both cardiovascular adaptations such as increases in cardiac output (Knoepfli-Lenzin et al., 2010) and muscle changes (Bangsbo, 1994d). Adaptations in skeletal muscle following exercise include morphological and metabolic changes that increase the rate of energy production from both aerobic and oxygen-independent pathways (Hawley et al., 2006). Such alterations include long-term changes in mitochondrial enzyme activities in skeletal muscle, such as *3-hydroxyacyl co-enzyme A dehydrogenase (HAD)* and *citrate synthase (CS)* (Bangsbo & Miznuno, 1988; Bangsbo, Nielsen et al., 2009; Krustrup et al., 2009).

Mitochondrial synthesis, which is frequently termed *mitochondrial biogenesis,* is a fundamental factor in facilitating mitochondrial function and substrate oxidation in skeletal muscle. These changes are important in underpinning the improved exercise capacity and greater resistance to fatigue observed in trained individuals (Adhihetty et al., 2003). The regulation of mitochondrial biogenesis appears to be controlled by complex molecular process that require numerous transcriptional factors and transcriptional co-activators (Coffey & Hawley, 2007). Peroxisome proliferator-activated receptor γ coactivator-1α (*PGC-1α*), a versatile transcription coactivator, plays a key role in regulating such mitochondrial adaptations to prolonged steady state exercise(Pilegaard & Richter, 2008; Yan, 2009). Protein kinases such as *AMPK and p38MAPK* are also suggested to increase the promoter activity of *PGC-1α* and to up-regulate its transcriptional activity (Wu et al., 2002; Hardie, 2004; Wright, Geiger et al., 2007; De Filippis et al., 2008; Yan, 2009; Egan et al., 2010). Changes in these transcription factors that are important for mitochondrial biogenesis are elicited by alterations in various mechanical and metabolic characteristics associated with single exercise bouts such as fluctuations in cytoplasmic calcium (Ca^{2+}) levels, energy reserves, oxygen consumption and reactive oxygen species (ROS) in skeletal muscle (Ljubicic et al., 2010).

It has been suggested that the molecular adaptations to training are highly specific to the type of training stimulus that is provided with factors such as volume, intensity, duration and mode of exercise important (Booth & Thomason, 1991; Coffey & Hawley, 2007). These parameters are probably important as a consequence of the importance of disruptions to cellular metabolism in driving transcription (Norrbom et al., 2004). Recent years has seen a move towards sports-specific conditioning rather than traditional athletic type training especially in sports such as soccer where the competitive demands restrict training time (Impellizzeri et al., 2006). This sports-specific conditioning frequently takes the form of structured technical and tactical practices that enable athletes to "train as you play" (Bishop, 2009). The physiological responses to these types of activities have been characterised to some extent over recent years (Jeong, Reilly, Morton, Bae & Drust, 2011) though it is currently unclear what impact such soccer-specific training has on the complex signaling networks that may underpin the molecular changes in skeletal muscle associated with exposure to this type of exercise. It is likely that the relatively unique mechanical and metabolic responses to this type of exercise could initiate a different series of molecular events during and following exercise to those associated with other exercise patterns frequently investigated in human skeletal muscle.

The aim of the present study was therefore to investigate the acute signaling responses associated with mitochondrial biogenesis in skeletal muscle of healthy men to a single bout of soccer-specific intermittent exercise. We hypothesised that performing soccer-specific intermittent exercise would induce increases in the phosphorylation of important protein kinases, thereby leading to activation of *PGC-1α* expression.

5.2 Method

5.2.1 Subjects

Nine recreationally active males participated in the study (mean ± SD: age, 25 ± 4 yr; body mass, 75 ± 7 kg; height, 1.75 ± 0.04 m). The experimental procedures and potential risks related to the study were explained verbally to all subjects. Written participant information was also given during a familiarization session. Informed consent was obtained from all subjects prior to participation.

Subjects refrained from strenuous exercise at least 48 h before the exercise trial. None of the subjects had any current medical problems that were neurological and/or musculoskeletal or were under pharmacological treatment during the course of the study. The study was approved by the Ethics Committee of Liverpool John Moores University.

5.2.2 Assessment of physiological fitness

All participants completed a $\dot{V}O_{2max}$ test using an incremental exercise test performed on a motorised treadmill (HP cosmos Pulsar® 4.0, h/p/cosmos, Germany). Oxygen uptake was measured continuously during exercise using an on-line gas analysis system (Cortex Metamax, Leipzig, Germany). The test began with a 3-min warm up stage at a treadmill speed of 10 $km \cdot h^{-1}$ followed by 3 min stages at 12 $km \cdot h^{-1}$, 14 $km \cdot h^{-1}$ and 16 $km \cdot h^{-1}$. Upon completion of the 16 $km \cdot h^{-1}$ stage, the treadmill was inclined by 2 % every 3 min until volitional exhaustion. The $\dot{V}O_{2max}$ was taken as the highest $\dot{V}O_2$ value obtained in any 10-sec period and was stated as being achieved by the following end-point criteria: 1) heart rate within 10 $b \cdot min^{-1}$ of age-predicted maximum, 2) respiratory exchange ratio > 1.1, and 3) plateau of oxygen consumption despite increased workload. The mean ± SD of $\dot{V}O_{2max}$ of the sample of subjects was 59 ± 6 $ml \cdot kg^{-1} \cdot min^{-1}$.

5.2.3 Experimental Design

An overview of the experimental design is shown in **Figure 5.1**. At least 3 days after the initial assessment of maximal oxygen consumption ($\dot{V}O_{2max}$), subjects performed a single bout of the laboratory-based soccer-specific training simulation (LSSTS) which consisted of 3 identical blocks of exercise performed on the treadmill. Heart rate (HR) was measured continuously during the exercise protocols (Polar S610i, Kempele, Finland). Ratings of perceived exertion (RPE) were measured every 20-min throughout exercise and 30-min after completion of the LSSTS. Blood samples were obtained pre-exercise, every 20-min during exercise, post-exercise and 3 hr after exercise. Muscle biopsies were taken from the vastus lateralis pre-, post- and 3-h after LSSTS. During the recovery period between the post-exercise biopsy and 3-h biopsy, subjects remained seated in the laboratory.

Figure 5.1 The schematic design of the study. LSSTS; laboratory-based soccer-specific training simulation, Pre; pre-exercise, Post; post-exercise, 3-h; 3-h after a bout of exercise

5.2.4 Dietary control

To calculate macronutrient intake, subjects were instructed to complete a 2-day food diary preceding the LSSTS. Subsequent dietary analysis was performed by the computer software programme Microdiet. A breakdown of the subjects' macronutrient intake can be seen in **Table 5.1.** Subjects refrained from intaking alcohol and caffeine for at least 48 h prior to the testing session. An overnight fast was also required prior to attending the laboratory in the morning. In the 30 min preceding exercise, subjects consumed a volume of water (5 $ml \cdot kg^{-1}$). During exercise subjects were not allowed to drink. Only plain water was provided during the recovery period between the post-exercise and 3 h biopsies. This was calculated to compensate the loss of body fluid during exercise (1.5L x loss of body mass (kg)).

Table 5.1 Macronutrient intake of subjects

CHO (%)	Fats (%)	Protein (%)	MJ
47 ± 9	34 ± 8	19 ± 5	7.1 ± 4.0

5.2.5 Exercise protocols

The laboratory-based soccer-specific training simulation for the study was devised for a motorized treadmill (HP cosmos Pulsar® 4.0, h/p/cosmos, Germany). The treadmill was controlled by a computer-based programme. The movements included in the simulation consisted of walking, jogging, high speed running and sprinting. Static pauses were also included. During this period, subjects remained stationary on the treadmill. The speeds of each movement on the treadmill were based on previous observations obtained during match play (Mohr et al., 2003). The relevant speeds utilised for walking, jogging, cruising and sprinting were 6 $km \cdot h^{-1}$, 12 $km \cdot h^{-1}$, 19 $km \cdot h^{-1}$ and 23 $km \cdot h^{-1}$ respectively. The total time for a bout of LSSTS was around 60 min. A single block of exercise that lasted 20 min 36 s incorporated 93 discrete activities. These included 26 static pauses, 28 walks, 17 jogs, 16 high speed runnings and 6 sprints. High-intensity activities were separated by low-intensity recovery periods to replicate the acyclic nature of the movement patterns observed in the training session. Details about this protocol are presented in ***Chapter 3 and 4***. The intensity of the protocol was evaluated by measuring HR, oxygen consumption, and session-RPE.

5.2.6 Muscle biopsies

Muscle biopsies were obtained from separate incision sites (2-3 cm apart) from the lateral portion of the vastus lateralis muscle pre-, post- and 3-h after the exercise protocol using a Bard Monopty Disposable Core Biopsy Instrument 12 gauge x 10 cm length (Bard Biopsy Systems, Tempe, AZ, USA). Samples were obtained (30-40 mg) under local anaesthesia (0.5 % Marcaine, Astrazeneca, USA). They were immediately frozen in liquid nitrogen and stored at –80 °C for later analysis. The procedures of muscle biopsies were performed by a qualified medical doctor and were presented in **Figure 5.2**. Samples were analysed for protein contents of *AMPK*, *p38MAPK* and *GAPDH* and *mRNA* contents of *PGC1α*.

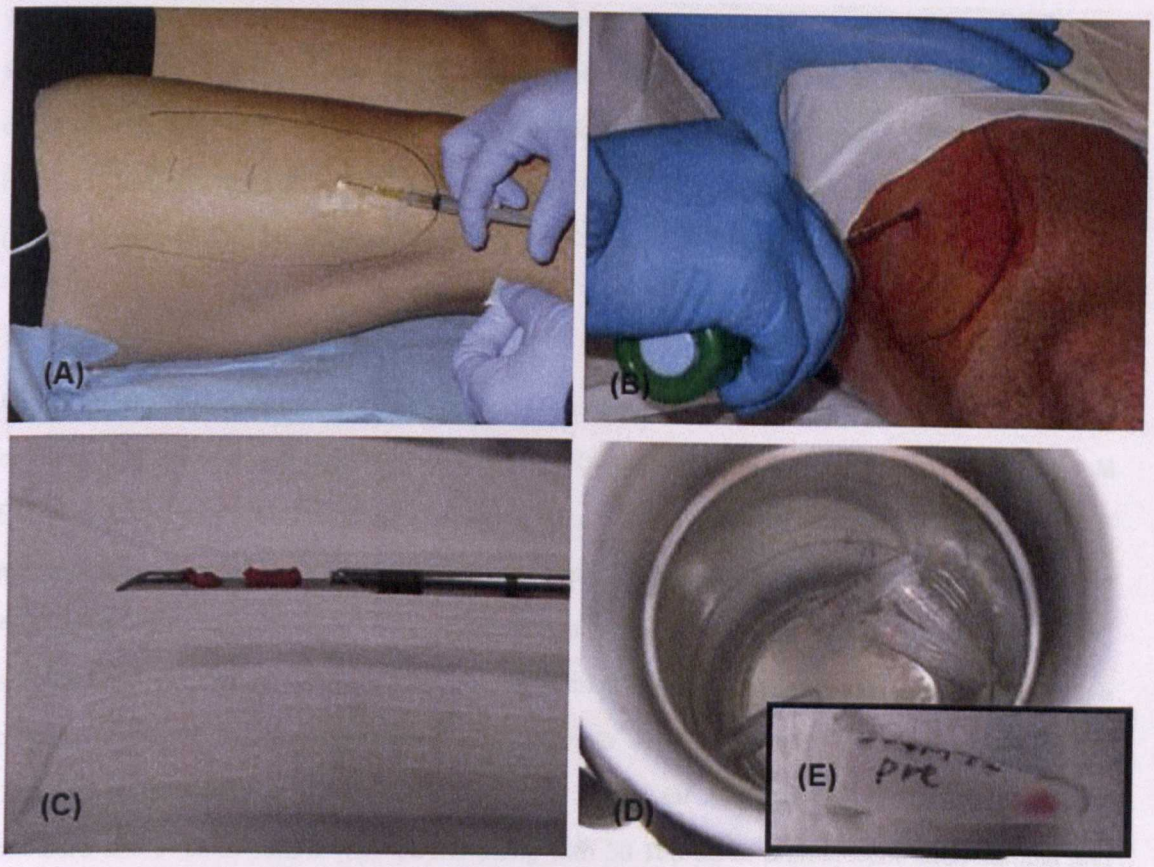


Figure 5.2 Schematic representation of the procedure of muscle biopsy. (A) The infiltration of local anaesthetics after demarcating the Vastus Lateralis, (B) insertion of a biopsy needle, (C) muscle tissues taken by the needle, (D) the temporary storage of the muscle in liquid nitrogen and (E) a container for the muscle.

5.2.7 Muscle analysis

5.2.7.1 Muscle Glycogen

Approximately 2-3 mg of freeze dried sample was powdered and subsequently hydrolyzed by incubation in 500 µl of 1 M HCl of 3-4 hr at 100°C. After cooling to room temperature, samples were neutralized by the addition of 250 µl 0.12 mol·L^{-1} Tris-2.1 mol·L^{-1} KOH saturated with KCl. Following centrifugation, 150 µl of supernatant was analysed in duplicate for glucose concentration according to the hexokinase method using a commercially available kit (GLU-HK, Randox Laboratories, Antrim, UK). Glycogen concentration is expressed as mmol·kg^{-1} dry weight (dw), and intra-assay coefficients of variation was < 5%.

5.2.7.2 Western blotting

Approximately 20-30mg piece of frozen muscle was ground to powder and homogenised in 120 µl of ice cold lysis buffer (25 mM Tris/HCl [pH 7.4], 50 mM NaF, 100 mM NaCl, 5 mM EGTA, 1 mM EDTA, 10 mM Na-Pyrophoshatase, 1 mM Na_3VO_4, 0.27 M sucrose, 1 % Triton X-100, 0.1 % 2-mercaptoethanol) and supplemented with a protease inhibitor tablet (Complete mini, Roche Applied Science, West Sussex, UK). Homogenates were centrifuged at 14,000 g for 10 min at 4°C and the supernatant was collected. The protein content of the supernatant was determined using a bicinchoninic acid assay (Sigma, UK). Each sample was diluted with an equal volume of 2X Laemmli buffer (National Diagnostics, USA) and boiled for 5-min at 100 °C. For each blot, a standard and internal control was loaded along with 50-100 µg of protein from each sample and then separated in Tris-glycine running buffer (10 X Tris/Glycine, Geneflow Ltd, Staffordshire, UK) using self-cast 4% stacking and 10 % separating gels (National Diagnostics, USA). Gels were transferred semi-dry onto nitrocellulose membrane (Geneflow Ltd, Staffordshire, UK) for 2-h at 200 V and 45 mA per gel in transfer buffers (anode 1; 0.3 M Tris, 20 % methanol, pH 10.4; anode 2; 0.25 M Tris, 20 % methanol, pH 10.4; cathode; 0.4 M 6-amino hexanoic acid, 20 % methanol, pH 7.6). After transfer, membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBST: 0.19 M Tris pH 7.6, 1.3 M NaCl, 0.1 %Tween-20) with 5% non-fat milk. The membranes were then washed for 3 x 5 min in TBST before being incubated overnight at 4°C with phospho-specific anti-bodies for $AMPK^{Thr172}$ and $p38MAPK^{Thr180/Tyr182}$ (all from Cell Signaling, UK) as well as total proteins of GAPDH (all from Cell Signaling, UK) all at concentrations of 1:1000 in 1 X TBST. The next morning, membranes were washed for a further 3 x 5 min in TBST and

subsequently incubated with anti-species horseradish peroxidise-conjugated secondary antibody (Bio-Rad or Dako, UK) for 1-h at room temperature. After a further 3 x 5 min washes in TBST, membranes were exposed in a chemiluminescence liquid (SuperSignal, Thermo Fisher Scientific, Rockford, IL, USA) for 5-min. Membranes were visualised using a Bio-Rad Chemi-doc system, and band densities were determined using Image Lab image-analysis software. All raw densitometry data were used for statistical analysis purposes so as to compare within-subject responses. However, because it is technically incorrect to compare densitometry data between gels (and hence, between subjects), for graphical purposes each subject's pre-exercise values was normalised to 1 (hence no error bars are shown for this time point) such that values at post-exercise and 3-h post-exercise are subsequently expressed as fold change relative to pre-exercise values. This approach has been used previously by Morton and colleagues (Morton et al., 2009) and other researchers (Perry et al., 2010). GAPDH was used for normalisation to phosphorylated proteins where appropriate.

