ผลของการเสริมกลูตามีนต่อจำนวนเม็ดเลือดขาวและความเมื่อยล้าหลังแบบจำลองการแข่งขันฟุตบอลในนักกีฬาฟุตบอลเยาวชนไทย

นางสาว ณัฐกฤตา อิ่มกระจ่าง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเวชศาสตร์การกีฬา
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

EFFECTS OF GLUTAMINE SUPPLEMENTATION ON LEUCOCYTES NUMBER AND FATIGUE AFTER SIMULATED SOCCER MATCHES IN THAI YOUTH SOCCER PLAYERS

Miss Natkrita Imkrajang

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Sports Medicine

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Thesis Title	Effects of glutamine supplementation on leucocytes number and fatigue after simulated soccer matches in Thai youth soccer players
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Field of Study	Sports Medicine
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ณัฐกฤตา อิ่มกระจ่าง : ผลของการเสริมกลูตามีนต่อจำนวนเม็ดเลือดขาวและความเมื่อยล้าหลังแบบจำลองการแข่งขันฟุตบอลในนักกีฬาฟุตบอลเยาวชนไทย. (EFFECTS OF GLUTAMINE SUPPLEMENTATION ON LEUCOCYTES NUMBER AND FATIGUE AFTER SIMULATED SOCCER MATCHES IN THAI YOUTH SOCCER PLAYERS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร. วิไล อโนมะศิริ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ.ดร. อัชฌาสัย ศิริตันติกร, 66 หน้า.

การศึกษาวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมกลูตามีนต่อจำนวนเม็ดเลือดขาวและความเมื่อยล้าหลังแบบจำลองการแข่งขันฟุตบอลในนักกีฬาฟุตบอลเยาวชนชายไทย อายุระหว่าง 15 – 17 ปี จำนวน 12 คน สุ่มให้ผู้เข้าร่วมงานวิจัยได้รับเครื่องดื่มผสมกลูตามีนหรือเครื่องดื่มหลอกต่อเนื่องกัน 5 วัน เว้นระยะเวลาห่างกัน 2 สัปดาห์จึงได้รับเครื่องดื่มอีกชนิดหนึ่ง โดยในวันที่ 5 ได้รับเครื่องดื่มก่อนการทดสอบ Ball-sports Endurance and Sprint Test ซึ่งเป็นการจำลองแบบการแข่งขันฟุตบอล ทำการเจาะเลือดและวิเคราะห์ Complete blood count ซึ่งประกอบด้วยเม็ดเลือดขาว นิวโทรฟิลส์ ลิมโฟไซต์ โมโนไซต์ อีโอสิโนฟิลส์ และเบโซฟิลส์ ฮีมาโตคริต ปริมาตรของเม็ดเลือดแดงแต่ละเซลล์ ความเข้มข้นของแอมโมเนีย กรดยูริก ยูเรีย และแลคเตท ก่อนการทดสอบ หลังการทดสอบทันที และหลังการทดสอบ 1 ชั่วโมง

ผลการศึกษาพบว่า เมื่อได้รับเครื่องดื่มทั้งสองชนิด มีการเพิ่มขึ้นของจำนวนเม็ดเลือดขาว นิวโทรฟิลส์ โมโนไซต์ ฮีมาโตคริต ความเข้มข้นของแอมโมเนีย กรดยูริก ยูเรีย และแลคเตท หลังการทดสอบทันที เมื่อเทียบกับก่อนการทดสอบอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 การเพิ่มขึ้นของจำนวนเม็ดเลือดขาว นิวโทรฟิลส์ และยูเรีย ยังคงอยู่ถึงช่วงพักภายหลังการทดสอบแล้ว 1 ชั่วโมง ในขณะที่ โมโนไซต์ ฮีมาโตคริต ความเข้มข้นของแอมโมเนีย และแลคเตทมีการลดลง ผลของการเสริมกลูตามีนในการศึกษานี้ทำให้เห็นแนวโน้มการเพิ่มขึ้นสูงกว่าเครื่องดื่มหลอกของจำนวนเม็ดเลือดขาว นิวโทรฟิลส์ และโมโนไซต์ของระบบภูมิคุ้มกัน ซึ่งเป็นเซลล์ที่เกี่ยวข้องกับการอักเสบจากการออกกำลังกาย นอกจากนี้ยังพบว่าการเสริมกลูตามีนมีการลดลงของปริมาณแลคเตทต่ำกว่าเครื่องดื่มหลอกและการเพิ่มขึ้นอย่างมีนัยสำคัญของยูเรียในกลุ่มที่ได้รับการเสริมกูลตามีน เป็นข้อสังเกตว่า มีการลดลงของความเมื่อยล้าและการเพิ่มการกำจัดแอมโมเนียหลังการทดสอบแบบจำลองการแข่งขันฟุตบอลทันที

สาขาวิชา เวชศาสตร์การกีฬา ลายมือชื่อนิสิต ณัฐกฤตา อิ่มกระจ่าง

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม

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KEYWORDS : GLUTAMINE SUPPLEMENTATION / LEUCOCYTES / FATIGUE / SIMULATED SOCCER MATCHES

NATKRITA IMKRAJANG : EFFECTS OF GLUTAMINE SUPPLEMENTATION ON LEUCOCYTES NUMBER AND FATIGUE AFTER SIMULATED SOCCER MATCHES IN THAI YOUTH SOCCER PLAYERS. THESIS ADVISOR : ASSOC.PROF. WILAI ANOMASIRI, Ph.D., THESIS CO-ADVISOR : ASSIST.PROF. ATCHASAI SIRITANTIKORN, Ph.D., 66 pp.

The purpose of this study was to investigate the effects of glutamine supplementation on leucocytes number and fatigue after simulated soccer matches in Thai youth soccer players aged 15 - 17 years old. Twelve participants were randomized to receive glutamine or placebo drink for 5 days with 2 weeks washout period. On the fifth day, subjects performed the simulation soccer matches (the Ball-sports Endurance and Sprint Test protocol) and blood specimens were collected for complete blood count: white blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils, haematocrit, mean corpuscular volume, ammonia, uric acid, urea and lactate at pre test, immediate post test and 1 h post test.

The results showed that subjects received both drinks had the increases of WBC, neutrophils, monocytes, haematocrit, ammonia, uric acid, urea and lactate immediately post test as compared to pre test ($p < 0.05$). Increases of WBC, neutrophils and urea were maintained after test for 1 hour whereas monocytes, haematocrit, ammonia and lactate were decreased. Some intriguing points were observed at the immediate post test. Although there was no significant different between glutamine supplementation and placebo, glutamine supplementation tended to increase higher than placebo in WBC, neutrophils and monocytes of the innate immune system which was responsible for inflammation process from exercise induced injury. In addition, there was lower blood lactate in glutamine supplementation and significant higher blood urea in glutamine supplementation group, suggesting less fatigue and faster ammonia clearance after simulated soccer matches.

Field of Study : Sports Medicine Student's Signature Natkrita Imkrajang

Academic Year : 2009 Advisor's Signature Wilai Anomasiri

Co-Advisor's Signature Atchasai Sirithantikorn

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I wish to thank the Office of Sports and Recreation Development, Bureau of Sports Science for allowing me in using Newtest Powertimer to analyze speed. I also wish to thank the Sports Authority of Thailand, Department of Sports Science for allowing me in using Swift Yardstick to analyze high of vertical jump in this study.

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LIST OF ABBREVATIONS

BEAST	Ball-sports Endurance And Sprint Test
bpm	Beat per minute
BW	Body weight
CBC	Complete Blood Count
CNS	Central nervous system
EDTA	Ethylene Diamine Tetraacetic Acid
Gln	Glutamine
GXT	Graded exercise test
Hct	Haematocrit
HR	Heart rate
HR_{max}	Maximum heart rate
LIST	Loughborough Intermittent Shuttle Test
MCV	Mean corpuscular volume
mM	Millimolar (millimole per liter)
mmHg	Millimeters of mercury
mmol	Millimole
PL	Placebo
ROS	Reactive oxygen species
RPE	Rate of Perceived Exertion
rpm	Revolutions per minute
SSEP	Soccer-Specific Exercise Protocol
URTI	Upper Respiratory Tract Infections
VO_{2max}	Maximal oxygen uptake

CHAPTER I
INTRODUCTION

Soccer is classified as a high intensity intermittent team sport (Bangsbo, 1994; Cameron et al., 2007). During a 90 minute game, soccer players encounter numerous explosive bursts of activity, such as, jumping, kicking, tackling, turning, sprinting, changing pace and sustaining forceful contraction to maintain and control of the ball against defensive pressure (Bangsbo, 1994; Reilly and Williams, 2003). Exercise intensity in soccer closed to that observed during marathon running (70% – 80% of maximal oxygen uptake) (Bangsbo, 1994; Helgerud, Engen, Wisloff et al., 2001; Reilly and Williams, 2003; Van Gool, 1988). Following competitive soccer match play, an average intensity was closed to the anaerobic threshold, being 80% – 90% of maximal heart rate (HR_{max}) or 70% – 80% of maximal oxygen uptake (VO_{2max}) (Helgerud, 2001; Reilly, 1994; Van Gool, 1988). Average blood lactate concentrations of 2 to 10 mM have been observed during soccer matches (Bangsbo, Laia and Krustrup, 2007). Nevertheless, the findings of high blood lactate concentrations indicated that the rate of muscle lactate production was high during match play (Krustrup and Mohr, 2006).

Although there have been many team sport simulation protocols designed, there are none that have included and completely replicated the intensity (HR, VO_{2max}), duration, distance, ball-skills work, movement patterns and general soccer skills that are required or observed in a soccer match. Most studies have replicated only a few of these qualities in their protocols. William, Abt and Kilding (2009) has come out with a field-based simulated protocol which was composed of different repeated sprint test, as well as a sport-specific performance measure (i.e. 90 minutes of soccer-specific intermittent exercise). It had categories of activity during soccer match-play (i.e. walking, jogging, cruising submaximally (striding), sprinting, moving backwards and moving in possession of the ball). They named this protocol as “Ball-sports Endurance and Sprint Test protocol or BEAST”. This protocol was used in this study.

The interactions between physical activity stress and the immune responses provide a unique opportunity to evaluate the role of underlying stress and immunophysiological mechanisms. It has been suggested that exercise represents a

quantiflable model of physical stress (Hoffman-Goetz and Pederson, 1994). Gleeson et al., (2004) showed that intense exercise, heavy training and competition leaded to upper respiratory tract infections (URTI). Changes in the numbers and types of cells in immune system were also reported (Castell, 2003; Hoffman-Goetz and Pederson, 2000; Malm, 2004; Nieman et al., 1998).

Effects of exercise on immune function could either be positive or negative. Nieman et al. (1998) showed relationship of exercise intensity and infection risk that moderate exercise stimulated immune function. However, prolonged high intensity exercise was impaired immune function and increased the rate of infection. Therefore, exercise enhances or reduces immune function, depending on its frequency, duration, and intensity (Malm, 2004; Lagranha et al., 2004). Exercise-induced leucocytosis can be attributed mainly to neutrophilia and to a lesser extent to lymphocytosis and monocytosis. After prolonged exercise, leucocyte numbers may decrease below the numbers at rest. The source of leucocytes appearing in circulation after physical exercise can be blood vessel walls, lung, gastrointestinal tract and spleen (Malm, 2004).

Alterations in metabolism and metabolic factors contribute to exercise-associated change in immune function. In 2007, Gleeson demonstrated that immune function was depressed after each high intensity exercise due to decreases of glutamine in blood. Reductions in plasma glutamine concentrations due to muscular exercise have been hypothesized to influence lymphocyte function (Newsholme and Parr-Billings, 1990). Altered plasma glucose has also been implicated in decreasing stress hormone levels and thereby influencing immune function (Nieman and Pedersen, 1999). Furthermore, free oxygen radicals and prostaglandin released by the elevated number of neutrophils and monocytes may influence the function of lymphocytes and contribute to the impaired function of the later cells. However, glutamine is used at a high rate by lymphocytes and macrophages. It is oxidized as a fuel and for the synthesis of DNA and RNA (Venkatraman and Pendergast, 2002). Thus nutritional supplementation with glutamine, carbohydrate, antioxidants or prostaglandin inhibitors may in principle influence exercise associated immune function (Pedersen and Hoffman-Goetz, 2000).

The supplements in the immune response and infection on high intensity and prolonged exercise include zinc, glutamine and vitamin C. Glutamine is the most abundant free amino acid in human muscle and plasma and is utilized at very high rates by leucocytes to provide energy and optimal conditions for nucleotide biosynthesis. Prolonged exercise is associated with a fall in the plasma concentration of glutamine (Gleeson et al., 2004; Kargotich et al., 2005). Previous studies have been suggested that glutamine supplementation increased plasma glutamine concentration and hence glutamine might prevent leucocytes apoptosis after intense and prolonged exercise (Krzywkowski et al., 2001). In addition, glutamine improved a response of immune in immunity by increase ammonia removal from muscle (Lagranha et al., 2004; Cameron et al., 2007).

Even so, high intensity and prolonged exercise influences to change in number of leucocytes and induce fatigue. Thus, glutamine supplementation might prevent changes of leucocytes as immune system, reduce fatigue and improve athletes performance.

Therefore, the aim of this study focused on effects of glutamine supplementation on leucocytes number and fatigue after simulated soccer matches in Thai youth soccer players.

1.1 Research Question

1. Does glutamine supplementation induce changes of leucocytes number after simulated soccer matches in Thai youth soccer players?

2. Does glutamine supplementation decrease fatigue after simulated soccer matches in Thai youth soccer players?

1.2 Objective of this study

To investigate the effects of glutamine supplementation on leucocytes number and fatigue after simulated soccer matches in Thai youth soccer players.

1.3 Assumptions

1. Glutamine supplementation induced changes of leucocytes number after simulated soccer matches in Thai youth soccer players.