5.2.7.3 Real time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RNA isolation and cDNA synthesis Total RNA was isolated from small muscle biopsies (20-30 mg) using Trizol reagent (Invitrogen), according to the manufacturer's protocol. RNA quality and quantity were determined using Implen Nanophotometer (Implen, Munchen, Germany) and the RNA was stored at -80°C. cDNA was synthesised using random hexamers (Applied Biosystems) and Superscript III enzyme (Invitrogen), using manufacturer's protocol.

Gene expression analysis by RT-qPCR Gene specific expression data was obtained using probes selected from Human Universal Probe Library (Roche Diagnostics) with compatible oligonucleotide primers (MWG Eurofins). One µl of each sample were analysed in duplicates with negative controls using AB 7500 Real-Time Quantitative PCR instrument (Applied Biosystems) and Agilent Brilliant II qPCR Master Mix with Low ROX (Agilent Technologies). One microliter of cDNA, 500 nM of primer and 200 nM of probe were used for each 20µl reaction **(Table 5.2)**. The following cycling parameters were used: 50°C for 2 minutes, initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/elongation at 60°C for 1 minute. Data was collected and analysed using AB SDS 1.43 Software (Applied Biosystems, Foster City, USA). Changes in mRNA content were calculated according to the 2-ΔΔCt method

where GAPDH was used as the housekeeping gene (Heid, Stevens, Livak & Williams, 1996).

Table 5.2 Primer and probe sequences used for real-time PCR

Gene	Forward primer	Reverse primer	Probe
GAPDH	GCTCTCTGCTCCTCCTGTTC	ACGACCAAATCCGTTGACTC	60
PGC-1α	TGAGAGGGCCAAGCAAAG	ATAAATCACACGGCGCTCTT	13

5.2.8 Venous blood samples and biochemical analysis

Blood samples were obtained pre- and post exercise and drawn from a superficial vein in the antecubital crease of the forearm using standard venepuncture techniques (Vacutainers Systems, Becton, Dickinson). Samples were collected into vacutainers containing EDTA and lithium heparin and were stored on ice until centrifugation at 2,000 rpm for 15 min at 4°C. Following centrifugation, aliquots of plasma were stored at -80°C for later analysis. Samples were analyzed for plasma glucose, lactate, glycerol and NEFA concentration using commercially available kits (Randox Laboratories, Antrim, UK). All post-exercise blood samples were corrected for plasma volume changes according to Dill and Costill (1974).

5.2.9 Statistical analysis

The responses of blood metabolites, muscle glycogen, protein and mRNA contents to the laboratory-based soccer-specific training simulation were evaluated using a one-way analysis of variance (ANOVA) for repeated measures. The Least Significant Difference (LSD) post-hoc test for multiple comparisons was performed in order to examine the effect of time (*i.e.*, pre-, 20-min, 40-min, post-exercise and 3-h after exercise). Results are presented as means ± standard deviation (SD). *P*-values <0.05 were considered significant.

5.3 Results

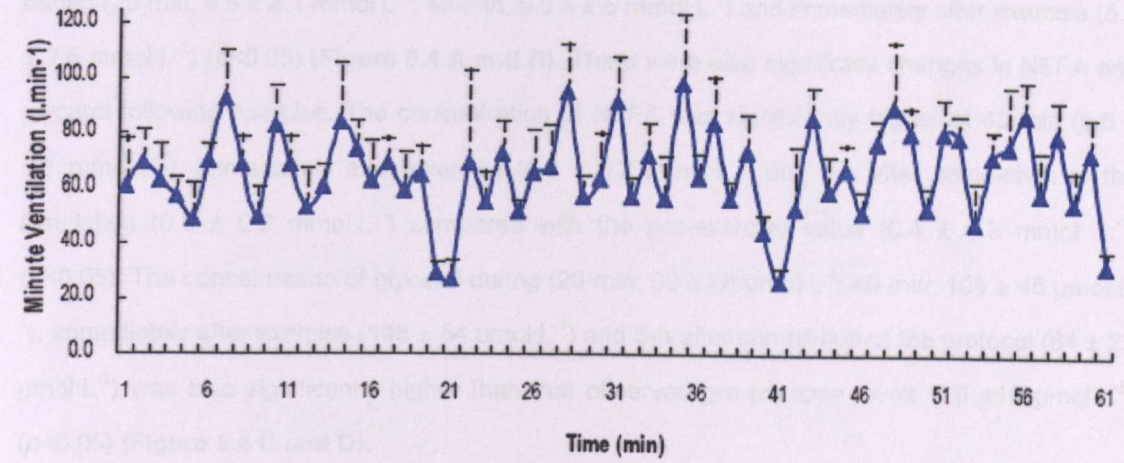
5.3.1 Physiological responses

The physiological responses to the laboratory-based soccer-specific training simulation are presented in **Table 5.3**. The LSSTS elicited intermittent fluctuations in ventilation (minute ventilation, 65 ± 17 $L \cdot min^{-1}$) and oxygen consumption (2.4 ± 0.6 $L \cdot min^{-1}$) during exercise **(Figure 5.3).**

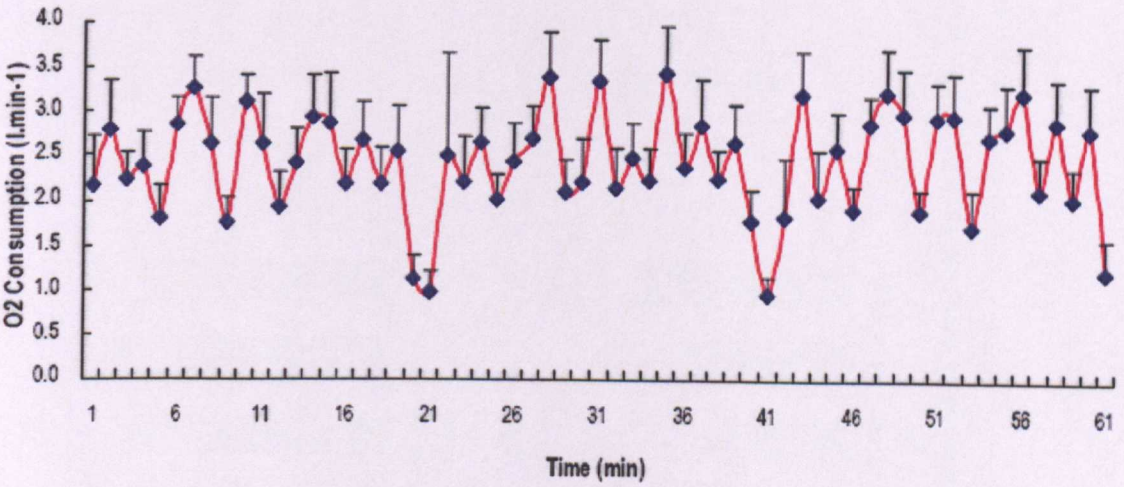
Table 5.3 Physiological responses during the LSSTS

	Means ± SD
HR ($b \cdot min^{-1}$)	150 ± 12
Minute ventilation ($L \cdot min^{-1}$)	65 ± 17
Oxygen consumption ($L \cdot min^{-1}$)	2.4 ± 0.6
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	32 ± 3
% $\dot{V}O_{2max}$ (%)	55 ± 6
RER (AU)	1.0 ± 0.1
Session-RPE (AU)	6 ± 1

HR-heart rate, $\dot{V}O_{2max}$ –maximal oxygen consumption, RPE-ratings of perceived exertion, RER-respiratory exchange ratio



(A)



(B)

Figure 5.3 Minute ventilation (A) and oxygen consumption (B) during the LSSTS.

5.3.2 Blood metabolites

Blood glucose concentration did not change either during or following the LSSTS. Compared with pre-exercise (1.6 ± 0.6 mmol·L^{-1}), the blood lactate concentration was significantly increased during (20-min, 4.6 ± 2.1 mmol·L^{-1}; 40-min, 5.0 ± 2.5 mmol·L^{-1}) and immediately after exercise (5.3 ± 2.6 mmol·L^{-1}) (p<0.05) **(Figure 5.4 A and B)**. There were also significant changes in NEFA and glycerol following exercise. The concentration of NEFA was significantly higher at 40-min (0.5 ± 0.1 mmol·L^{-1}), immediately after exercise (0.6 ± 0.2 mmol·L^{-1}) and 3-h after completion of the simulation (0.8 ± 0.2 mmol·L^{-1}) compared with the pre-exercise value (0.4 ± 0.3 mmol· L^{-1}) (p<0.05). The concentration of glycerol during (20-min, 69 ± 28 µmol·L^{-1}; 40-min, 105 ± 46 µmol·L^{-1}), immediately after exercise (145 ± 54 µmol·L^{-1}) and 3-h after completion of the protocol (64 ± 22 µmol·L^{-1}) was also significantly higher than that observed pre-exercise levels (29 ±19 µmol·L^{-1}) (p<0.05) **(Figure 5.4 C and D).**

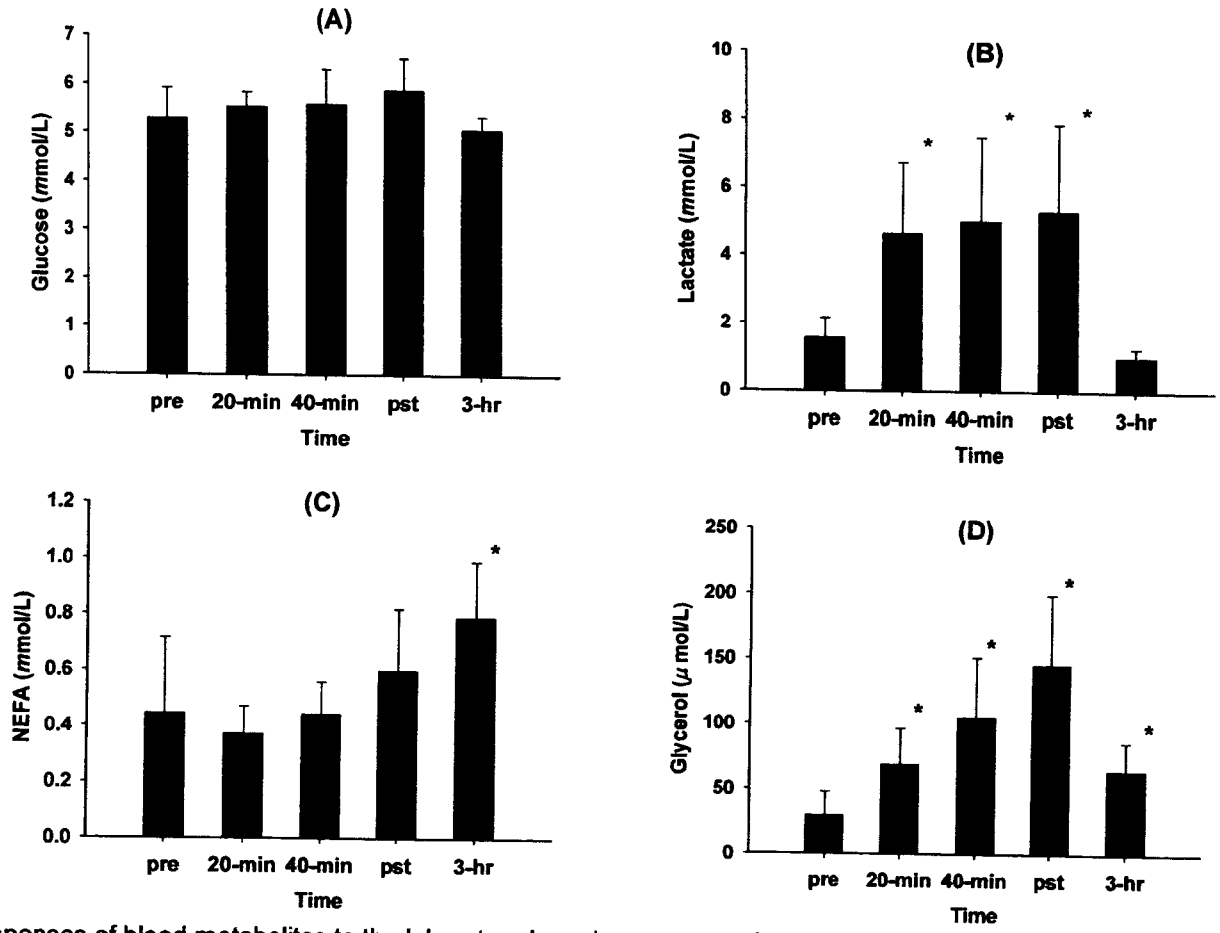


Figure 5.4 The responses of blood metabolites to the laboratory-based soccer-specific intermittent protocol; (A) glucose, (B) lactate, (C) NEFA and (D) glycerol. Values mean ± standard deviation. * $p<0.05$, significant difference compared to pre-exercise.

5.3.3 Muscle Glycogen

There was a significant reduction in muscle glycogen levels after the laboratory-based soccer specific training when compared to the pre-exercise muscle glycogen concentrations (pre, 397 ± 86 mmol·kg^{-1} dw Vs post, 344 ± 64 mmol·kg^{-1} dw; $p < 0.05$) **(Figure 5.5)**.

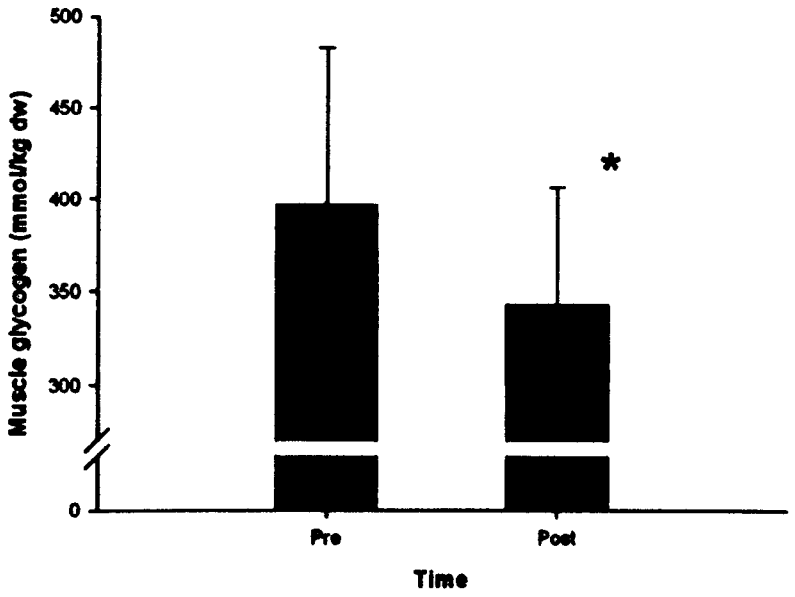


Figure 5.5 Muscle glycogen changes pre- and post-exercise. There were significant decreases in muscle glycogen levels following a single bout of the laboratory-based soccer specific training simulation. Values mean ± standard deviation. * $p<0.05$, significant difference compared to pre-exercise.

5.3.4 Phosphorylation of protein kinases associated with mitochondrial biogenesis

AMPK and p38MAPK phosphorylation There was no significant change in the phosphorylation of *AMPK and p38MAPK* at the end of exercise or 3-h after exercise compared to rest (p>0.05) **(Figure 5.6)**.

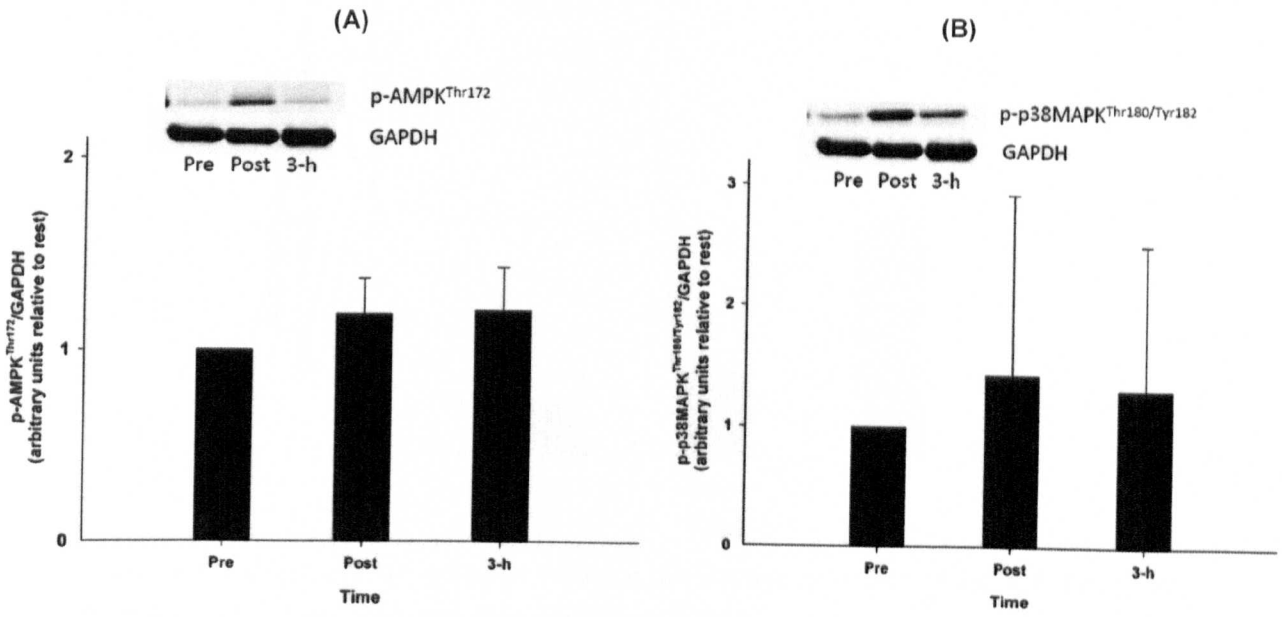


Figure 5.6 Phosphorylation of *AMPK* (A) and *p38MAPK* (B) following the laboratory-based soccer-specific training simulation (n=6). *AMPK and p38MAPK* phosphorylation was normalised to GAPDH protein contents and expressed relative to the rest value. Values are mean ± standard deviation.

5.3.5 Expression of *PGC-1α mRNA*

PGC-1α mRNA content was significantly elevated by around 5-fold and 3-fold 3-hr after exercise compared with pre- and post-exercise levels, respectively ($p<0.05$) **(Figure 5.7)**.

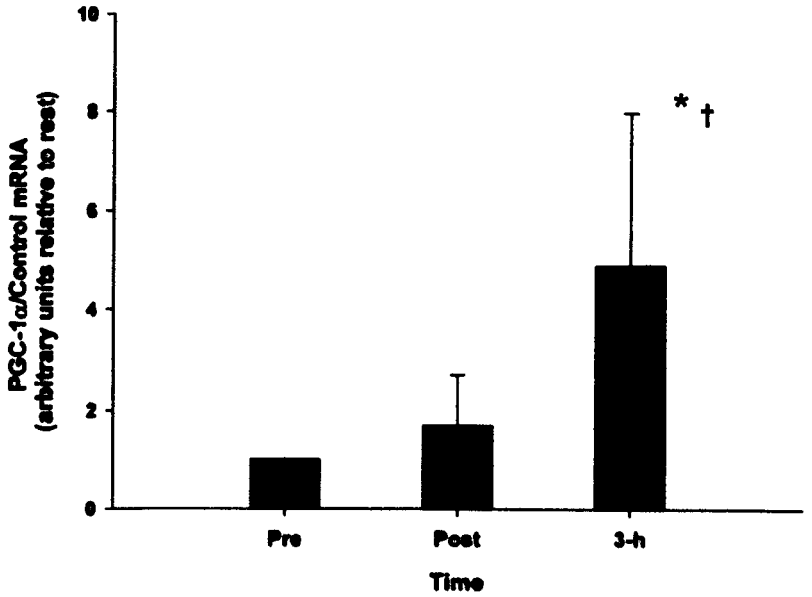


Figure 5.7 *PGC-1α mRNA* contents following the laboratory-based soccer-specific training simulation (n=6). There were significant increases in *PGC-1α mRNA* contents 3-h after a single bout of exercise relative to levels of pre- (*) and post-exercise (†), respectively ($p<0.05$). Values are mean ± standard deviation.

5.4 Discussion

The primary aim of the present study was to test the hypothesis that soccer-specific intermittent exercise would induce increases in the phosphorylation of key signaling kinases and activation of *PGC-1α* mRNA expression. The main findings of the present study were that soccer-specific exercise enhanced the expression of *PGC-1α mRNA* in human skeletal muscle after exercise. This is the first study to examine *PGC-1α mRNA* responses to intermittent exercise patterns that are soccer-specific. There was, however, no significant change in phosphorylation of *AMPK* and *p38 MAPK*. These results suggest that soccer-specific training can lead to adaptations associated with mitochondrial biogenesis in skeletal muscle although the signaling pathways associated with *AMPK* and *p38 MAPK* do not seem play a major role in this adaptation.

In chapter 4, the physiological responses to the laboratory-based soccer-specific training simulation were evaluated and were found to be similar to those recorded in the actual training sessions of the elite professional players. No significant differences between the mean values for HR and RPE-based training load were observed between the two conditions. The time spent in high intensity HR zones (80~100% of HR_{max}) was also equivalent between both groups. Such similarities in the physiological responses between the actual training session and the simulation would seem to be indicative of the suitability of the protocol to act as a recreation of the locomotor pattern of a real world training session in the laboratory. The present data examining blood and muscle metabolites demonstrated the activation of the glycolytic energy system in the early stages of exercise. These findings suggest that muscle glycogenolysis provide a large portion of the energy required during soccer-specific training. Increases in fat utilisation, as illustrated by increases in free fatty acids and glycerol seemed to follow the increases in muscle glycogenolysis. This may indicate that fat utilisation also plays an important role in supporting the energy requirements of soccer training. Such responses are similar to the patterns observed in match-play (Bangsbo et al., 1991; Bangsbo, 1994a; Krustrup et al., 2006), model-matches (Bangsbo, 1994d) and match-specific simulations (Clarke et al., 2005). These comparisons would as expected suggest that soccer training stimulates the specific metabolic systems that are used to support energy provision in match-play.

The current data provides novel information for the literature in that it demonstrates that soccer-specific intermittent exercise can enhance the expression of *PGC-1α* mRNA 3-h after exercise. These increases in *PGC-1α mRNA* content seem to be similar to the adaptive responses observed in cycling protocols (Gibala et al., 2009; Cochran et al., 2010; Little et al., 2010; Little, Safdar, Bishop, Tarnopolsky & Gibala, 2011) and running exercise (Harber et al., 2009) in terms of time-course and magnitude. Such an over-expression of *PGC-1α* mRNA would be sufficient to enhance the oxidative capacity of skeletal muscle and thus increase the capacity for both fat and carbohydrate utilisation during exercise (Pilegaard & Richter, 2008), thereby leading to improvements in performance (Calvo et al., 2008). These findings suggest that the global effect observed in soccer-specific training such as small-a-sided games (Reilly & White, 2005; Sassi et al., 2005; Impellizzeri et al., 2006) and interval dribbling (McMillan et al., 2005) may be partly mediated by mitochondrial biogenesis in skeletal muscle.

The upregulation of *PGC-1α* mRNA observed in the current study was not linked to an activation of *AMPK* despite other evidence illustrating a relationship between *AMPK* phosphorylation and *PGC-1α*. In individual analysis, only one data set on *AMPK* phosphorylation seemed to be associated with upregulation of *PGC-1α mRNA*. The failure to highlight such a relationship in the current study may be associated with the intensity of the protocol employed in the study (around 55 ± 6% of $\dot{V}O_{2max}$). The long recovery period between discrete high-intensity activities such as sprints and high speed running also appears to cause low overall intensity of exercise. Such intensity of exercise seems to be the major determinant of the cellular energy status (e.g. free AMP and glycogen levels).

This factor seems to be more important than the peak intensities that are attained for relatively short times associated with dynamically changing exercise intensities as in intermittent protocols (Drust et al., 2007). For example, free AMP contents in human skeletal muscle did not change after low intensity cycling exercise (35~65% of $\dot{V}O_{2max}$) but was significantly increased at higher intensities (90% of $\dot{V}O_{2max}$) in the same exercise pattern (Howlett et al., 1998). Muscle glycogen utilisation also seems to change in an intensity-dependent manner (Gollnick, Piehl & Saltin, 1974; Egan et al., 2010). The soccer-specific training simulation here utilised just 15% of pre-exercise muscle glycogen levels, whereas there was a 30~45% of muscle glycogen utilisation after running

exercise on a treadmill which averaged 70~75% of $\dot{V}O_{2max}$ (Tsintzas, Williams, Boobis & Greenhaff, 1995; Arkinstall, Bruce, Nikolopoulos, Garnham & Hawley, 2001; Harber et al., 2009). Match-play, with similar intensity of around 70~75% of $\dot{V}O_{2max}$ (Bangsbo, 1994d; Bangsbo, 1994c; Reilly, 1997), also reduces muscle glycogen concentration by around 40~75% of resting muscle glycogen level (Saltin, 1973; Krustrup et al., 2006). Such large changes in muscle free AMP and glycogen could represent an important metabolic stress to stimulate *AMPK*. The available data from previous studies (Wojtaszewski et al., 2000; Chen et al., 2003; Wojtaszewski et al., 2003; Lee-Young et al., 2008) indicated that a threshold intensity of exercise would be around 60% of $\dot{V}O2_{max}$ for the activation of *AMPK* in response to acute cycling exercise. These findings may implicate that the low overall intensity of the current protocol may limit the rate of *AMPK* phosphorylation.

Potential signaling factors that could mediate increases in *PGC-1α* expression following exercise include hypoxia, redox flux and mechanical stretch in addition to the pathways associated with metabolic availability and energy perturbation mentioned above (Ljubicic & Hood, 2009). This suggests that exercise induced increases in *AMPK* do not completely explain the increases in *PGC-1α* mRNA (Hashimoto et al., 2007; Terada et al., 2004). For example, the *MAPK* pathways are able to convert both mechanical/biochemical stimuli into increases in muscle gene expression of *PGC-1α* (Koulmann & Bigard, 2006). The phosphorylation of *p38MAPK* seems to be a key component of the general molecular response to all forms of exercise in vivo (Nader & Esser, 2001). For example, Egan and colleagues (2010) reported the activation of *p38MAPK* following a bout of cycling exercise. The present study also demonstrated a 1.5-fold increase above resting levels in *p38MAPK* in response to the soccer-specific training simulation although this increase was not statistically significant. This result could be due to temporal factors related to the timing of the muscle biopsies and insufficient statistical power associated with the relatively small number of subjects may explain the failure to establish statistical significance for the findings of the current study. Three individual data sets on *p38MAPK* phosphorylation, however, showed 2.2-fold increase above resting levels immediately after exercise. This finding would suggest that acute bout of soccer-specific intermittent exercise may potentially stimulate *PGC-1α mRNA expression through p38MAPK*. Lactate produced by glycolysis during exercise may also be one of the potential signals stimulating the transcription of genes involved in mitochondrial biogenesis

(Hashimoto, Hussien, Oommen, Gohil & Brooks, 2007). In an *in vivo* study, Hashimoto et al. (2007) illustrated that cell incubation with exogenous lactate increased *PGC-1α* mRNA expression indicating that such changes in *PGC-1α mRNA* may be associated with lactate signaling cascades. Hashimoto and colleagues (2007) suggested that this response may be a consequence of lactate increasing cytosolic Ca^{2+} signaling, which in turn may lead to activation of *PGC-1α* expression. That mechanism may be partly mediated by changes in reactive oxygen species increasing intracellular Ca^{2+} flux and raising *CaMK* activity (Hashimoto et al., 2007; Ljubicic & Hood, 2008; Ljubicic & Hood, 2009). Although these findings have debates in terms of *in vitro* study, this proposal may suggest that increases in lactate levels (~5.3 ± 2.6 $mmol \cdot l^{-1}$) following soccer-specific training may stimulate cellular adaptations associated with mitochondrial biogenesis in skeletal muscle. The present study did not, however, examine this hypothesis as we did not examine the phosphorylation of *CaMK* due to the limited availability of human skeletal muscle tissue from our samples. Further study is necessary in order to investigate adaptive responses of such potential signaling pathways to soccer-specific intermittent exercise in human skeletal muscles.

5.5 Conclusion

The present data illustrate that simulated soccer-specific training activates the expression of *PGC-1α mRNA* in human skeletal muscle. This would suggest that the global effect of soccer-specific intermittent exercise on aerobic performance may be partly mediated by adaptations associated with mitochondrial biogenesis in skeletal muscle. Future investigation should aim at examining molecular links between upstream activators such as protein kinases and *PGC-1α* expression in response to diverse intensity of soccer-specific intermittent exercise. It is necessary to assess changes in biological markers related to mitochondrial function and density following soccer-specific intermittent exercise.

CHAPTER 6

THE INFLUENCE OF A PRIOR BOUT OF EXERCISE ON THE ACUTE MOLECULAR RESPONSES TO SOCCER-SPECIFIC INTERMITTENT EXERCISE

6.1 Introduction

The training programmes of professional soccer teams can combine multiple daily sessions (Bangsbo et al., 2006). Such training organisation has been used to integrate the large volume of training or the various types of exercise needed to prepare the players for the high-standards of performance required in the sport. Recently it has been suggested that these training programmes may be especially effective as they amplify the physiological adaptations that occur (Ronsen et al., 2001; Baar & McGee, 2008; Yeo et al., 2008). This change may not just be a consequence of the additional training volume but a function of changes in the exercise stimulus as a result of the completion of a prior bout of exercise (Stich et al., 2000; Ronsen et al., 2001; Goto, Ishii, Mizuno et al., 2007). For example, repeated training sessions can alter the magnitude of the exercise-induced changes in glycogen depletion (Yeo et al., 2008), fat availability (Stich et al., 2000; Goto, Ishii, Mizuno et al., 2007) and the neuroendocrine response to exercise (Ronsen et al., 2001; Goto, Ishii, Mizuno et al., 2007) when compared to a single bout of exercise.

These repeated training sessions in a day may also promote enhanced molecular adaptations than those associated with the completion of a single session between days (Yeo et al., 2008). For example, the transcription of *PGC-1α* in human skeletal muscle is influenced under different metabolic states (Pilegaard et al., 2005). Pilegaard and colleagues (2005) demonstrated that the elevation of *PGC-1α* mRNA content following exercise was up-regulated for longer time periods in skeletal muscles with low-glycogen compared to normal glycogen during the recovery period. These findings suggest that the change in the intracellular energy state following the prior bout of muscle contraction plays an important role in the initiation of the acute molecular response to exercise. That is to say that the perturbation of the ATP: AMP: ADP balance (Winder, Taylor & Thomson, 2006) and changes in muscle glycogen levels (Hawley et al., 2006; Baar & McGee, 2008) can affect the regulation of signaling pathways. For example, dynamic changes in muscle glycogen levels during and after exercise can regulate proteins that contain glycogen-binding domains (Baar & McGee, 2008) and subsequently lead to changes in diverse cellular functions such as gene transcription and substrate oxidation (Hargreaves, 2004; Baar & McGee, 2008). Recent studies illustrated that the low muscle glycogen level activated protein kinases such as *AMPK* (Wojtaszewski et al., 2003; Steinberg et al., 2006) and *p38MAPK* (Chan et al., 2004;

Cochran et al., 2010) in cycling exercise.

Nevertheless, it is still unclear whether such specific regulatory mechanisms lead to enhancements in the training effects of training programmes that are relevant to soccer. Understanding acute molecular responses to the different daily organisation of soccer-specific intermittent exercise may, therefore, suggest practical insights into the optimal soccer training programmes improving fitness level of players. The aim of the present study was to elucidate the effect of training organisation on the acute signaling responses associated with mitochondrial biogenesis in skeletal muscle of healthy men. This study also aimed to investigate the acute effect of prior soccer-specific training on the physiological and metabolic responses following two consecutive bouts of soccer-specific intermittent exercise. We thus hypothesised that the training organisation with two consecutive bouts of soccer-specific intermittent exercise in a day would induce a more pronounced physiological and metabolic response and greater signaling activation in relation to mitochondrial biogenesis than that with one bout of soccer-specific exercise performed on two consecutive days.