2. Glutamine supplementation decreases fatigue after simulated soccer matches in Thai youth soccer players.

1.4 Conceptual Framework

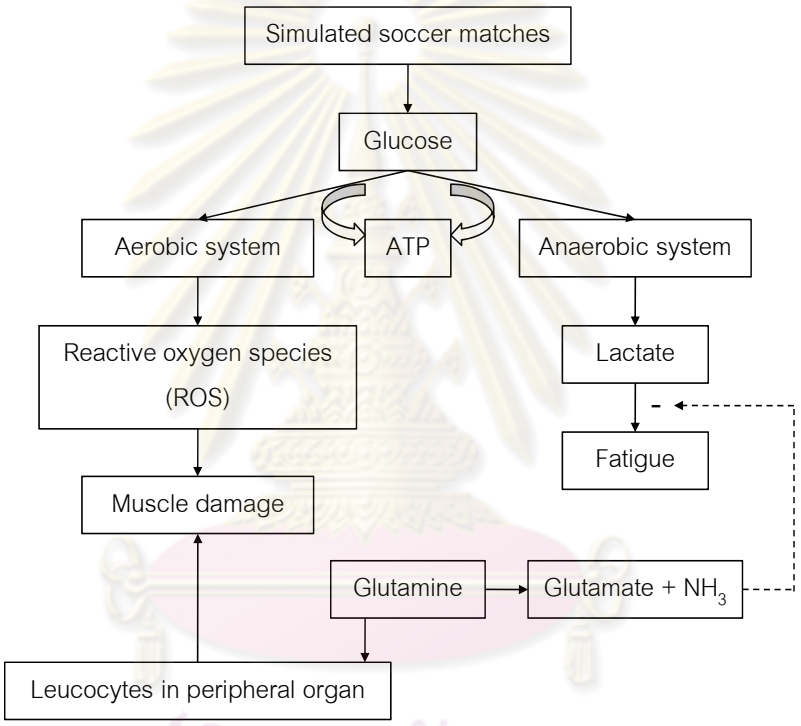

Figure 1.1 Conceptual framework

1.5 Limitations

1. The result of study may not be refered to the general population in all ages or the other kinds of sports/ exercise.

2. Mental fatigue and relaxation of participants could not be controlled.

3. The number of subjects is limited.

1.6 Operational Definitions

1. Glutamine supplementation is the drink containing 100 mg glutamine per kilogram body weight in 500 ml water.

2. Placebo is the drink containing 500 ml water.

3. Leucocytes are white blood cells including neutrophils, lymphocytes, monocytes, eosinophils and basophils.

1.7 Expected Benefits of the study

1. To gain knowledge of the effects of glutamine supplementation on leucocytes number and fatigue after simulated soccer matches in Thai youth soccer players.

2. To try out the BEAST protocol for physical performance of soccer players in simulated match.

3. The result of this study would provide some suggestions for future research on glutamine and immune response in exercise.

CHAPTER II
REVIEW LITERATURES

Soccer is probably the most widely practical sport in the world and its popularity is continually to increase. It is considered a physically demanding sport, which required a high degree of technical skill, strength, agility and endurance. Exercise intensity in soccer ranges from standing, walking, and sprinting (Helgerud, 2001). It is estimated that the mean distance covered during the game about 8 – 13 km (Bangsbo, 1991; Helgerud, 2001; Reilly and Williams, 2003; Withers, 1982) at an average intensity comparable to that was observed during marathon running (70 – 80% of maximal oxygen uptake (VO_{2max})). This closes to the anaerobic threshold or the lactate threshold (LT) which is equivalent to 80 – 90% of maximal heart rate (HR_{max}). (Helgerud, 2001; Reilly, 1994; Van Gool, 1988). During a 90 min game, the overall distance covered by outfield players consists of 24% walking, 36% jogging, 20% cruising submaximally (striding), 11% sprint, 7% moving backwards and 2% moving in possession of the ball (Reilly and Williams, 2003).

2.1 Soccer simulation protocols

2.1.1 Lab-based soccer simulation protocols

Drust, Reilly and Cable (2000) designed a soccer-specific protocol performed on a motorized treadmill. Seven male university players were recruited for this study and each visited the laboratory on three separate occasions six days apart. The soccer-specific intermittent protocol consisted of different exercise intensities observed during soccer match-play (e.g. walking, jogging, cruising and sprinting etc). The proportions of activities incorporated were similar to those reported by Reilly and Thomas, 1976. A recovery period was also included in the protocol, in which the subjects stood stationary on the treadmill for 71 s. The speeds chosen for all subjects for each activity were as follows: walking 6 $km.h^{-1}$, jogging 12 $km.h^{-1}$, cruising 15 $km.h^{-1}$, and sprinting 21 $km.h^{-1}$. The total duration of the test representing one half of a soccer match was 46 min 11 s. The protocol consisted of two 22.5 minute cycles separated by a static recovery period of 71 s. Each cycle integrated 23 distance bouts of activity: six bouts of walking, six

bouts of jogging, three cruises, and eight sprints. Mean HR for the protocol was 168 ± 10 $b.min^{-1}$ (~83% HR_{max}).

In a similar study, Abt et al. (2003) designed a non-motorised treadmill test to replicate the speeds of team sport activity (especially that of soccer). Five male recreation team sport players followed an activity profile on a monitor placed at eye level which displayed a target speed together with their current speed. The players were instructed to change speed by audio bleeps and verbal commands generated by a computer over two 45 minute "halves". During the 90 minute protocol players walked 37%, jogged 24%, ran fifty percent of maximum 14%, and ran seventy percent of maximum 4%, sprinted 3% and stood stationary 18%. Players covered a mean total distance of 10196 ± 403 m.

Thatcher and Batterham (2004) used six male professional English soccer players to run a soccer-specific exercise protocol (SSEP). The SSEP consisted of two bouts of 9 x 5 min repeated cycles on a non-motorised treadmill, separated by at 15 min rest period (half-time). All subjects were given a visual cue via a computer that displayed both the target speed and treadmill speed. The distance of the SSEP (9924 m) was similar to that observed during an English premier soccer match (9741 ± 882 m to 10274 ± 609 m). Mean HR responses in the SSEP were; first half: 166 ± 13 bpm and second half: 166 ± 11 bpm.

One clear advantage that Abt et al. (2003) and Thatcher et al.' s (2004) protocols had over the protocol designed by Drust et al. (2000) was that they were both performed on a non-motorised treadmill, not on a motorised treadmill. Subjects using a non-motorised treadmill can change speeds at their own pace, which can make it easier for researchers, coaches and trainers to observe a fatiguing effect within the subjects over the course of the protocol. The target speeds can also be reached a lot quicker when using a non-motorised treadmill, as there is sometimes a long time delay when changing speeds on a motorised treadmill.

Another advantage that these two studies had over Drust et al. (2000) was that total distance covered during the protocols were both similar to the distances covered during a soccer match (Bangsbo and Lindquist, 1992; Bangsbo et al., 1991; Helgerud et al., 2001; Krustrup et al., 2005; Mohr et al., 2003; Rienzi et al., 2000; Thatcher and

Batterham, 2004; Van Gool et al., 1998; Withers et al., 1982). However, it should be acknowledged that Drust et al. (2000) did highlight their protocol was designed to represent one half of a soccer match (~45 min). It is unknown if Abt et al.' s (2003) treadmill protocol had similar mean HR values to those found in a soccer game as they were not reported. Drust et al. (2000) and Thatcher et al.' s (2004) protocols both had similar mean HR values to those previously found in soccer matches (Ali and Farrally, 1991; Bangsbo, 1994b; Krustrup et al., 2005; Krustrup et al., 2006; Mohr et al., 2004; Strøyer et al., 2004; Thatcher and Batterham, 2004; Van Gool et al., 1998).

One positive aspect of lab-based soccer-specific protocols is that they can replicate the speeds and distances found during a soccer match. Consequently the physiological load is specific to a soccer game. Standardization of the lab environment (consistent air temperature, humidity, barometric pressure etc) is another positive aspect of lab-based interventions. A clear limitation of all three lab-based protocols is that no reliability studies or validity studies have been undertaken on them; therefore we cannot determine the amount of error present in each protocol.

However, one study by Hughes, Doherty, Tong, Reilly and Cable (2006) examined the reliability of repeated sprint exercise in non-motorised treadmill ergometry and found maximal speed was highly reliable (CV = 2.75%). Due to these tests being performed on treadmills, subjects were restricted to straight line running only; therefore no agility/ change of direction movements or ball-skills were performed throughout the test, which are major characteristics of team sports, especially that of soccer, and these are likely to change the demands of the task and the effect on muscle fatigue.

Another example of lab based protocol was designed by Sugiura and Kobayashi (1998). They used a cycle ergometry protocol to determine the effects on sprint performance of glucose and fructose ingestion during a 15-min rest period half way through 90 min of intermittent exercise. The procedure of protocol following: subjects cycled at 60 rpm to adjust 65% of VO_{2max} and then sprinted for 30 s at 90 rpm to maintain 100% of VO_{2max} every 2.5 min. After 45 min of intermittent exercise (the first half) was over, they took a 15-min rest and were instructed into the second half of exercise (Figure 2.1). This protocol used a 30 second high intensity sprint for every 2.5 min which would be highly exhausted for the leg muscle to finish the protocol.

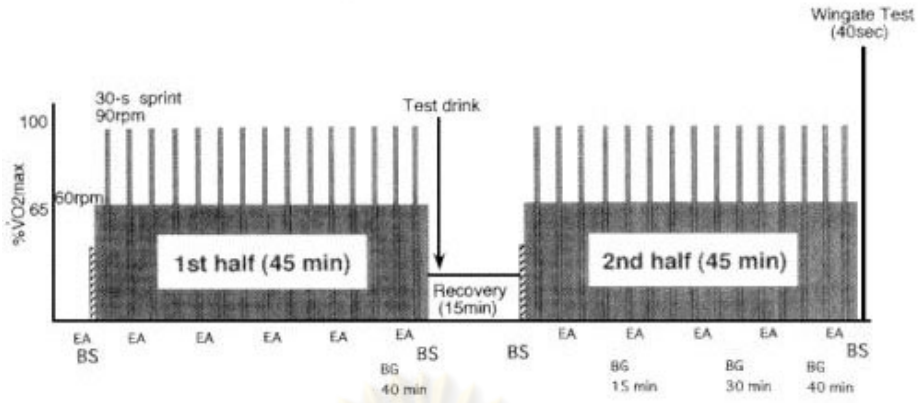

Figure 2.1 Cycle ergometry protocol. EA, expired air; BS, blood sample; BG, blood glucose; [square with upper right to lower left fill], warm-up; [dotted square], exercise; [black small square], Wingate test.

2.1.2 Field-based soccer simulation protocols

The second type of soccer-specific simulation protocol designed is the field-based protocol. Bangsbo and Lindquist (1992) designed a soccer-specific performance protocol (Figure 2.2) that consisted of forward, backwards and sideways running, and weaving in and out of slalom flags. The test lasted 16.5 minutes, during which players alternated between 40 bouts of high-intensity exercise each lasting fifteen seconds, and 40 bouts of low-intensity exercise each lasting ten seconds. During the high-intensity periods, subjects followed an outlined circuit around a penalty area of a soccer field. Players ran 40 m forward, 8.25 m backwards, 8.25 m forward and then through a 120 degree angled slalom, 8.25 m sideways while facing away from the centre of the circuit, and 8.25 m sideways while facing the centre of the circuit. During the low-intensity periods, players jogged to the centre of the circuit and back to the last cone marked position they reached at the end of the previous high-intensity period. Despite the soccer-specific movements involved in this study (high and low-intensity running, backwards movements, agility etc), there were still no integrated ball-skill activities (such as shooting, passing etc), any reported replication of soccer match distance or duration.

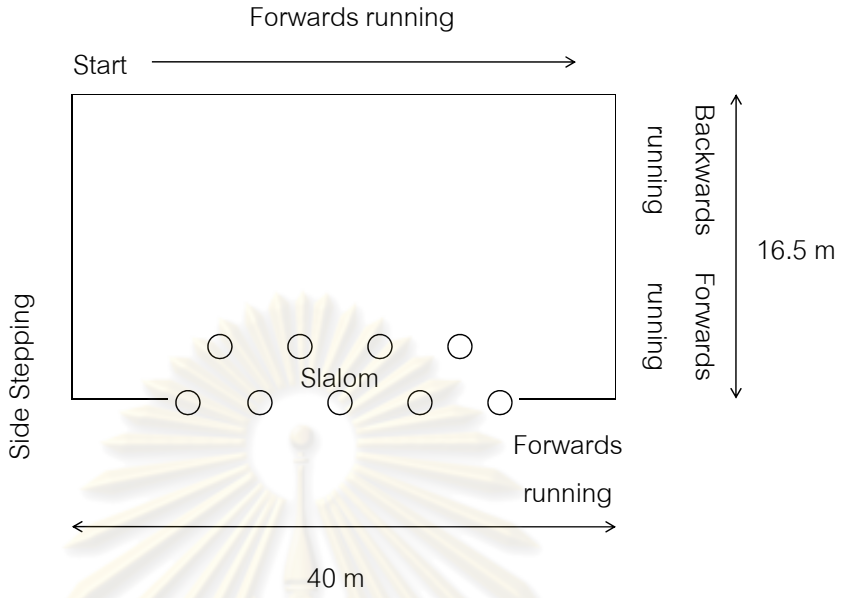

Figure 2.2 Bangsbo's Interval Field Test

Muller, Kornex and Leienstorfer (1992) designed a similar soccer-specific endurance protocol to that of Bangsbo and Lindquist (1992), which was based off a previous design by Binz (1986). The protocol had a total distance of 205 m and took approximately 76 s per lap, for a total duration of 10.08 minutes. The protocol included jogging, sprinting, walking, jumping hurdles (80 cm), and backwards running. There was also sprinting with soccer balls and shooting accuracy integrated which made the protocol more soccer-specific; however no agility movements (i.e. slalom movements) or jumping movements were performed (unlike Bangsbo and Lindquist's protocol). Muller et al. (1992) did well to replicate many of the movement patterns and skills found in a soccer game, as every movement pattern performed had a time limit. For example, subjects had to walk 20 m in 13 s; jog 40 m in 12 s; and sprint 13 m in 2 s, etc. This idea was supposed to control the pace of the subjects, however the authors did note there was a limitation of this, stating "the planned running intensity and time were not exactly maintained during the test", possibly due to motivation levels and/ or fatigue. Another similarity of this protocol to Bangsbo and Lindquist (1992) was the minimal distance covered (205 m) and time taken to complete (~10 minutes). One advantage that Muller

et al.'s (1992) protocol had over Bangsbo and Lindquist's (1992), was that it was described by the authors as being highly reliable (product-moment correlation coefficient r = 0.90), whereas Bangsbo and Lindquist (1992) did not illustrate any reliability results or studies undertaken on their field test.