6.2 Method

6.2.1 Subjects.

Eight recreationally active males participated in the study (mean ± SD: age, 22 ± 2 yr; weight, 75 ± 7 kg; height, 177 ± 6 cm; $\dot{V}O2_{max}$, 62 ± 8 $ml \cdot kg^{-1} \cdot min^{-1}$). The experimental procedures and potential risks related to the study were explained verbally, along with written information, during a familiarization session. Consent was obtained from all subjects prior to participation. Subjects refrained from strenuous exercise at least 48 h before each exercise trial. None of the subjects had any current medical problems of the neurological and/or the musculoskeletal systems or was under any pharmacological treatment during the course of the study. The study was approved by the Ethics Committee of Liverpool John Moores University.

6.2.2 Assessment of physiological fitness.

All participants were initially assessed for $\dot{V}O2_{max}$ using an incremental exercise test performed on a motorised treadmill (HP Cosmos, Germany). Oxygen uptake was measured continuously during exercise using an on-line gas analysis system (Metamax, Cortex). Details about these procedures are presented in ***Chapter 5.***

6.2.3 Experimental design.

An overview of the experimental design is shown in Figure 6.1. At least 3 days after the initial assessment of maximal oxygen consumption ($\dot{V}O2_{max}$), subjects performed one of two experimental trials, *BETWEEN DAY* and *WITHIN DAY*. The BETWEEN DAY trial consisted of one bout of soccer-specific intermittent exercise in a day. In the WITHIN DAY trial, two consecutive bouts of soccer-specific intermittent exercise were performed in a day. The second bout of exercise was followed 24 h after the first bout in the BETWEEN DAY trial and after 2-h in the WITHIN DAY trial respectively. After at least 1-week following the first trial, subjects completed the alternative exercise trial. The order of experimental trials was randomly allocated. Each bout of exercise in these trials consisted of a laboratory-based soccer-specific training simulation (LSSTS) as described in Chapter 4. Heart rate (HR) was measured continuously during exercise protocols (Polar S610i, Kempele, Finland). Ratings of perceived exertion (RPE) were measured every 20-min during and 30-min after completion of LSSTS. Blood samples were obtained pre- and post exercise and at the same time as the RPE. Muscle biopsies were taken from the vastus lateralis pre-, post- and 3-h after the second bout of LSSTS. During the recovery period between the post-exercise biopsy and 3-h biopsy, subjects remained seated in the laboratory.

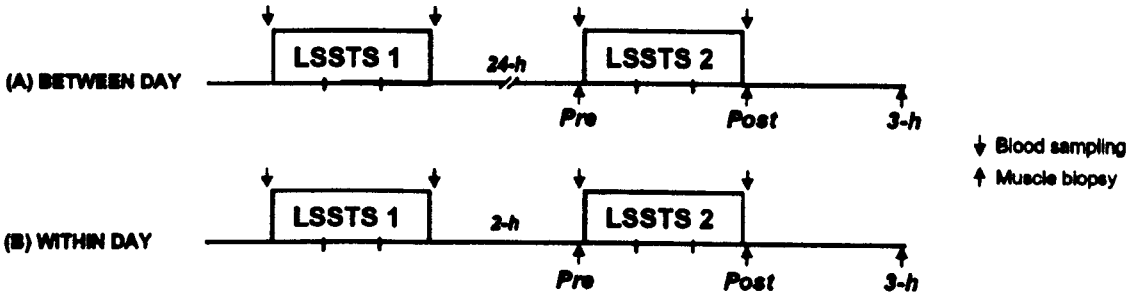


Figure 6.1 The schematic design of the repeated bouts of exercise (A) *BETWEEN DAY*; two bouts of exercise with 24-h recovery period, (B) *WITHIN DAY*; two bouts of exercise with 2-h recovery period, LSSTS 1 and 2; the first and second bout of a laboratory-based soccer specific training simulation. Pre; pre-exercise, Post; post-exercise, 3-h; 3-h after exercise

6.2.4 Dietary control

To ensure that subjects repeated the same dietary intake prior to each trial, subjects were instructed to complete a 2-day food diary preceding the first bout of LSSTS in the first trial. The dietary intake recorded in the diary was then repeated 2 days before the first bout of LSSTS in the second trial. After completing the first bout of exercise in the BETWEEN DAY trial, subjects consumed a high-carbohydrate (CHO) diet in order to load glycogen (CHO ingestion rate of 10g per body weight (Kg)). Subsequent dietary analysis was performed using the computer software programme Microdiet. A breakdown of the subjects' macronutrient intake can be seen in **Table 1**. Subjects refrained from alcohol and caffeine for at least 48-h prior to the testing session. An overnight fast was also required prior to attending the laboratory in the morning. In the 30-min preceding exercise, subjects consumed water (5 ml/kg). During exercise subjects were not allowed a drink. Only plain water was provided during the recovery window between post-exercise and the 3-h biopsies and during the 2-h recovery between sessions in the WITHIN DAY (1.5L/ loss of body weight (kg)).

Table 6.1 Macronutrient intake of subjects

	CHO (%)	Fat (%)	Protein (%)	MJ
General diet	47 ± 8	34 ± 6	18 ± 4	7.6 ± 3.9
High-CHO diet	81	10	9	25

6.2.5 Exercise protocols

The laboratory-based soccer-specific training simulation for the study was devised for a motorized treadmill (HP cosmos Pulsar® 4.0, h/p/cosmos, Germany). The treadmill was controlled by a computer-based programme. Details about this protocol are presented in ***Chapter 3 and 4***. The intensity of the protocol was evaluated by measuring HR and session-RPE.

6.2.6 Muscle biopsies

Muscle biopsies were obtained from separate incision sites (2-3 cm apart) from the lateral portion of the vastus lateralis muscle pre-, post- and 3-h after the second bout of exercise of each trial using a Bard Monopty Disposable Core Biopsy Instrument 12 gauge x 10 cm length (Bard Biopsy Systems, Tempe, AZ, USA). Details about procedures are presented in ***Chapter 4***.

6.2.7 Muscle analysis

6.2.7.1 Muscle glycogen

Approximately 2-3 mg of freeze dried sample was powdered and subsequently hydrolyzed by incubation in 500 µl of 1 M HCl of 3-4 hr at 100°C. After cooling to room temperature, samples were neutralized by the addition of 250 µl 0.12 mol·L^{-1} Tris-2.1 mol·L^{-1} KOH saturated with KCl. Following centrifugation, 150 µl of supernatant was analysed in duplicate for glucose concentration according to the hexokinase method using a commercially available kit (GLU-HK,

Randox Laboratories, Antrim, UK). Glycogen concentration is expressed as mmol·kg^{-1} dry weight (dw), and intra-assay coefficients of variation was < 5%.

6.2.7.2 Western blotting

Approximately 20-30mg piece of frozen muscle was ground to powder and homogenised in 120 µl of ice cold lysis buffer (25 mM Tris/HCl [pH 7.4], 50 mM NaF, 100 mM NaCl, 5 mM EGTA, 1 mM EDTA, 10 mM Na-Pyrophoshatase, 1 mM Na_3VO_4, 0.27 M sucrose, 1 % Triton X-100, 0.1 % 2-mercaptoethanol) and supplemented with a protease inhibitor tablet (Complete mini, Roche Applied Science, West Sussex, UK). Homogenates were centrifuged at 14,000 g for 10 min at 4°C and the supernatant was collected. The protein content of the supernatant was determined using a bicinchoninic acid assay (Sigma, UK). Each sample was diluted with an equal volume of 2X Laemmli buffer (National Diagnostics, USA) and boiled for 5-min at 100 °C. For each blot, a standard and internal control was loaded along with 50-100 µg of protein from each sample and then separated in Tris-glycine running buffer (10 X Tris/Glycine, Geneflow Ltd, Staffordshire, UK) using self-cast 4% stacking and 10 % separating gels (National Diagnostics, USA). Gels were transferred semi-dry onto nitrocellulose membrane (Geneflow Ltd, Staffordshire, UK) for 2 h at 200 V and 45 mA per gel in transfer buffers (anode 1; 0.3 M Tris, 20 % methanol, pH 10.4; anode 2; 0.25 M Tris, 20 % methanol, pH 10.4; cathode; 0.4 M 6-amino hexanoic acid, 20 % methanol, pH 7.6). After transfer, membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBST: 0.19 M Tris pH 7.6, 1.3 M NaCl, 0.1 %Tween-20) with 5% non-fat milk. The membranes were then washed for 3 x 5 min in TBST before being incubated overnight at 4°C with phospho-specific anti-bodies for AMPK^{Thr172} and p38MAPK$^{Thr180/Tyr182}$ (all from Cell Signaling, UK) as well as total proteins of GAPDH (all from Cell Signaling, UK) and PGC-1α (Calbiochem, Merck Chemicals, UK) all at concentrations of 1:1000 in 1 X TBST. The next morning, membranes were washed for a further 3 x 5 min in TBST and subsequently incubated with anti-species horseradish peroxidise-conjugated secondary antibody (Bio-Rad or Dako, UK) for 1-h at room temperature. After a further 3 x 5 min washes in TBST, membranes were exposed in a chemiluminescence liquid (SuperSignal, Thermo Fisher Scientific, Rockford, IL, USA) for 5-min. Membranes were visualised using a Bio-Rad Chemi-doc system, and band densities were determined using Image Lab image-analysis software. All raw densitometry data were used for statistical analysis purposes so as to compare within-subject responses. However, because it is technically incorrect to compare

densitometry data between gels (and hence, between subjects), for graphical purposes each subject's pre-exercise values was normalised to 1 (hence no error bars are shown for this time point) such that values at post-exercise and 3-h post-exercise are subsequently expressed as fold change relative to pre-exercise values. This approach has been used previously by Morton and colleagues (Morton et al., 2009) and other researchers (Perry et al., 2010). GAPDH was used for normalisation to phosphorylated proteins where appropriate.

6.2.7.3 Real time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RNA isolation and cDNA synthesis Total RNA was isolated from small muscle biopsies (20-30 mg) using Trizol reagent (Invitrogen), according to the manufacturer's protocol. RNA quality and quantity were determined using Implen Nanophotometer (Implen, Munchen, Germany) and the RNA was stored at -80°C. cDNA was synthesised using random hexamers (Applied Biosystems) and Superscript III enzyme (Invitrogen), using manufacturer's protocol.

Gene expression analysis by RT-qPCR Gene specific expression data was obtained using probes selected from Human Universal Probe Library (Roche Diagnostics) with compatible oligonucleotide primers (MWG Eurofins). One µl of each sample were analysed in duplicates with negative controls using AB 7500 Real-Time Quantitative PCR instrument (Applied Biosystems) and Agilent Brilliant II qPCR Master Mix with Low ROX (Agilent Technologies). One µl of cDNA, 500 nM of primer and 200 nM of probe were used for each 20µl reaction **(Table 6.2)**. The following cycling parameters were used: 50°C for 2 minutes, initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/elongation at 60°C for 1 minute. Data was collected and analysed using AB SDS 1.43 Software (Applied Biosystems, Foster City, USA). Changes in mRNA content were calculated according to the 2-ΔΔCt method where GAPDH was used as the housekeeping gene (Heid et al., 1996).

Table 6.2 Primer and probe sequences used for real-time PCR

Gene	Forward primer	Reverse primer	Probe
GAPDH	GCTCTCTGCTCCTCCTGTTC	ACGACCAAATCCGTTGACTC	60
PGC-1α	TGAGAGGGCCAAGCAAAG	ATAAATCACACGGCGCTCTT	13

6.2.8 Venous blood samples and biochemical analysis

Blood samples were obtained pre- and post exercise from a superficial vein in the antecubital crease of the forearm using standard venepuncture techniques (Vacutainers Systems, Becton, Dickinson). Samples were collected into vacutainers containing EDTA and lithium heparin and were stored on ice until centrifugation at 2,000 rpm for 15 min at 4°C. Following centrifugation, aliquots of plasma were stored at -80°C for later analysis. Samples were analyzed for plasma glucose, lactate, glycerol and NEFA concentration using commercially available kits (Randox Laboratories, Antrim, UK). All post-exercise blood samples were corrected for plasma volume changes according to Dill and Costill (1974).

6.2.9 Statistical analyses

The effects of time and trials (*BETWEEN DAY* and *WITHIN DAY*) on the responses of physiological variables, blood metabolites, muscle glycogen, protein and mRNA contents were evaluated using two-way analysis of variance (ANOVA) for repeated measures. One-way ANOVA for repeated measures was used to evaluate the effect of time for each trial. The Least Significant Difference (LSD) *post hoc* test for multiple comparisons was performed in order to examine differences between pre- and post-exercise values in each bout of exercise. A paired t-test was used to evaluate differences between values of both trials at the same time point. Results are presented as means ± standard deviation (SD). *P*-values <0.05 were considered significant.

6.3 Results

6.3.1 Physiological responses

In the WITHIN DAY trial, all of the physiological responses such as HR, time spent in the HR zones of 80~100% HR_{max} and session-RPE were significantly increased during the second bout of exercise (HR, 149 ± 11 $b \cdot min^{-1}$; % HR_{max}, 79 ± 4 %; time spent in HI zone, 53 ± 12 %; session-RPE, 7 ± 1 AU) than those observed in the first bout of exercise (HR, 141 ± 12 $b \cdot min^{-1}$; % HR_{max}, 74 ± 4 %; time spent in HI zone, 38 ± 14 %; session-RPE, 6 ± 1 AU) ($p<0.05$). There were also significant increases in such physiological responses during the second bout of exercise in the WITHIN DAY trial, compared to those obtained in the BETWEEN DAY trial (HR, 142 ± 13 $b \cdot min^{-1}$; % HR_{max}, 75 ± 5%; time spent in HI zone, 39 ± 16 %; session-RPE, 5 ± 1 AU). There was, however, no difference in physiological responses between the first and second bout of exercise in the BETWEEN DAY trial **(Table 6.3)**.

Table 6.3 Physiological responses during the bout of exercise in each trial

	BETWEEN DAY		WITHIN DAY	
	1st bout	2nd bout	1st bout	2nd bout
HR ($b \cdot min^{-1}$)	142 ± 13	142 ± 13	141 ±12	149 ± 11*‡
% HR_{max} (%)	75 ± 4	75 ± 5	74 ± 4	79 ± 4*‡
Time spent in the HI zones (80~100% HR_{max}) (%)	39 ± 14	39 ± 16	38 ± 14	53 ± 12*‡
Session-RPE (AU)	5 ± 1	5 ± 1	6 ± 1	7 ± 1*‡

Values are means ± standard deviation. HR-heart rate, HR_{max}-maximal heart rate, RPE-ratings of perceived exertion * p <0.05, significant difference between the first and second bouts within the same trial, ‡ p <0.05 significant difference between both trials.