In regards to longer duration (>75 minutes) soccer-specific field protocols, only two to date have been devised (Cox, 2002; Nicholas et al., 2000). Firstly, Nicholas et al. (2000) devised the Loughborough Intermittent Shuttle Test (LIST) to simulate the activity patterns common to soccer performance, without any contact (Figure 2.3). Seven trained healthy male soccer and rugby players were used as subjects. This protocol consisted of two parts, A and B. Part A was of fixed duration and consisted of five 15 minute exercise periods separated by three minutes of recovery. Part A consisted of bouts of walking, and running at speeds corresponding to 55 and 95% of an individual's pre-determined VO_{2max} over a 20 m distance. Part A was as follows: 3 x 20 m at walking pace, 1 x 20 m at maximal running speed, 4 s recovery, and 3 x 20 m at running speed corresponding to 55% of individual VO_{2max}, 3 x 20 m at a running speed corresponding to 95% of individual VO_{2max}. Part B was an open-ended period of intermittent shuttle running, designed to exhaust the participants within ten minutes. The duration of the LIST was approximately 75 minutes and the total distance covered was 12400 m. The mean HR for trial one and trial two for part A was 169 and 171 bpm and part B was 175 and 176 bpm. The distance and mean HR values were similar to those calculated in professional soccer matches (Bangsbo et al., 1991; Reilly and Thomas, 1976; Van Gool et al., 1988). The duration of the LIST was 15 minutes shorter than a soccer match.

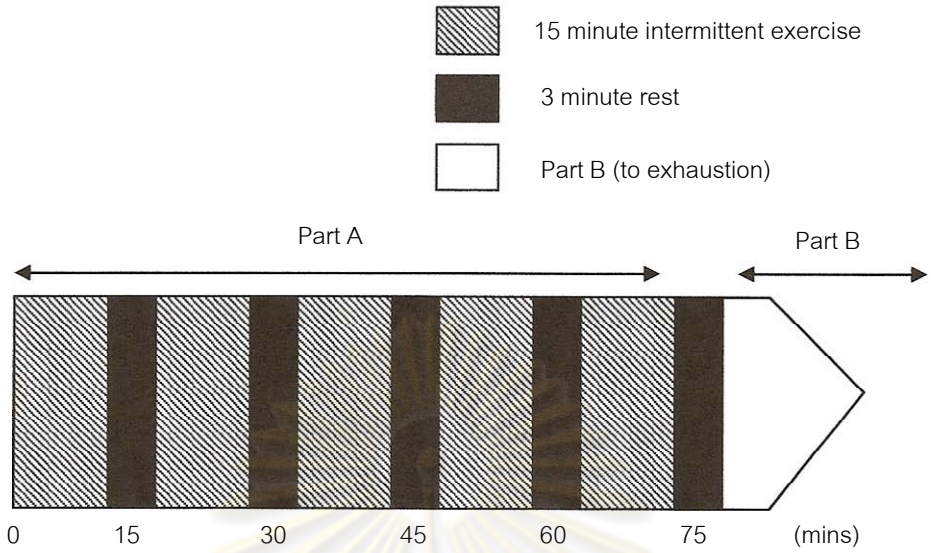

Figure 2.3 Loughborough Intermittent Shuttle Test

Cox et al. (2002) used 14 elite female soccer players from the Australian National soccer team. Subjects participated in to performance testing session separated by seven days. This soccer protocol consisted of five 12 minute exercise testing blocks (11 minutes of exercise and minute of recovery) based on activity patterns previously observed in female soccer players. Each of these testing blocks included eleven all-out twenty meter sprints, two agility runs, and one precision ball kicking drill, separated by several by standardized recovery twenty meter walks, jogs and runs. The test lasted approximately 60 minutes which was also less than a soccer match (~30 min) and 15 min shorter than the LIST. The mean HR of 174 bpm was also similar to that observed in a soccer match (Bangsbo et al., 1991; Reilly and Thomas, 1976; Van Gool et al., 1988) and similar to the mean HR documented in the LIST. The distance covered during Cox's (2000) protocol was not reported. One advantage of Cox's (2000) soccer-specific protocol had over the LIST was Cox included ball-skills, whereas the LIST had no ball-skill work. Cox (2000) did not report whether a reliability study had performed on his protocol, whereas the authors of the LIST did note reliability was within the "observed limits".

Although there have been many team sport simulation protocols designed, there are non that have included and completely replicated the intensity (HR, VO_2), duration, distance, ball-skills work, movement patterns and general soccer skills that are required/ observed in a soccer match. Most studies have replicated only a few of these qualities in their protocols. Having a soccer-specific protocol that can simulate the duration and demands of a soccer game can benefit the athletes, trainers and coaches as it illustrates where exactly in a game (first 30 minutes or last 15 minutes), and what/ where the athlete's weaknesses are (jumping ability, shooting etc), without the athlete having to play and be analyzed during an actual competitive soccer game. If a soccer performance protocol is inadequate in duration and does not replicate an entire soccer game, it makes it very difficult to determine whether there are any effects from a training or nutritional intervention on an athlete entire 90 minute performance. Clearly, a closely controlled, sport-specific soccer simulation protocol that reflects the most recent temporal analysis of soccer match-play (e.g. number of sprints, turns, game distance, duration / distance of sprints, recovery duration, HR, game duration etc) will help to accurately determine the potential performance enhancing effects of glutamine supplementation. Williams, Abt and Kilding in 2009 has published a 90 minute soccer specific simulation protocol which is a combination of field-based and soccer skills performance. They name this protocol as Ball-sports Endurance and Sprint Test (BEAST) protocol.

2.2 The Ball-sports Endurance and Sprint Test (BEAST) protocol

The BEAST protocol (Williams, Abt and Kilding, 2009) simulated soccer match play in terms of time, movement pattern, physical demands (volume and intensity) and distance. It could be used in studies that wish to determine the effects of training or nutritional intervention on prolonged intermittent physical performance.

The BEAST is a soccer-specific protocol designed to simulate the physical demands of a typical competitive soccer game (i.e. it is run continuously over a 90 minute period consisting of two 45 minute halves, with a half-time break of fifteen minutes). Only a single subject can run the BEAST at any given time.

The BEAST protocol consisted of two laps (Appendix B), which made up one circuit. Each circuit was continuously repeated for the duration of the half time (45 minutes), followed by a half-time recovery (fifteen minutes); then repeated for a further 45 minutes (second half). One circuit has a distance of 380.4 m. Sprinting, backwards jogging, walking, jogging/ decelerating, and forwards running (75% of at maximum effort), make up 8.4%, 8.4%, 9.7%, 24.5% and 39% of the total distance covered per circuit respectively. Reliability of measure over 90 minutes ranged from 0.9 – 25.5% (% typical error), suggesting good reliability. The BEAST protocol could be used in studies that wish to determine the effects of training or nutritional interventions on prolonged intermittent physical performance.

2.3 Fatigue and exercise

Athletic performance in soccer is affected by the subject's energy-transducing capacity (Madsen, MacLean and Christensen, 1996). The average work intensity in soccer is close to the lactate threshold (equivalent to 80 – 90% of HR_{max}) (Helgerud et al., 2001). However, the matches are characterized by intermittent short periods and situations of high intensity activity where accumulation of lactate takes place with periods of low-intensity activity to remove lactate from the working muscles (Stolen et al., 2005). In addition, ammonia has been used as an indicator of metabolic activity during exercise because it is associated with the requirement and production of ATP. Several studies have shown that high intensity and prolonged exercises are correlated to ammonia appearance in blood. Furthermore, it has been suggested that high ammonia levels can be toxic to both muscles and the central nervous system (CNS), and induce peripheral and central fatigue (Banister and Cameron, 1990; Castell and Newsholme, 1997; Eriksson et al., 1985; Graham et al., 1997; Sahlin et al., 1990).

The intense exercise periods during a soccer match play lead to high anaerobic-energy turnover with an associated accumulation of lactate and lowering of pH in the exercised muscles. In addition, after 90 minutes of standard training was significantly increased blood lactate (blood lactate of pre exercise was 1.45 (0.96) mmol/l and post exercise was 3.60 (1.41) mmol/l) (Karakoc et al., 2005). Intermittent exercise was probably caused by different turnover rates of muscle and blood lactate during the 2

types of exercise, with the rate of lactate clearance being significantly higher in muscle than in blood. (Bangsbo et al., 2007). In addition, excess lactic acid accumulation inhibits further ATP production. Although the excess lactic acid causes fatigue during exercise, this inhibitory effect is a protective response, as excess acidity can lead to cell death (Abernethy, 1997). Moreover, in order to decrease the toxic effect caused by increased ammonia, the CNS enhances glutamine (Gln) synthesis to buffer free ammonia (Cameron et al., 2007).

Borg developed a psychophysical scale (Borg scale) that linked the experienced sensations of exertion to the performed exercise intensity. These scales contain two variables, a 'physical component' and the 'perceived magnitude'. The latter is a psychological component and it represents the intensity of the perceived sensations during the exercise performance. The psychophysical scale represents the relationship between these two variables. Two parameters estimate the physical properties of exercise, the type of exercise performed and the endurance time. In dynamic exercise there is a linear relationship between workload, represented as VO_{2max}, and heart rate. To obtain rate of perceived exertion (RPE) during exercise testing, researcher used the original (6 to 20) RPE scale (Figure 2.4) (Ament and Verkerke, 2009; Heyward, 1997).

Borg's RPE Scale	
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (Heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Figure 2.4 The original Borg's RPE scale (Heyward, 1997)

2.4 Immune effects

The acute phase response is a common reaction to a range of threats to homoeostasis including inflammatory diseases (Kushner and Rzewnicki, 1994) and prolonged exercise (Fallon et al., 1999). Exercise-induced reactive oxygen species (ROS) are also thought to modulate acute phase inflammatory responses (Cannon and Blumberg, 2000).

The strong muscle contractions during exercise may cause micro-tears in both muscle and the vascular endothelium, which increases the migration of white blood cells into the muscle, including acute phase inflammatory reactions. They have shown that muscle injury markers appear in an early window with leucocytosis during intermittent exercise (Bassini-Cameron et al., 2007).

Our immune system is composed of two main features known as leucocytes and lymphoid tissues. The former groups are commonly known as white blood cells (WBC). White blood cells perform a wide range of tasks in the immune system. Five general categories of WBC exist. These are: basophils, eosinophils, neutrophils, lymphocytes, and monocytes (Venom, 2008). White blood cells and neutrophils number have been significantly increased after training (Bessa et al., 2008; Perez et al., 2001; Rebelo, 1998; Sureda et al., 2009). While monocytes number have been increased or not change during and after acute exercise and may be decreased during prolonged periods of intense endurance training (Mackinnon, 1999; Malm, 2004; Woods et al., 2000). In addition, exercise increases the concentration of lymphocytes in peripheral blood (Bessa et al., 2008; Castell, 1997; Nielsen, 2003); however, 1 – 2 hours into the recovery, the lymphocytes number decreases to, or even to below, the pre-exercise level (Castell, 1997; Nielsen, 2003). However, Keen et al., 1995 showed that during a major multi-stage race in the evening, those of individual leucocyte classes (neutrophils, lymphocytes, monocytes, eosinophils and basophils) were all inside the normal range both in the morning and in the evening. Evening counts (after the race) were 30-50% higher than morning counts (before the race), for all classes except eosinophils, it is likely that the samples were taken as the count was still falling in the morning, and rising again in the evening, leaving the observed values little different. Moreover, Haematocrit (Hct) and Mean corpuscular volume (MCV) have been significantly decreased after 90

minutes of soccer training (Karakoc et al., 2005). Whereas, Hct have been significantly increased after simulated soccer match (Bassini-Cameron et al., 2007). In the other hand, Bessa et al. (2008) and Sureda et al. (2009) reported that Hct did not change during the high intensity endurance exercise.

2.5 Metabolic changes

Several recent studies showed that blood ammonia concentration increases during endurance exercise mainly due to myokinase activity and deamination by muscle (Banister and Cameron, 1990), and has been proposed as a cause for both peripheral and central fatigue (Carvalho-Peixoto, Alves and Cameron, 2007; Nybo and Dalsgaard, 2005; Nybo and Secher, 2004). Also, metabolites such as uric acid and urea increase during high-intensity exercise in response to the IMP and ammonia clearance demand (Boyum et al., 2002). Moreover, Smith and Norris (2000) showed the concentration of glutamine significant decrease after heavy training of endurance sports, such as cross-country skiing, swimming and speed skating. Nevertheless, Kargotich et al. (2005) founded that 95% interval training session (ITS) decreased plasma glutamine concentration significantly lower than 70% ITS and pre-exercise. Periods of very heavy training are associated with a chronic reduction in plasma concentrations of glutamine and it has been suggested that this may be partly responsible for the immunodepression apparent in many endurance athletes (Parry-Billings et al., 1992). In addition, prolonged exercise is associated with a decrease in the intramuscular and plasma concentrations of glutamine and it has been hypothesized that this decrease in glutamine availability could impair immune function (Castell, 2003; Parry-Billings et al., 1992).