6.3.2 Blood metabolites

The concentrations of glucose (BETWEEN DAY, 6.2±0.7 mmol·L^{1} and 6.4±0.9 mmol·L^{-1}; WITHIN DAY, 6.2 ± 0.7 mmol·L^{-1} and 6.2 ± 0.5 mmol·L^{-1}) and lactate (BETWEEN DAY, 3.3 ± 1.6 mmol·L^{-1} and 3.8 ± 1.6 mmol·L^{-1}; Trial 2, 4.2 ± 2.0 mmol·L^{-1} and 3.2 ± 2.0 mmol·L^{-1}) immediately after the bout of exercise were significantly greater than those observed pre-exercise in both trials (glucose, BETWEEN DAY, 5.5 ± 0.4 mmol·L^{-1} and 5.6 ± 0.5 mmol·L^{-1}; WITHIN DAY, 5.3 ± 0.5 mmol·L^{-1} and 5.6 ± 0.5 mmol·L^{-1}, lactate, BETWEEN DAY, 1.4 ± 0.3 mmol·L^{-1} and 1.9 ± 0.9 mmol·L^{-1}; WITHIN DAY, 1.9 ± 1.0 mmol·L^{-1} and 1.5 ± 0.3 mmol·L^{-1}) (p<0.05). There was no difference between trials in the glucose level following the second bout of exercise. The lactate level following the second bout of exercise was, however, significantly less in WITHIN DAY than that observed in BETWEEN DAY **(Figure 6.2 A and B).** There was a more pronounced increase in NEFA and glycerol in the WITHIN DAY trial compared with the BETWEEN DAY trial post-exercise following the second bout of exercise (BETWEEN DAY, NEFA, 0.8 ± 0.3 mmol·L^{-1}, glycerol, 90 ± 48 µmol·L^{-1}; WITHIN DAY, NEFA, 1.7 ± 0.3 mmol·L^{-1}, glycerol, 260 ± 87 µmol·L^{-1}; p<0.05). Prior to the second bout of exercise in the WITHIN DAY trial, the NEFA concentration was still elevated (p<0.05) **(Figure 6.2 C and D).**

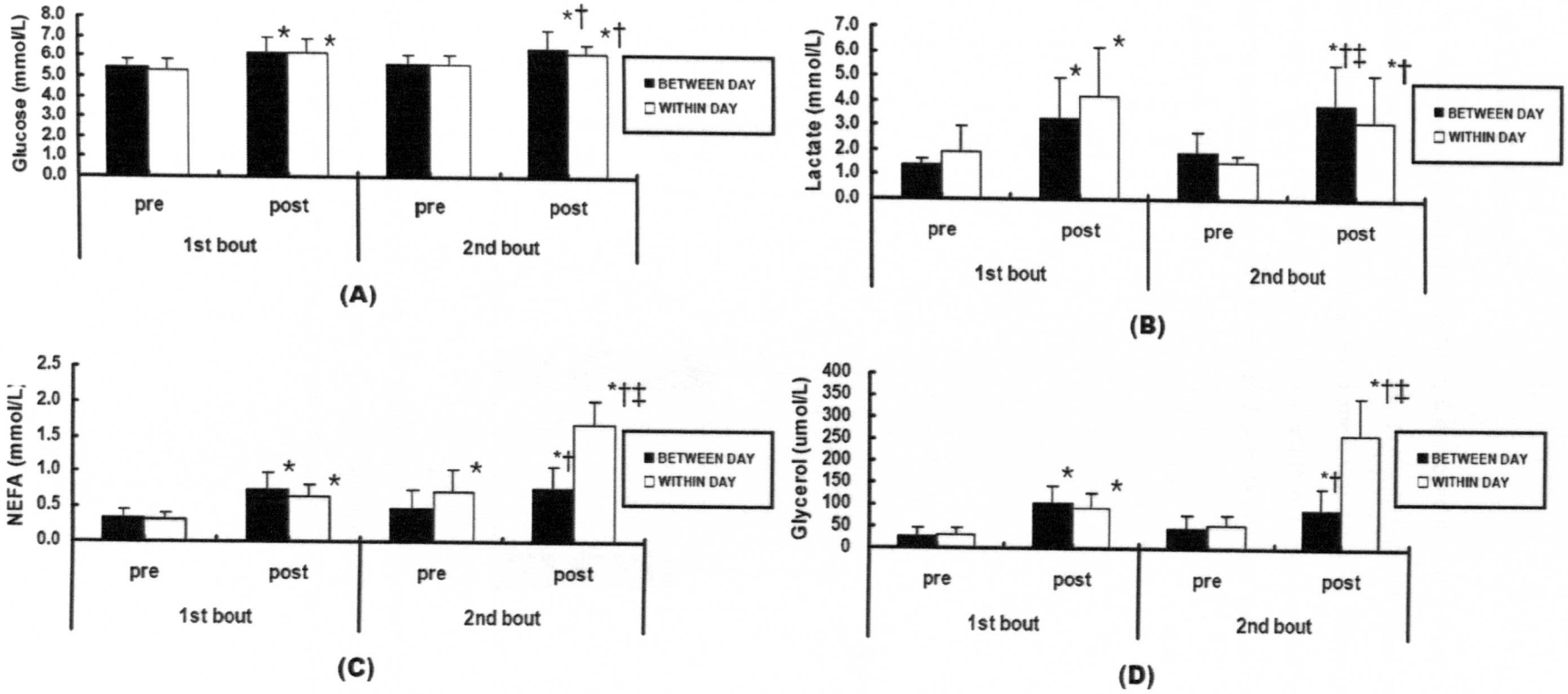


Figure 6.2 Responses of blood metabolites to the repeated bouts of the lab-based soccer-specific training simulation (LSSTS); (A) Glucose, (B) lactate, (C) NEFA and (D) glycerol. * $p<0.05$, significant difference compared with pre-exercise in the first bout of the LSSTS; † $p<0.05$, significant difference compared with pre-exercise in the second bout of the LSSTS; ‡ $p<0.05$, significant difference between both trials at the same time point.

6.3.3 Muscle glycogen

The level of muscle glycogen following the second bout of the LSSTS significantly decreased by approximately 17% of pre-exercise values in the WITHIN DAY trial (pre, 359 ± 88 mmol·kg^{-1}; post, 297 ± 102 mmol·kg^{-1}, $p<0.05$), whereas there was around a 7% reduction in the BETWEEN DAY trial (pre, 375 ± 118 mmol·kg^{-1}; post, 350 ± 115 mmol·kg^{-1}, $p>0.05$). There was no difference in muscle glycogen concentration pre- and post-exercise of the second bout between both trials, respectively **(Figure 6.3).**

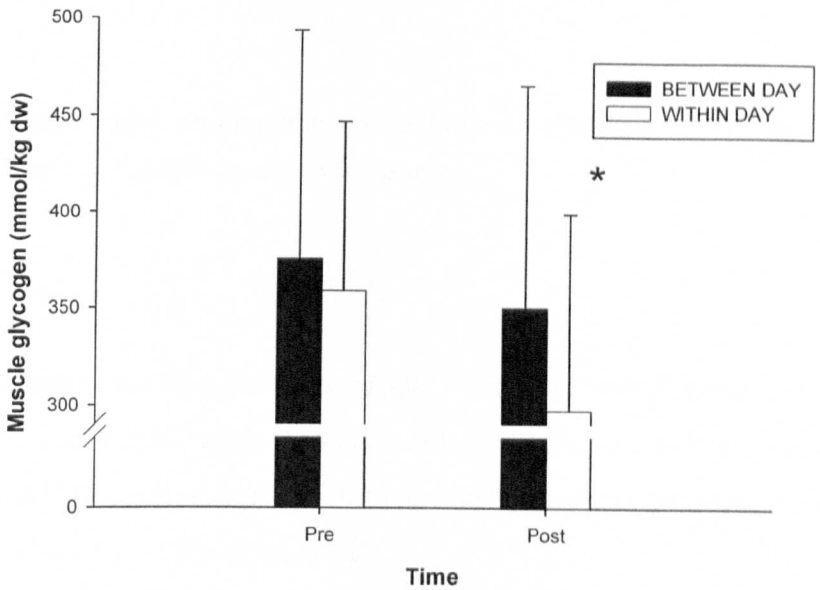


Figure 6.3 Muscle glycogen changes following the second bout of the laboratory-based soccer specific training simulation. Muscle glycogen significantly decreased post-exercise only in the WITHIN DAY trial. Values mean ± standard deviation. * *p<0.05*, significant difference compared to pre-exercise.

6.3.4 Protein kinases associated with mitochondria biogenesis

Representative Western blots are shown in **Figure 6.4**.

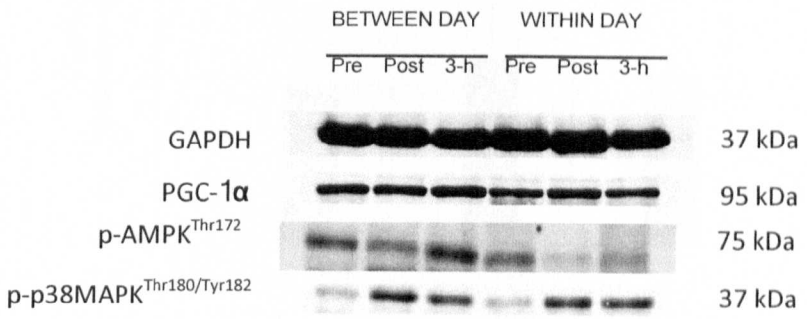


Figure 6.4 Representative Western blots before (Pre), immediately after (Post) and 3-h after (3-h) the second bout of the LSSTS. p-, phosphorylated.

AMPK phosphorylation Phosphorylation of AMP^{Thr172} did not change immediately post-exercise or 3-h after exercise (BETWEEN DAY, p=0.686; WITHIN DAY, p=0.777) compared to pre-exercise values. There was no significant difference in this response between trials in accordance with time (time x trial, p = 0.280) **(Figure 6.5).**

p38MAPK phosphorylation There was no difference in the phosphorylation of p38MAPKThr180/Tyr182 post- exercise and 3-h after exercise compared to pre-exercise levels (BETWEEN DAY, p=0.818; WITHIN DAY, p=0.841). There was also no difference in this response in either trial in accordance with time (time x trial, p=0.596) **(Figure 6.6).**

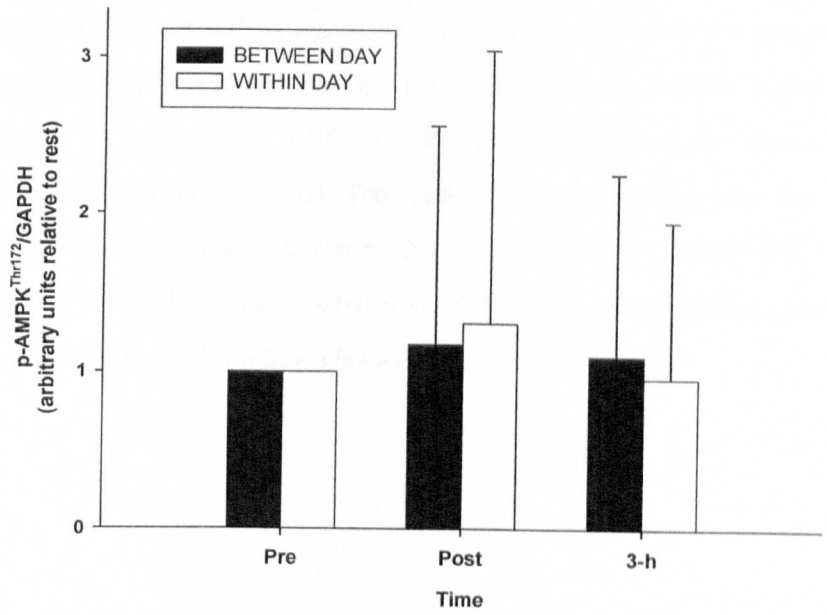


Figure 6.5 Phosphorylation of AMPK following repeated bouts of the laboratory-based soccer-specific training simulation.

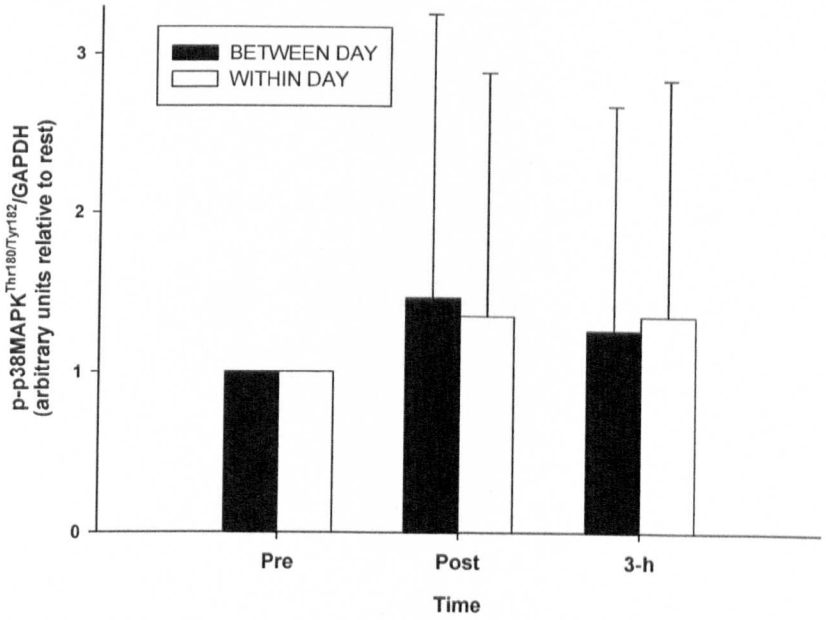


Figure 6.6 Phosphorylation of p38MAPK following repeated bouts of the laboratory-based soccer-specific training simulation. .

6.3.5 Expression of PGC-1α protein and mRNA

The expression of *PGC-1α* mRNA significantly increased approximately 4-fold at 3-hr following exercise compared to pre- and post-exercise values, respectively ($p<0.05$). There was, however, no difference in the expression of *PGC-1α mRNA* between either trial in accordance with time (time x trial, p=0.321) **(Figure 6.7 B)**. The levels of *PGC-1α* protein were not different post-exercise and 3-h after exercise compared to pre-exercise levels (BETWEEN DAY, p=0.984; WITHIN DAY, p=0.846). Exercise in either trial did not change total *PGC-1α* protein content in accordance with time (p=0.648) **(Figure 6.7 A)**.

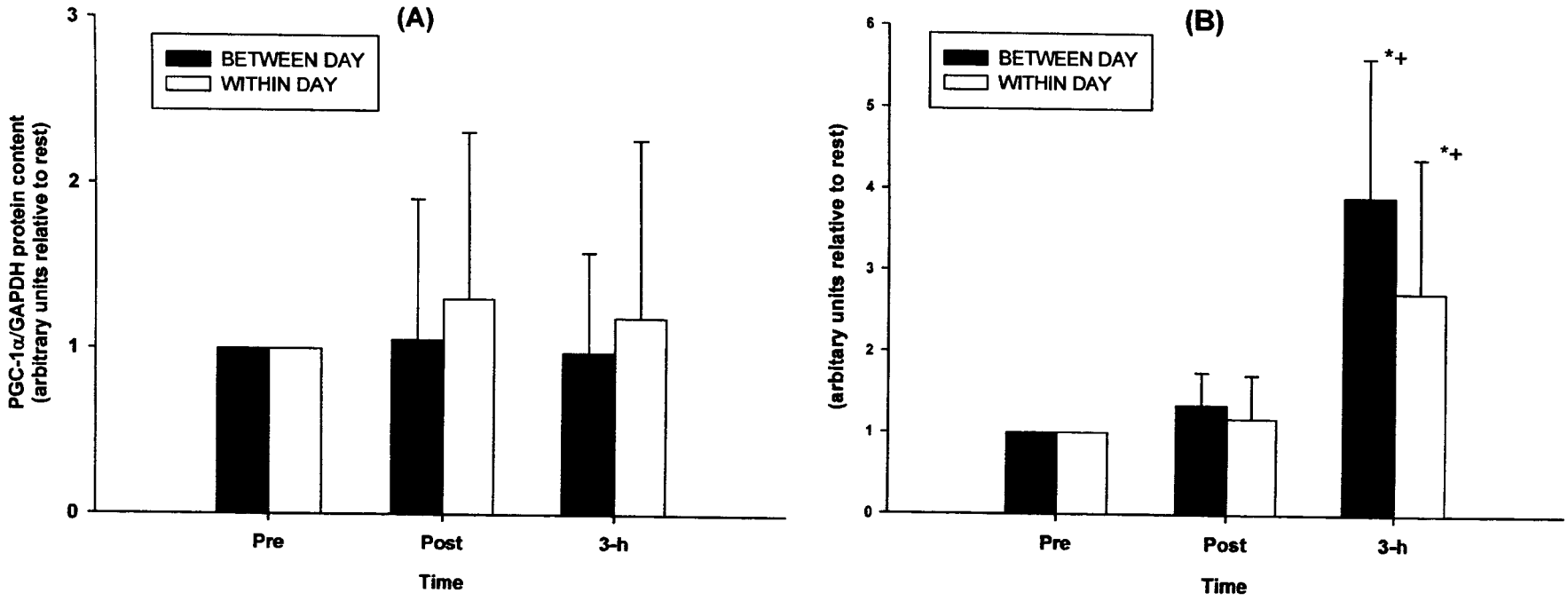


Figure 6.7 The protein content (A) and *mRNA* expression (B) of *PGC-1α* following repeated bouts of the laboratory-based soccer-specific training simulation. Significant different compared to pre-exercise (·) and post-exercise (†) ($p < 0.05$).