2.6 Glutamine

2.6.1 Glutamine metabolism

Glutamine (also known as L-Glutamine) is a naturally occurring nonessential neutral amino acid. It is important as a constituent of proteins and as a means of nitrogen transport between tissues (Watford, 2008). It is also important in acid-base regulation, gluconeogenesis, and as a precursor of nucleotide bases and the

antioxidant glutathione. Glutamine is the most abundant free amino acid in human muscle and plasma. In adult humans, following an overnight fast, the normal plasma glutamine concentration is 550–750 μmol/L and the skeletal muscle glutamine concentration is ~20 mmol/kg wet weight (Jonnalagadda, 2007). Skeletal muscle is the major tissue involved in glutamine synthesis and is known to release glutamine into the circulation at ~50 mmol/h in the fed state. Its alleged effects can be classified as anabolic and immunostimulatory. Glutamine is utilized at high rates by leukocytes (particularly lymphocytes) to provide energy and optimal conditions for nucleotide biosynthesis and hence, cell proliferation. Indeed, glutamine is considered important, if not essential, to lymphocytes and other rapidly dividing cells, including the gut mucosa and bone marrow stem cells. Unlike skeletal muscle, leukocytes do not possess the enzyme glutamine synthetase, which catalyses the synthesis of glutamine from ammonia (NH_3) and glutamate, and therefore leukocytes are unable to synthesize glutamine (Ardawi and Newsholme, 1983). Consequently, leukocytes are largely dependent on skeletal muscle glutamine synthesis and release into the blood to satisfy their metabolic requirements.

Glutamine supplementation effectively counteracts the decline in protein synthesis and muscle wasting from repeated glucocorticoid use. In one study of female rat, infusing glutamine for 7 days inhibited the downregulation of myosin (muscle contractile protein) synthesis and atrophy in skeletal muscle that normally accompanies chronic glucocorticoid administration. Data also indicate that increasing glutamine availability by supplementation modulates glucose homeostasis during and after exercise in a direction that facilitates post-exercise recovery. It also promotes muscle glycogen accumulation in human muscle in recovery, perhaps by serving as gluconeogenic substrate in the liver (McArdle et al., 2009).

2.6.2 Glutamine supplementation

Many previous study of glutamine supplementation were reported about dosage to receive by oral ingestion. Athletes consuming beverages containing glutamine following rowing competitions and marathons by 5g glutamine after the activity and two

hours later (Castell, 2002; Castell et al., 1996; Castell et al., 1997). After 60 minutes of prolonged exercise, the subjects consumed 3.5 g of glutamine in an aqueous solution (Krzywkowski et al., 2001) as well as an oral supplementation (5 g in 330 ml water) consumed immediately after and 2 h after marathon (Castell et al., 1996). However, several recent glutamine feeding intervention studies indicated that although the plasma glutamine concentration could be kept constant during and after prolonged strenuous exercise, the glutamine supplementation did not prevent the post-exercise changes in several aspects of immune function (Gleeson, 2008). Moreover, studies that have ingested glutamine before prolonged exercise approximately 1 hour were showed that 100 $mg.kg^{-1}$ body weight in aqueous solution of glutamine. Glutamine supplementation before exercise partially prevented the exercise induced apoptosis. (Lagranha et al., 2004). Also, Cameron et al. (2007) provided 100 $mg.kg^{-1}$ body weight of glutamine dissolved in a non-nutritional beverage before intermittent high intensity prolonged exercise. The result showed that glutamine supplementation prior to exercise partially prevents the hyperammonemia observed after intermittent exercise. Again, no signs of toxicity and side effects were reported. Thus, this study provided 100 $mg.kg^{-1}$ body weight of glutamine in 500 ml drinking water before simulated soccer matches 1 hour.

2.7 Glutamine and immune function

In humans, glutamine (Gln) is the most abundant circulating amino acid. It is synthesized in large amounts by the muscles, takes part in carbon transport and is a non-toxic ammonia carrier. The concentrations of plasma Gln decrease as a function of exercise intensity because of the increase in gluconeogenesis and urea synthesis. It has been widely shown that oral Gln supplementation increases its plasma concentration and its efficiency as an energy substrate during rest or metabolic stress. The use of amino acids as energy substrates may increase the pool of ammonia from amino acid deamination (Cameron et al., 2007). Glutamine changes the immune response,

including leucocytosis, lymphocytosis and neutrophilia along with an increase in metabolic rate (Krzywkowski et al., 2001).

It has generally been accepted that cells of the immune system obtain their energy by metabolism of glucose. However, it has been established that glutamine is also an important fuel for lymphocytes and macrophages (Newsholme and Parry-Billings, 1990). Several lines of evidence suggest that glutamine is used at a very high rate by these cells, even when they are quiescent. It has been proposed that the glutamine pathway in lymphocytes may be under external regulation, due partly to the supply of glutamine itself (Newsholme, 1994). Skeletal muscle is the major tissue involved in glutamine production and known to release glutamine into the bloodstream at a high rate. It has been suggested that the skeletal muscle plays a vital role in maintenance of the key process of glutamine utilization in the immune cells. Consequently, the activity of the skeletal muscle may directly influence the immune system. According to the “glutamine hypothesis,” under intense physical stress, such as exercise, the demands on muscle and other organs for glutamine are such that the lymphoid system may be forced into a glutamine debt. Thus factors that directly or indirectly influence glutamine synthesis or release could theoretically influence the function of lymphocytes and monocytes (Newsholme, 1990; Newsholme, 1994). After intense long-term exercise and other physical stress disorders, the glutamine concentration in plasma declines (Essen et al., 1992; Keast et al., 1995; Lehmann et al., 1995; Parry-Billings, 1992), and low glutamine levels have been reported to be associated with overtraining (Rowbottom et al, 1996; Rowbottom et al, 1996). Although there is evidence that glutamine has an important role in lymphocyte function in vitro, recent placebo-controlled glutamine intervention studies found that glutamine supplementation after the exercise abolished the post-exercise decline in plasma glutamine without influencing post-exercise immune impairment (Rohde, 1998). There is little experimental support to the hypothesis that post-exercise decline in immune function is caused by a decrease in the plasma glutamine concentration.

CHAPTER III
MATERIALS AND METHODS

3.1 Research design

This cross over research study was designed to investigate the effects of glutamine supplementation and placebo on leucocytes number and fatigue after simulated soccer matches in Thai youth soccer players. The healthy youth soccer players gave their written informed consent for the experiment, which had been approved by the Faculty of Medicine, Chulalongkorn University Committee for Ethics in Human Experimentation.

3.2 Population and sample

The target population was youth Thai male soccer players who were 15-18 years old.

The sample population was twelve Thai male soccer players at Surasakmontree School, who were recruited according to the following inclusion criteria.

3.2.1 Eligibility criteria

Inclusion criteria

1. Participated in last year soccer match.
2. Soccer training for 3-6 days per week.
3. Given informed consent to participate in this study.

Exclusion criteria

1. The volunteers were sick or injured that were difficult to training, competition and participation in this study.
2. History of liver, kidney or heart disease.