6.4 Discussion

The aim of the current study was to evaluate the effect of prior soccer-specific exercise on the physiological, metabolic and molecular responses to subsequent bouts of soccer-specific intermittent exercise. This study provides novel data by demonstrating that two consecutive bouts of soccer-specific intermittent exercise in a day result in higher physiological responses (such as HR and RPE) and higher FFA availability than one bout of the same exercise session in a day. However, contrary to our hypothesis, acute molecular signaling pathways regulating mitochondrial biogenesis were not different between the two conditions irrespective of the number of bouts of soccer-specific intermittent exercise within a day. There was also no difference in phosphorylation of protein kinases as a consequence of training organisation. Based on these observations it would seem that different approaches to training organisation may be more important for the acute physiological responses to soccer-specific intermittent exercise than the molecular changes underpinning chronic adaptations.

The present data on the physiological responses demonstrated that repeated bouts of exercise in a day provide a more intense training stimulus than exercise performed once a day. This more intense physiological strain may be a consequence of the progressive decrease in the carbohydrate availability (i.e., glycolytic flux) in blood during two consecutive bouts of exercise in a day (Coyle, Jeukendrup, Wagenmakers & Saris, 1997; Sidossis, Gastaldelli, Klein & Wolfe, 1997). For example, fat availability showed different responses in response to the different training organisations. The prior bout of exercise in the training twice a day model induced a higher fat availability. Interestingly, the enhancement in NEFA levels following the first bout of exercise was also significantly maintained during the recovery period between both bouts of exercise when training twice a day. The recovery periods between bouts of exercise seem to allow for a significant blood flow to adipose tissue, thereby promoting the release of those metabolites to the blood (Krustrup et al., 2006). In addition, we provided data on decline in lactate production following the repeated bouts of training in the same day compared with the lactate level observed in a single bout in a day. Although our glycogen data does not accurately reflect carbohydrate availability, this would indicate that the prior bout of exercise slowed glycolysis. These results

supported data from the study that prior intense exercise substantially modified the carbohydrate metabolism to subsequent whole body intermittent exercise (Bangsbo, 1994d; Christmass, Dawson, Goodman & Arthur, 2001). Such metabolic responses to repeated bouts of exercise seem to be associated with exercise-induced rise of adrenaline and lower plasma insulin level (Ronsen et al., 2001; Goto, Ishii, Mizuno et al., 2007; Cochran et al., 2010). These hormones may contribute to the activation of fat availabilty following prior intermittent exercise, thereby leading to impeding glycolytic flux during the subsequent bout of exercise (Christmass et al., 2001). The current findings in the present study suggest that training organisation with two consecutive bouts of soccer-specific exercise in a day can induce greater metabolic stress than those observed in daily once training programme.

As opposed to the systemic responses of blood metabolites such as NEFA, glycerol and lactate, the data from the present study demonstrated that no difference was observed in molecular responses between both conditions. These results extend previous findings that increases in expression of *PGC-1α mRNA* does not appear to be associated with altered metabolites including NEFA, glycerol and lactate during the recovery period after cycling (Cluberton, McGee, Murphy & Hargreaves, 2005) and running exercise (Bartlett et al., 2012). Indeed, increased circulating FFA during exercise and subsequent recovery has no effect on metabolic gene expression such as *pyruvate dehydrogenase kinase 4 (PDK4)* and *PGC-1α* (Tunstall, McAinch, Hargreaves, van Loon & Cameron-Smith, 2007). Taken together, the present data confirms that systemic changes in circulating metabolites such as NEFA, glycerol and lactate may not acutely influence intracellular signaling pathways associated with mitochondrial biogenesis in skeletal muscle.

Changes in cellular energy balance and mechanical stretch associated with *AMPK* and *p38MAPK* signaling have been implicated in the gene expression response to exercise (Widegren et al., 2001b; Wojtaszewski et al., 2003). These energetic and mechanical stimuli in the present study were expected to intensify due to the repeated bouts of soccer-specific exercise in a day. Such experimental design may therefore induce greater energy deficits and mechanical stress on a cellular level, compared to the single bout of exercise used in this investigation. Increases in *PGC-1α mRNA* expression with no significant change in phosphorylation of *AMPK* and *p38MAPK* in the present study were similar to those observed in the earlier study presented in Chapter 5. These

novel results would support the suggestion that the signaling pathways associated with *AMPK* and *p38 MAPK* may not play a major role in mitochondrial adaptations to the soccer-specific intermittent simulation with low overall intensity of exercise.

Although *AMPK* and *p38MAPK* are shown to be putative stress signals that converge on *PGC-1α* (Akimoto et al., 2005; Jäger, Handschin, St.-Pierre & Spiegelman, 2007; Wright, Geiger et al., 2007), we have shown in the present study no change in *AMPK* and *p38MAPK* despite a 4-fold increase in expression of *PGC-1α mRNA*. A potential explanation for this finding may be related to the low average intensity of the soccer-specific exercise protocol (Chapter 5). Furthermore, the low muscle glycogen utilisation in the present study seems to result in little change in the signaling responses of these protein kinases (Wojtaszewski et al., 2003; McBride et al., 2009; Yeo et al., 2010) supposedly due to the glycogen binding domains existent on *AMPK* and *p38MAPK* (Chan et al., 2004). The other possible scenario to explain no detectable changes in *AMPK* and *p38MAPK* is that heterogeneity of muscle tissues sampled in the present study may not determine changes in the levels of muscle glycogen between both conditions. That is, mixed-muscle analysis employed in the present study can be argued. The magnitude of glycogen utilisation induced by exercise could be different in accordance with the contribution of the types of muscle fibres within samples (Tsintzas et al., 1995). For example, Tsintzas et al. (1995) reported that running exercise on a treadmill (60 min at 70% of $\dot{V}O_{2max}$) resulted in a greater depletion of muscle glycogen in type I fibres (66 ± 3% of pre-exercise value) compared with type II fibres (20 ± 4% of pre-exercise value). The similarity of pre-exercise muscle glycogen level between both trials in the current study may not allow differences in phosphorylation of an energy sensing protein in skeletal muscle to be detected. However, the 4-fold increase in *PGC-1α mRNA* in this study cannot be explained by such molecular changes as a consequence may be related to circulating hormones such as epinephrine and norepinephrine. These hormones can increase gene expression associated with mitochondrial biogenesis (Watt et al., 2004). Although the present study did not characterise responses of such hormones to this type of soccer-specific intermittent exercise, it may be tempting to speculate this concept that may contribute to the increased expression of *PGC-1α mRNA*.

6.5 Conclusion

In summary, the current study demonstrates novel findings that two consecutive bouts of a soccer-specific training session stimulate a more intense physiological strain and altered metabolic response than training performed once a day. Data from the present study supports that soccer-specific intermittent exercise significant induces molecular responses associated with mitochondrial biogenesis although *AMPK* and *p38MAPK* may not play a role in such adaptations. The organisation of training does not seem to play a major role in these molecular responses.

CHAPTER 7

SYNTHESIS OF FINDINGS

7.1 Introduction

This chapter aims to review and integrate the experimental findings from the experimental studies. This analysis will provide a basis for an evaluation of the successful completion of the aims and objectives of the thesis. The final section relates to the recommendations for future research

7.2 Achievement of Aims and General Discussion

Aim 1: To characterize the physical demands of professional soccer training by quantifying and comparing the physiological training loads and activity profiles of professional players during the pre-season and in-season periods.

This aim was achieved through the completion of study 1 in Chapter 3. Study 1 quantified the physiological loads and movement profiles of Korean professional players during pre-season and in-season training. This was the first attempt to investigate the demands of programmed soccer training in this way for a professional team at different times of the annual plan. The present data demonstrated that the overall physiological load of the weekly training schedule was significantly greater in 'pre-season' compared with 'in-season' training. Interestingly, these differences in training intensity were largely associated with the increased physiological demands induced by the technical and tactical specific training sessions during the 'pre-season' training phase. Such variations in training load are highly likely to be a direct function of the specific aims and objectives of the coaches during particular training periods.

Aim 2: To develop a laboratory based treadmill simulation that represents the physiological requirements and activity profiles of professional soccer training.

This aim was achieved through the completion of study 2 in Chapter 4. In study 2, data from study 1 was utilised to develop a laboratory-based treadmill simulation that represented the physiological requirements and activity profiles of professional soccer training. The laboratory-based soccer specific training simulation successfully replicated both the activity profile and the duration of each discrete activity observed in actual training. The current simulation, however, did include differences in the total number of discrete activity bouts and excluded the utility

movements and soccer-specific technical elements seen in training. Such omissions of these activity categories is a direct consequence of the technological limitations and safety issues associated with the treadmill. Despite these limitations the simulation elicited similar physiological responses to those obtained in professional soccer training albeit in another population of participants. These data together would seem to suggest that the simulation is a valid model for laboratory-based investigations into the physiological responses and the molecular adaptations of skeletal muscle to the soccer-specific intermittent exercise.

Aim 3: To examine the acute effect of a single bout of soccer-specific intermittent exercise on metabolic stress and molecular responses associated with mitochondrial biogenesis in human skeletal muscle.

This aim was achieved through the completion of study 3 in Chapter 5. Study 3 investigated the hypothesis that soccer-specific intermittent exercise would induce increases in the phosphorylation of important signaling protein kinases and the activation of *PGC-1α mRNA* expression. The soccer-specific intermittent exercise enhanced the expression of *PGC-1α mRNA* in human skeletal muscle 3-h after soccer-specific training simulation with these changes similar to the adaptive responses in terms of magnitude and time-course observed with continuous exercise (Gibala et al., 2009; Harber et al., 2009; Cochran et al., 2010; Little et al., 2011). This activation of *PGC-1α mRNA* is likely to enhance the oxidative capacity of skeletal muscle. No significant changes in the phosphorylation of *AMPK and p38MAPK* were observed. The mechanism mediating these changes in *PGC-1α mRNA* expression may be calcium related signaling pathways although this has not been assessed in the current study. These results suggest that the global effect of soccer-specific intermittent exercise on aerobic performance may be partly mediated by increases in mitochondrial biogenesis in skeletal muscle although the specific signaling pathways associated with this adaptation is not clear.

Aim 4: To evaluate the effect of prior soccer-specific training on the physiological, metabolic and molecular responses to single and consecutive bouts of soccer-specific intermittent exercise.

This aim was achieved through the completion of study 4 in Chapter 6. Study 4 hypothesized that the prior bout of soccer-specific intermittent exercise would induce greater signaling responses

associated with mitochondrial biogenesis in human to the subsequent bout of exercise skeletal muscle than those obtained in one bout of soccer-specific exercise in a day. Significant increases in the expression of *PGC-1α mRNA* at 3-hr were observed after soccer-specific intermittent exercise irrespective of the number of bouts of soccer-specific intermittent exercise within a day. Such expression of *PGC-1α mRNA* was not however different when training organisation was different. There was also no difference in phosphorylation of protein kinases as a consequence of training organisation. This study also provided novel data by demonstrating that two consecutive bouts of soccer-specific intermittent exercise in a day result in increased physiological load and higher fat availability than when one bout of training is performed on the same day. These metabolic responses seem to be associated with exercise-induced changes in hormones (e.g., increase in epinephrine and decrease in insulin). These findings do not identify the major effect of different training organisations on intracellular adaptive responses of *PGC-1α mRNA* and signaling protein kinases but such differences in training organisations can be enough to affect physiological and metabolic responses.

7.3 Synthesis of Findings

This thesis has covered an extensive range of areas, attempting to investigate the area of training adaptation from the field to the cell. The observations from the field study provided insights on the real training of professional football players during pre-season and in-season. Based on the findings of the field study, the seasonal variations of physiological loading demonstrate that soccer training is specifically periodised in accordance with the macro cycle of the annual plan. For example, this study clearly illustrates that the programmes of pre-season training are organised to provide a greater level of physiological stress than similar types of training completed in the in-season. Such increased physiological demands might be expected to be caused by the focus on physical preparation work in this phase of training. Novel data from this study suggests that the higher intensities arise from the technical/tactical specific training sessions during the pre-season period rather than any physiological differences between physical training sessions at different times of the year. The available data can also be analysed in relation to the organisation of micro-cycles. In terms of a specific micro-cycle the results of this study illustrate that the training

programmes on field are organised with a specific pattern during the week. That is, training in pre-season was typically programmed by multiple sessions in a day, whilst a single session was usually completed in a day during in-season. Such patterns of training organisation will theoretically peak and taper physiological loadings across days, thereby optimizing adaptations to soccer-specific training on the body. These differences in the patterns of physiological loadings and organisation in professional soccer training are compatible with the theoretical concepts of training purposes: pre-season training usually focuses on the rebuilding of fitness levels in players and in-season training frequently aims to maintain the specific capacities developed during pre-season (Bangsbo, 1994b).

Experimental investigations and/or the depth of understanding associated with laboratory-based analytical procedures are based on the application of controlled laboratory conditions to exercise (Drust et al., 2000). A valid laboratory-based simulation could provide useful information to accurately describe the physiological requirements of the sport. A laboratory-based ergonomic model of soccer training was developed in this thesis. This laboratory-based soccer-specific simulation was devised according to observations from two chapters in the current thesis. The validity of the simulation was evaluated by a statistical analysis that compared the physiological loads associated with the protocol to those observed during the actual training of players. Such systematic processes, with multiple steps, supported the efficacy of the protocol as a suitable experimental model to apply to the investigation of the physiological responses and the intracellular adaptations of skeletal muscle to soccer-specific intermittent exercise **(Figure7.1)**. This protocol is also a novel simulation that attempts to replicate the physiological demands and activity patterns of soccer training of professional players. This is unlike other protocols that replicate the exercise patterns observed during match-play using both motorised (Drust et al., 2000; Gregson, 2002) and non-motorised treadmills (Clarke et al., 2005). These protocols included less frequency of activities and longer durations of specific movements due to technological issues of the treadmills used at the time. Similarly, the simulation in the present thesis also omitted technical elements such as headers, kicks, passes and shots etc. and some specific utility movements including change-direction, side-way and backward running. Those explosive activities omitted in the present simulation could greatly induce energy perturbations and/or mechanical stimuli on cells in skeletal muscle, compared to those provoked in straightly

forward-running activities. This limitation of the simulation may influence the findings that the specific signaling pathways such as *AMPK* and *p38MAPK* were not activated. This therefore implicates that data sets on the molecular studies from the current thesis would be carefully translated into practical applications.

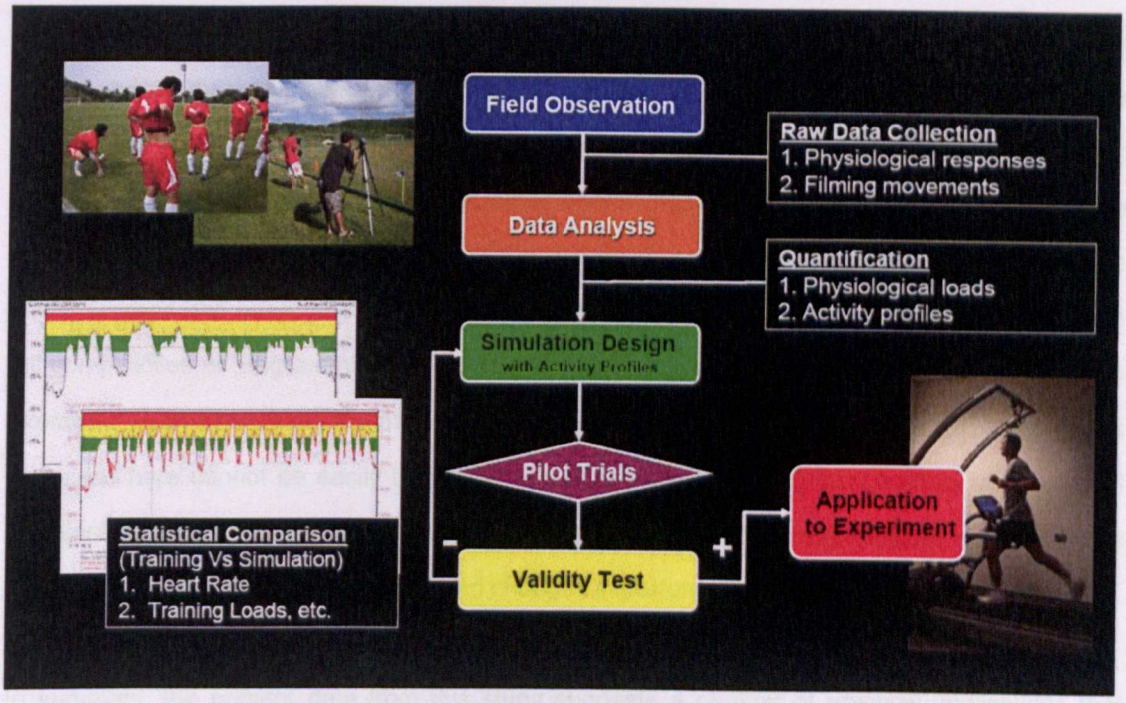


Figure 7.1 The general scheme for the development of a simulation.

Differences in training organisation between in-season and pre-season periods could have implications for the physiological and metabolic responses to the training sessions. The experimental approaches in the current study simulated patterns of soccer-specific training organisation. That is, the study design generally represented pre-season and in-season soccer-specific training; training twice a day represented the pre-season period while training once a day represented in-season training. Data from the present study indicates that pre-season training organised into two sessions per day probably stimulates more intense physiological strain and greater metabolic stress than the in-season soccer-specific training. One interesting finding of the

present thesis is that we could not detect a major effect of specific-training organisation on the intracellular adaptive responses of signaling pathways in human skeletal muscle despite significant differences in the physiological and metabolic responses. This may indicate that training organisation may not be a major determinant of the extent of adaptations associated with mitochondrial biogenesis in human skeletal muscle. Considering that a similar magnitude of adaptations associated with mitochondrial biogenesis has been induced by a single bout of soccer-specific intermittent exercise in the present thesis, two consecutive bouts of the same exercise in a day may not be beneficial to training adaptation. This may suggest that all single training bouts that are sufficiently intense to disturb the physiological homeostasis could be regarded as enough of a stimulus to provoke and initiate training adaptations (Hassmén, 1998). It is, however, not clear what the effect of other approaches to training organisation (e.g. training sessions combined with two different types of exercise) on the same signaling pathways is. This would suggest that other studies should be carried out regarding training organisation before the full complexities of programming for the soccer player can be optimised. Caution should also be exercised when interpreting the data from this thesis as the acute changes in signaling pathways examined here cannot be easily translated into observations on long-term training effects. It still therefore remains important to examine the chronic effect of such training programmes on chronic adaptations in human skeletal muscle and changes in performance in soccer players.

In summary, the present data from this study suggests a number of practical implications that could be applied to soccer-specific training. *Firstly*, soccer-specific training that enables players to "train as you play" should be emphasised during the pre-season period. For example, technical/tactical sessions including practice matches could induce high physiological loads on the body, thereby leading to improvements in the physiological conditioning of soccer players. *Secondly*, soccer-specific sessions completed twice a day would be beneficial to players as they have the potential to induce greater physiological and metabolic responses than single sessions in a given day. They may also change the substrate provision used to support the exercise, e.g. increase the utilisation of fat as opposed to carbohydrate. *Thirdly*, a daily single soccer-specific training session would seem to be enough of a training stimulus to initiate and provoke training adaptations associated with endurance performance especially if the bout of exercise is intense enough to disturb physiological homeostasis. This would suggest that the quality of training is

more crucial in the induction of training adaptations than training organisation.

7.4 Recommendations for Future Research

There are several potential areas of future research which have become apparent as a consequence of critically analyzing the experimental chapters in this thesis. These are outlined below.

7.4.1 Recommendation 1

Attempt to evaluate and coordinate a combination of data that includes both physiological loads and specific patterns of movements and sports-specific actions.

The physiological responses that are provoked from each activity are more likely to be a consequence of the specific pattern or combination of those different activities within a session. The total or average amount of physiological stress may not represent the physiological responses to the specific pattern of intermittent exercise completed during a soccer training session. The updated tracking technologies such as global position system (GPS) and local position system (LPS) could allow collecting mass data during longer periods of a competitive season, thereby leading to evaluating data combined physiological loads and specific patterns of movements and sports-specific actions. Such a combination of data would provide data on optimizing the intensity and organisation of soccer training programmes.

7.4.2 Recommendation 2

Investigation to elucidate responses of signaling pathways associated with protein kinases to an actual soccer-specific training session.

Signaling pathways associated with protein kinases could play potential roles as upstream activators to stimulate *PGC-1α* expression. The present findings in study 3 could not demonstrate that the upregulation of *PGC-1α mRNA* was linked to an activation of protein kinases such as

AMPK and *p38 MAPK*. The laboratory-based exercise protocol employed in the study had some of technological limitations that could cause differences from a real training session in energetic perturbation and mechanical strain on skeletal muscle. For example, the protocol did not incorporate all of the specific types of movements such as jumps, headers, kicks, sideway running and backward running etc. The number of activity change in the current simulation was also less than those observed in the real training session of professional players. A future study designed with the actual soccer training therefore will elucidate the relationship between protein kinases and *PGC-1α mRNA* activation in human skeletal muscles. This approach may also allow explaining other potential signaling pathways such as intracellular Ca^{2+} flux and CaMKs, thereby leading to clarifying the adaptive mechanism of soccer-specific intermittent exercise.

7.4.3 Recommendation 3

Need to identify the chronic effect of different soccer-specific training organisations on signaling responses associated with mitochondrial biogenesis.

The present findings in study 4 could not detect the significant differences in expression of *PGC-1α mRNA* between two training organisations (training once Vs training twice a day) following acute bout of soccer-specific intermittent exercise. Nevertheless, the transient signaling responses to acute bout of exercise would probably be converted to more permanent changes in the structure and function of organelles when the stress induced by exercise is chronically repeated by training. The recent studies demonstrated that soccer-specific training organisation with repeated sessions in a day induced greater mitochondrial enzyme activities including CS, β-HAD and SDH than those observed in a single session in a day. However, no research has been attempted to elucidate the chronic effect of different soccer-specific training organisations on molecular responses associated with mitochondrial biogenesis. Such investigation will provide useful information to practitioners in order to optimise training programmes in soccer context.

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APPENDICES

APPENDIX 1

Consent form and participant information sheet for the study1 & 2

LIVERPOOL JOHN MOORES UNIVERSITY

CONSENT FORM 연구 참가 동의서

(FORM OF CONSENT TO TAKE PART AS A SUBJECT IN A MAJOR PROCEDURE OR RESEARCH PROJECT)

연구 제목 (Title of project/procedure):
프로 축구선수들의 훈련 부하와 훈련 중 일률 분석
Analysis of Training Loads and Work-rates of Professional Soccer Players

I, ...(연구 대상자 이름)*는 상기 연구 참가에 동의하며, 이에 대하여 충분한 설명을 서면으로 받았음을 확인함. **(Subject's full name) agree to take part in the above named project/procedure, the details of which have been fully explained to me and described in writing.**

서명(Signed) ..... 날짜 (Date).....
연구 대상자 (Subject)

I,.....(연구자 이름)*는 상기 연구에 대하여 충분히 설명하였고, 연구 대상자가 이해하도록 충분한 설명을 서면으로 하였음을 확인함. **(Investigator's full name)* certify that the details of this project/procedure have been fully explained and described in writing to the subject named above and have been understood by him/her.**

서명 (Signed)..... 날짜 (Date).....
연구자 (Investigator)

I, ...(증인 이름)*는 연구 대상자가 상기 연구에 대하여 충분한 설명을 서면으로 받았음을 확인함. **(Witness' full name) certify that the details of this project/procedure have been fully explained and described in writing to the subject named above and have been understood by him/her.**

서명 (Signed)..... 날짜 (Date).....
증인 (Witness)

NB The witness must be an independent third party.

Participant Information Sheet

Name of Researcher: Tae-Seok Jeong

Address of Academic Location:

[redacted]

Supervisors: Dr. Barry Drust and Professor Tom Reilly

Title of study/project: Analysis of Training Loads and Work-rates of Professional Soccer Players

Purpose of study: The aim of this study is to analyse the training loads (e.g. intensity, frequency, duration etc) and work-rates (e.g. sprinting, cruising, jogging, walking, standing etc) of professional soccer players during the pre-seasonal and in-seasonal training session.

Throughout this study the organization of training programmes are assessed according to fitness components such as aerobic, anaerobic, strengthening and technical/tactical training. Also, the training loads and work-rates of the English and Korean players are compared.

Procedures and Participants Role: You will be supported with a full explanation regarding this project during the induction session before signing on the consent form. This study will base observations on your routine activities of training for 1 week. This observation will be completed at two different times of the year to evaluate training loads and work-rates associated with both the pre-season and the competitive-season.

Measurements of heart rate (HR) and rating of perceived exertion (RPE) will be recorded during training sessions. To assess your maximal HR, we will perform Yo-Yo intermittent recovery test. You will be required to participate in the test or training wearing a chest belt for recording HR. To determine the exertion of each session, you will be asked as if how hard your training is 30 minutes after each training session. If we choose you as participant to evaluate work-rate profiles, you will be filmed using video camera. Before recording your training activities, you will be required to sprint, cruise, jog and walk 10 meters in order to calibrate the stride length at each category of activity. This calibration will be applied for calculation of your covered distance during training session. All of the recorded films are only used for this study and will be destroyed at the completion of the research.

All of data from you will be kept confidential and you will not be identified by name in any report of the research.

If you have any further questions, concerns or need for any clarity on the issues mentioned above, please ask the researcher.

Please note that you have the right to withdraw from this study at any time if you wish.

Thank you for your participation!

연구 참가자들을 위한 안내

연구자 (Name of Researcher): 정태석 (Tae-Seok Jeong)

소속 연구기관 주소 (Address of Academic Location):

[redacted]

연구 지도교수 (Supervisor): Dr. Barry Drust and Professor Tom Reilly

연구 제목 (Title of study): 프로 축구선수들의 훈련 부하와 훈련 중 일률 측정

연구 목적 (Purpose of study): 이 연구는 프로 축구 선수들이 프리시즌 및 시즌 동안 시행하는 훈련과정 중 훈련 부하(즉, 훈련 강도, 빈도, 기간 등)와 일률(즉, 스프린팅, 크루징, 조깅, 워킹, 스탠딩 등의 비율)을 분석을 목적으로 계획되었습니다. 이 연구를 통해 체력훈련 요소들(유산소 훈련, 무산소 훈련 근육훈련, 기술/전술 훈련 등)에 따라 훈련프로그램의 구성에 관한 분석이 이루어질 것이고, 잉글랜드와 한국 프로 축구선수들의 훈련 부하와 일률의 비교가 이루어질 것입니다.

연구 과정과 참여자들의 역할 (Procedures and Participants Role): 이 연구는 일주일 동안 평소 훈련을 관측하는 방식으로 진행되며, 프리시즌과 시즌 중에 각각 1회씩 시행될 것입니다. 이를 통해 훈련 부하와 일률을 평가를 위한 데이터가 모아지게 됩니다.

훈련 부하를 측정하기 위해 훈련기간 중 선수들의 심박수와 운동자각도를 기록할 것입니다. 선수들은 심박수 기록을 위해서 가슴 벨트를 차고 훈련에 참가하게 되는데, 이에 앞서 모든 선수들은 최대심박수의 평가를 위해' 요요 테스트' 가 시행됩니다. 또한 선수들은 운동자각도의 평가를 위해 한 세션이 끝날 때마다 그 훈련의 힘든 정도를 주어진 표에 따라 해당 점수를 표현해 주셔야 합니다.

일률 분석을 위한 대상자로 선정된 선수들 경우, 훈련 전 과정이 카메라로 촬영된 후 비디오 분석을 통해 평가가 이루어지게 될 것입니다. 카메라로 훈련 장면을 촬영하기에 앞서 간단한 ' 10m 테스트' 를 통해 스프린팅, 크루징, 조깅 및 워킹 동작 과정에서 보여주는 선수 개개인의 보폭을 측정하게 됩니다. 물론, 카메라로 촬영된 모든 내용은 연구 목적으로만 사용될 것이고, 연구가 완료되었을 때 폐기될 것입니다.

참가하는 모든 선수들은 연구자를 통해 연구 내용 및 과정에 관한 충분한 설명을 들은 후 참가 동의서에 서명하게 될 것입니다. 또한, 위에서 기술한 연구과정을 통해 얻어진 자료는 비밀이 보장되며, 참가자들의 개인 신상정보는 연구 보고서 어디에도 드러나지 않을 것입니다. 아울러, 연구에 관한 궁금한 사항이 있다면, 언제든지 연구자에게 문의해 주십시오.

모든 참가 선수들은 더 이상 연구에 참여하기를 원하지 않을 경우, 언제든지 동의한 내용을 철회할 수 있는 권리가 있음을 알려드립니다.

여러분의 적극적인 참여로 얻어진 고귀한 자료는 한국 축구를 더욱 발전시키는 훌륭한 밑거름이 될 것입니다. 감사합니다.

APPENDIX 2

Consent form and participant information sheets for the study 3 & 4

LIVERPOOL JOHN MOORES UNIVERSITY

CONSENT FORM

Project Title:

The Influence of Prior Soccer-Specific Training on the Physiological and Metabolic Response to the Soccer-Specific Intermittent Exercise

Researcher: Tae-Seok Jeong

Supervisory Team: Dr Barry Drust (Director of the project), Dr. James Morton, Dr Iain Campbell (Consultant for muscle biopsy)

School and Faculty: Research Institute for Sport & Exercise Sciences, Faculty of Science

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐
3. I understand that any personal information collected during the study will be anonymised and remain confidential ☐
4. I agree that any tissue samples collected in this study could be used for other additional analysis within a 6 month period. ☐
5. I agree that any photos taken during the investigations could be used in presentations at national and/or international conferences and/or in journal publications. ☐
6. I agree to take part in the above study ☐

Name of Participant	Date	Signature
Name of Researcher	Date	Signature
Name of Person taking consent	Date	Signature

Liverpool John Moores University

Muscle Biopsy Information Sheet

The muscle biopsy technique is a commonly employed technique within the exercise sciences. It is usually employed in studies that are examining the structure, metabolic and cellular state of a muscle in response to acute and chronic bouts of exercise. The following information is designed to inform all individuals interested in participating in a muscle biopsy study of the specific procedures and their associated risks and discomfort. Muscle biopsies are always carried out by fully qualified and experienced medical doctors.

The procedure of a muscle biopsy and possible associated discomfort

The muscle biopsy involves the removal of a small piece of muscle tissue from one of the muscles in your leg using a sterile hollow needle. The area over the outside of your lower thigh muscle (vastus lateralis muscle) and calf (gastrconemius muscle) will be carefully cleaned. A small amount of local freezing (anesthetic) will be injected into and under the skin. You will likely experience a burning sensation while the freezing is injected. Then a small, 4 – 5 mm incision will be made in your skin in order to create an opening for the biopsy needle. There is often a small amount of bleeding from the incision, but this is usually minimal. The biopsy needle will then be inserted through the incision into the thigh muscle and a small piece of muscle (20 – 50 mg) will be quickly removed and the needle taken out. During the time that the sample is taken (about 5 seconds), you may feel the sensation of deep pressure in the muscle. On some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise and daily activities. There may be some minimal bleeding when the needle is removed which may require application of pressure for a few minutes. Following the biopsy, the incision will be closed with sterile tape (steri-strips), and wrapped with a tensor bandage. Once the freezing wears off, your leg may feel tight and often there is the sensation of a deep bruise.

What to do following a muscle biopsy

After the procedure, you may feel some mild discomfort and possibly see some bruising. This often feels like the sensation of a 'dead leg' and your leg may feel discomfort when walking down the stairs etc. This is perfectly normal and should not cause you any undue concern. The tightness in the muscle usually disappears within 2 days. Seven to ten days after the biopsy you will be asked to visit the doctor who performed the biopsy at the **Research Institute for Sport and Exercise Sciences** for a formal assessment of how the biopsy site is healing.

Potential risks associated with muscle biopsies

The local freezing will likely result in a burning feeling in the muscle at the time of the injection. This will last only 5 – 10 seconds. There is an extremely low risk of allergic reaction to the local injection (1 in 1 million).The chance of a local skin infection in less than 1 in 1000. Carefully cleaning the skin and keeping the area clean until the skin heals will minimize this. Most subjects experience local soreness and stiffness in the leg for two or three days after the biopsy similar to a deep bruise. There is a very low risk of internal bleeding at the biopsy site which can result in more prolonged pain and stiffness in the leg. On occasions, a small lump of scar tissue may form under the site of the incision, but this normally disappears within 2-3 months, or within a few weeks if massaged. A small visible scar often remains from the biopsy incision. There is the possibility of a small area of numbness (about the size of a two pence) around the biopsy site. This usually resolves over 5 – 6 months. There is a very low risk (estimated at less than 1/5000) of damage to a small nerve branch to the muscle. This would result in partial weakness of the muscle and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

Problems or concerns

Infection can be serious and if you therefore experience a lot of bleeding from the biopsy site, swelling or infection around the biopsy site, faintness, light headedness, heart pain, chest pain or increasing pain in your leg which is not relieved by Paracetamol, you must contact the doctor who did the biopsy *right away*. However, if for some reason, you are not able to contact this physician then you should contact your family doctor or go to the Accident and Emergency Department.

MUSCLE BIOPSY SUBJECT SCREENING FORM

To help us ensure your safety and well-being please answer the following questions.

1. Have you ever had a negative or allergic reaction to local freezing (e.g. during dental procedures)?

   No ☐ Yes ☐

2. Do you have any tendency toward easy bleeding or bruising (e.g with minor cuts or shaving)?

   No ☐ Yes ☐

3. Are you currently taking any medications that may increase the chance of bleeding or bruising (e.g. Aspirin, Anti-inflammatories)?

   No ☐ Yes ☐

4. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?

   No ☐ Yes ☐

Subject Name (print) :_____

Subject Signature :_____

Date :_____

Signature of Person Conducting Assessment: _____

PARTICIPANT INFORMATION SHEET

Researcher: Tae-Seok Jeong

Supervisory Team:
Dr Barry Drust (Director of project), Prof. Tom Reilly and Dr. James Morton

Title of Project: The Influence of Prior Soccer-Specific Training on the Physiological and Metabolic Response to the Soccer-Specific Intermittent Exercise

General Guidance

You are being invited to take part in this study. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part in or not.

1. What is the purpose of the study?

The aim of the study is to investigate the influence of prior soccer-specific training on the physiological responses and the molecular adaptations of skeletal muscle to soccer-specific intermittent exercise.

2. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do you will be given this information sheet and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw will not affect your rights/any future treatment/service you receive.

3. What will happen to me if I take part?

This project will consist of 3 parts. You will be required to come to the laboratory on 5 occasions.

- **Part 1: Familiarization and Maximal Exercise Capacity Test (VO2max Test) -- 1 visit**

During this visit, the completion of a physical activity readiness questionnaire (PARQ) and a muscle biopsy screening form will determine your suitability as subject for the investigation. This will reduce the possibilities of any risks or discomforts that you may experience as a result of the procedures involved in this investigation. General procedures for muscle biopsy (**See the muscle biopsy information sheet for**

details), blood collection and insertion of rectal probe will be demonstrated by verbal explain. You will be required to perform a short session of running (10min) on the treadmill for familiarization with the exercise protocol as well.

You will be required to complete a test to determine your maximal exercise capacity. This test will carry out by experienced personnel in our laboratory on a daily basis and represent little more discomfort to you than is experienced during your normal training. You will be required to perform incremental treadmill exercise until volitional exhaustion. This test begins at 10 km· h^{-1} and increases by 2 km· h^{-1} every 2 min thereafter to 16 km· h^{-1}. If you successfully complete the 16 km· h^{-1} stage, the treadmill will then incline by 2% every 2 min until you can run no longer. The duration of the test should be between 10 and 15 min. The air that you breathe out during exercise will be collected via a small face mask worn throughout the test. The gas will be measured continuously using an online gas analysis system connected to the mask. Your heart rate will also be measured via a loose fitting band strapped around your chest. The total time for this part will be around 60 min.

- **Part 2: One bout of Soccer-Specific exercise --2 visits**

Totally this trial will take two consecutive days. During the 1st day, you will be required to stay for 5 hr at the lab. Then, you will stay for an hour during the subsequent day.

Prior to arriving at the laboratory you will have refrained from exercise, alcohol, and caffeine for a 24-hour period. On each visit you will be required to wear your normal training clothing with short pants.

On arrival, you will self-insert the rectal probe in a private changing room. After returning at lab, we will insert a needle into your arm to collect blood samples. A muscle biopsy will be also taken from your thigh and calf muscle for sampling muscle tissue pre-exercise. Additional blood samples will be obtained from an indwelling catheter of the forearm every 15 min during exercise as well. Immediately after completing a bout of exercise, blood samples will be collected again.

Just before exercise, you will be required to wear a face mask to measure expired gas during exercise. Following completion of a given warm up protocol, you will be required to complete a single bout of soccer-specific intermittent exercise on the treadmill during 60 min. During exercise your heart rate (HR), oxygen uptake and rate yours perceived exertion (RPE) will be obtained: Your HR will be measured every 5 s using short-range telemetry systems via a loose fitting chest belt. Your oxygen uptake will also be assessed using an on-line gas analysis system connected to the mask. Your feelings of how hard you are exercising will be measured by asking you to every 15 min.

At 3 h after exercise, additional muscle biopsies and blood sampling will be taken. Then, indwelling catheter will be removed from your forearm. At 24 h after exercise, you will be required to re-visit the

laboratory for the last muscle biopsy and blood sampling.

- **Part 3: Two bouts of soccer-specific exercise (2 visits)**

Totally two consecutive days will be required for this trial. You will get involved for 9 hours on the 1st visit and for 1 h on the 2nd visit in the laboratory. You will be required to visit the laboratory a week later after Part 2.

Prior to arriving at the laboratory you will have refrained from exercise, alcohol, and caffeine for a 24-hour period. On each visit you will be required to wear your normal training clothing with short pants.

On arrival, you will be required to prepare the experiment such as rectal probe self-insertion in a private changing room, indwelling catheter insertion in your forearm for blood collection in a same way as Part 2. Blood sampling and a muscle biopsy for sampling muscle tissue will be taken pre-exercise.

Just before exercise, you will be required to wear a face mask to measure expired gas during exercise. After completing given warm-up protocol, you will be required to perform two repeated bouts of soccer-specific intermittent exercise on the treadmill separated by 3 h rest. During both bouts of exercise, your HR, RPE, oxygen consumption and rectal temperature will be measured in a same manner as Part 2.

Blood samples will be obtained at 15-min intervals during exercise. Additional samples will be taken immediately after completing each bout of exercise, at 30-min interval during the time between each bout and at 3 h and 24 h after completing the 2nd bout of exercise. Additional muscle biopsies will be taken at 3 h after the 1st bout of exercise, at 3 h and 24 h after the 2nd bout of exercise. Completing blood sampling at 3h after the 2nd bout of exercise, indwelling catheter will be removed from your forearm. At 24 h after the 2nd bout of exercise, you will be required to re-visit the laboratory for the last muscle biopsy and blood sampling.

4. What are you going to do with my muscle tissue and blood samples obtained?

We will use your muscle tissue to analyze the protein and mRNA concentrations of different signalling proteins in your muscle and muscle glycogen. Blood samples will be used for analysis of acute metabolic and hormonal responses during and after exercise. This information will provide us with an understanding of the effects that the training programmes are having on your body.

5. Are there any risks / benefits involved?

- **Risks**

You may have an extremely low risk of allergic reaction to the local injection of anaesthetic (1 in 1 million). This risk will be minimized by the completion of the muscle biopsy screening form. There is also often a small amount of bleeding from the incision site to create an opening for the biopsy needle. It can be reduced by closing the incision site with sterile tape (steri-strips) and wrapping it with a surgical bandage. Following the biopsy, the incision will be risky to infection but closing the incision site with sterile tape, carefully cleansing the skin and keeping the area clean and dry until the skin heals will minimize this risk.

Even though it will be extremely rare, inserting the cannula may cause fainting. An infection to the puncture site can also occur following the prolonged use of an indwelling catheter for repeated blood collection. The use of surgical gloves and sterilisation of puncture site with medical swab will reduce a risk of infection.

There can be a potential risk with subjects losing balance and failing of the treadmill. This can be minimised by the familiarisation sessions. You can decide to stop the exercise trial at any time when they feel intolerable, injured or unwell.

- **Benefits**

Subjects will receive information on their current level of aerobic fitness and their physiological and molecular responses to soccer-specific exercise.

6. Will my taking part in the study be kept confidential?

In order to keep confidentiality, all data from participants will be encoded. Only the main investigator will have access to the coding system which will be protected by password. All human tissue will be stored in line with the Human Tissue Act 2004 regulations.

NB. You must freely volunteer to be a subject and are able to withdraw, without prejudice, at any time. Thank you for your time and interest in the study.

PARTICIPANT INFORMATION SHEET

Researcher: Tae-Seok Jeong

Supervisory Team:
Dr Barry Drust (Director of project), Dr. James Morton

Title of Project: The Influence of Prior Soccer-Specific Training on the Physiological and Metabolic Response to the Soccer-Specific Intermittent Exercise

General Guidance

You are being invited to take part in this study. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part in or not.

7. What is the purpose of the study?

The aim of the study is to investigate the influence of prior soccer-specific training on the physiological responses and the molecular adaptations of skeletal muscle to soccer-specific intermittent exercise.

8. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do you will be given this information sheet and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw will not affect your rights/any future treatment/service you receive.

9. What will happen to me if I take part?

This project will consist of 3 parts. You will be required to come to the laboratory on 4 occasions.

- **Part 1: Familiarization (1 visit)**

During this visit, the completion of a physical activity readiness questionnaire (PARQ) and a muscle biopsy screening form will determine your suitability as subject for the investigation. This will reduce the possibilities of any risks or discomforts that you may experience as a result of the procedures involved in

this investigation. General procedures for muscle biopsy (**See the muscle biopsy information sheet for details**), blood collection and insertion of rectal probe will be demonstrated by verbal explaination. You will be required to perform a short session of running (10min) on the treadmill for familiarization with the exercise protocol as well. At the end of this occasion, you will be randomly allocated to the first trial between Trial I and II for part 2 and 3.

You will be required to complete a test to determine your maximal exercise capacity. This test is carried out by experienced personnel in our laboratory on a daily basis and represents little more discomfort to you than is experienced during your normal training. You will be required to perform incremental treadmill exercise until volitional exhaustion. This test begins at 10 km· h^{-1} and increases by 2 km· h^{-1} every 2 min thereafter to 16 km· h^{-1}. If you successfully complete the 16 km· h^{-1} stage, the treadmill will then incline by 2% every 2 min until you can run no longer. The duration of the test should be between 10 and 15 min. The air that you breathe out during exercise will be collected via a small face mask worn throughout the test. The gas will be measured continuously using an online gas analysis system connected to the mask. Your heart rate will also be measured via a loose fitting band strapped around your chest. The total time for this part will be around 60 min.

- **Part 2 (Trial I) : One bout of soccer-specific exercise in a day across the 2 consecutive days (2 visits)**

When you participate in ***Trial I***, you will be required two bouts of exercise in 2 consecutive days. Totally this trial will take two consecutive days. On the first day, you will stay for 2 hr at the lab. On following day, you will be required to stay 5 hr at the lab.

Prior to arriving at the laboratory you will have refrained from exercise, alcohol, and caffeine for a 24-hour period. On each visit you will be required to wear your normal training clothing with short shorts.

On arrival on the first day, you will complete a given warm up protocol for 5 min. You will be required to perform a single bout of soccer-specific intermittent exercise on the treadmill lasting 61 min. During exercise your heart rate (HR) and ratings of perceived exertion (RPE) will be obtained: Your HR will be measured every 5 s using short-range telemetry systems via a loose fitting chest belt. Your feelings of how hard you are exercising will be measured by asking you every 20 min.

After completing the test of the first day, you will be provided a carbohydrate-rich drink and a dietary plan for lunch and dinner. You will also have refrained from exercise, alcohol and caffeine by the next visit.

On arrival on the following day, blood and muscle tissue will be taken before exercise. After completing given warm-up protocol, you will be required to perform one bout of soccer-specific intermittent exercise on the treadmill. Blood samples will be obtained immediately after completing the bout of exercise. Additional muscle biopsies will also be taken immediately after and 3 h after completing the bout of exercise. During exercise, your HR and RPE will be measured.

- **Part 3 (Trial II): Two bouts of soccer-specific exercise in a day (1 visit)**

If you are participating in ***Trial II***, you will be required to complete two bouts of exercise in a day separated by 3 hr-rest. Totally an entire day will be required for this trial. You will get involved for 9 hours on the day in the laboratory.

Prior to arriving at the laboratory you will have refrained from exercise, alcohol, and caffeine for a 24-hour period. On each visit you will be required to wear your normal training clothing with short shorts.

On arrival, you will complete a given warm up protocol for 5 min. You will be required to perform the first single bout of soccer-specific intermittent exercise on the treadmill lasting 61 min. During exercise your heart rate (HR) and ratings of perceived exertion (RPE) will be obtained: Your HR will be measured every 5 s using short-range telemetry systems via a loose fitting chest belt. Your feelings of how hard you are exercising will be measured by asking you to every 20 min.

After completing the first bout of exercise, passively you will be required to ***take a rest for 2 hr***. Plain water will also be provided during the rest.

When you come back to the laboratory, blood sampling and a muscle biopsy will be taken just before the second bout of exercise. Additional samples will be taken immediately after the bout of exercise. Additional muscle biopsies will also be taken immediately after and at 3 h after the second bout of exercise. During the second bout of exercise, your HR and RPE will be measured.

10. What are you going to do with my muscle tissue and blood samples obtained?

We will use your muscle tissue to analyze the protein and mRNA concentrations of different signalling proteins in your muscle and muscle glycogen. Blood samples will be used for analysis of acute metabolic responses after exercise. This information will provide us with an understanding of the effects that the training programmes are having on your body.

11. Are there any risks / benefits involved?

- **Risks**

You may have an extremely low risk of allergic reaction to the local injection of anaesthetic (1 in 1 million). This risk will be minimized by the completion of the muscle biopsy screening form. There is also often a small amount of bleeding from the incision site to create an opening for the biopsy needle. It can be reduced by closing the incision site with sterile tape (steri-strips) and wrapping it with a surgical bandage. Following the biopsy, intra- or inter-muscular bleeding may occur as a *rare* complication. This complication can cause swelling and bruise with soreness around the biopsy site but these symptoms will be recovered without any sequelae within 2~3 weeks. Its risk can be minimized by manual compression or elastic bandage with ice application on biopsy site. The incision will be risky to infection but closing the incision site with sterile tape, carefully cleansing the skin and keeping the area clean and dry until the skin heals will reduce this risk.

Even though it will be extremely rare, inserting the needle may cause fainting. An inflammation (e.g., phlebobitis) to the puncture site can also occur following the use of a needle for blood collection. The use of surgical gloves and sterilisation of puncture site with medical swab will reduce a risk of infection.

There can be a potential risk with subjects losing balance and falling of the treadmill. This can be minimised by the familiarisation sessions. You can decide to stop the exercise trial at any time when they feel intolerable, injured or unwell.

- **Benefits**

You will receive information on their current level of aerobic fitness and their physiological and molecular responses to soccer-specific exercise.

12. Will my taking part in the study be kept confidential?

In order to keep confidentiality, all data from participants will be encoded. Only the main investigator will have access to the coding system which will be protected by password. All human tissue will be stored in line with the Human Tissue Act 2004 regulations.

NB. You must freely volunteer to be a subject and are able to withdraw, without prejudice, at any time. Thank you for your time and interest in the study.

APPENDIX 3

A modified 10-point rating Borg scale (English and Korean version)

0 Rest

1 Very, very easy

2 Easy

3 Moderate

4 Somewhat hard

5 Hard

6

7 Very hard

8

9

10 Maximal

0 휴식 상태

1 아주, 아주 편함

2 편함

3 보통

4 약간 힘듦

5 힘듦

6

7 매우 힘듦

8

9

10 최고로 힘듦

APPENDIX 4

Mathematics for *Kappa (k)*

APPENDIX 5

Publication arising from work presented in the thesis