3.3 Materials

1. A weighting scale (Tanita TBF-531 A)
2. A scale for height measurement
3. Sphygmomanometer (ES-H5, Terumo Corporation, Tokyo, Japan)
4. Stopwatch (JS-609, FBT®, China)
5. Heart rate monitors (FS1, Polar electro, Finland)
6. Heart rate transmitter (T31 transmitter, Polar electro, Finland)
7. Rated Perceived Exertion (RPE) scale
8. Traffic cones
9. Vertical jump measurement (Yardstick, Swift Yardstick, Australia)
10. Speed timing (Powertimer, Newtest Powertimers, Finland)
11. Soccer balls
12. A ramp (1.50 m long, 1.15 m high)
13. A soccer goal (indoor soccer goal measured 4.5 m long x 3.0 m high)
14. A leather strap (20 cm wide, 90 cm long)
15. Activity boards
16. A lawn
17. Blood glucose meter (ACCU-CHEK® Performa, Roche Diagnostics, U.S.A.)
18. Blood lactate meter (Accutrend® Plus, cobas®, Roche Diagnostics, Germany)
19. Test strip for determination of glucose in blood (ACCU-CHEK® Performa, Roche Diagnostics, Germany)
20. Test strip for determination of lactate in blood (BM-Lactate, cobas®, Germany)
21. Blood collection tube 6 ml (K2 EDTA, BD Vacutainer®, U.S.A.)
22. 70% alcohol
23. Cotton
24. Plaster
25. Frozen gel pack
26. Surplug™ Needle-Free Connector with extension tube (SP*ET103LOSA, Terumo Corporation, Tokyo, Japan)

27. 10 ml syringe (SS+10S6, Terumo Corporation, Tokyo, Japan)
28. Surflo® I.V. Catheter Non-radiopaque/ etfe 22Gx1" (SR+OT2225C, Terumo Corporation, Philippines)
29. Disposable Needle 21x1.5 inch (AH2138, Nipro Corporation, Osakak, Japan)
30. Case record form
31. Determination of complete blood count (CBC), lactate and ammonia in blood by Center of Laboratory, King Chulalongkorn Memorial Hospital, Bangkok

3.4 Measurement

3.4.1 Body weight and height

Weight in kilogram was recorded with the individual wearing comfortable clothes without shoes.

The participant was standing barefoot with the heels together, then stretching upward to the fullest extent. Heels, buttocks, and upper back were touching a wall. The chin was not lifted. Height measurement was recorded in centimeter.

3.4.2 Resting heart rate

The participant had an adequate rest period of at least 5 minutes prior to the measurement. Adequate rest was indicated when the heart rate had stabilized at a low rate. The resting heart rate was measured with automatic sphygmomanometer (ES-H5, Terumo Corporation, Tokyo, Japan).

3.4.3 Resting blood pressure

The participant was sitting upright in a straight backed chair. Both feet were flat on the floor, and the left arm was resting on the table with the elbow flexed. Subject was relaxed for a few minutes in this position. Conversation was discouraged. The blood pressure was measured with sphygmomanometer (ES-H5, Terumo Corporation, Tokyo, Japan). The phase systolic pressure and diastolic pressure were recorded in millimeters of mercury (mmHg) as indicated on the sphygmomanometer scale.

3.4.4 Measurement of maximal oxygen uptake (VO_{2max})

Astrand Bicycle Ergometer Maximal Test Protocol

For the Astrand continuous test protocol (Figure 3.1), the initial power output is 600 kgm.min^{-1} (100 W) for men because the pedaling rate is 50 rpm, the resistance is 2 kg for men (2 kg x 6 m x 50 rpm = 600 kgm.min^{-1}). Subjects exercised at this initial work load for 2 minutes, then increase the power output every 2 to 3 minutes in increments of 300 kgm.min^{-1} (50 W) for men. Continue the test until the subject was exhausted or could no longer maintain the pedaling rate of 50 rpm. Use the ACSM metabolic equation for leg ergometry to estimate VO_2 from subject's power output during the last stage of the graded exercise test (GXT) (Heyward, 1997).

Metabolic calculations

Leg ergometer (ml.min^{-1}) = 3.5 ml.kg^{-1}.min^{-1} x kg BW + kgm.min^{-1} x 2

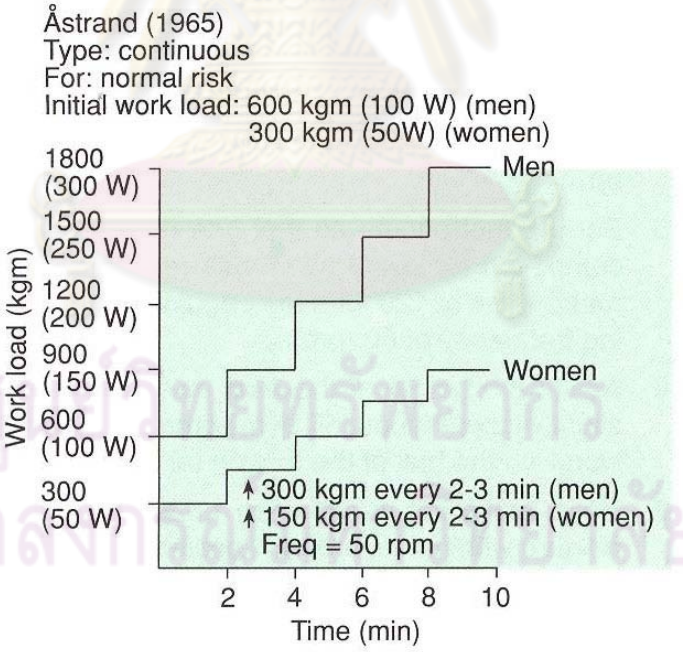

Figure 3.1 The Astrand continuous test protocol (Heyward, 1997).

3.4.5 Blood sample and analyses

Venipuncture was performed by a certified phlebotomist before and after the Ball-sports Endurance and Sprint Test. Before the test, an I.V. Catheter Non-radiopaque/ etfe 22Gx1" was placed in an antecubital vein to enable serial blood sampling throughout post test for 1 hour, and clotting was prevented by back flushing the catheter with 0.9% normal saline solution.

Six milliliters of whole blood sample were collected via puncture from the antecubital vein and immediately transferred to EDTA blood collection tube, then transferred to Center of Laboratory at King Chulalongkorn Memorial Hospital within 30 minute after blood collection for assay of CBC, ammonia, uric acid, urea and lactate.

3.4.6 Fatigue level

Fatigue scale was used to measure fatigue level. Fatigue scale was a line with 10 cm long. Participants were asked to mark within the line, which represent the magnitude of fatigue. Measurement was recorded in percent of fatigue level (0-100 %).

3.5 Methods

3.5.1 Subject Preparation

Prior to each test session, subjects were asked to abstain from caffeine, alcohol, and vigorous physical activity for 24 hours. In addition, food was not allowed within 2 hours prior to testing. Upon arrival to the laboratory, weight, height, resting heart rate, and resting blood pressure were recorded. Before test session started, all subjects performed stretching and warm up for 10 minutes. The subjects wore a heart rate monitor to determine their heart rate during all exercise tests.

3.5.2 Experimental Design

Block randomization method was used in order to provide the subject received opportunity with equivalent to receive either drink. The drinks were assigned into type A and B. Since this study was a cross-over research, subjects who received type A drink at the first time and then would get type B drink at the second time two weeks later. Each drink was received before training and testing.

Type of drinks included:

- Type A: Glutamine 100 mg per kilogram body weight in 500 ml drinking water (not provided energy).
- Type B: 500 ml drinking water.

On 1st to 4th day, all subjects received a drink 1 hour before training. On 5th day, they also received a drink 1 hour before testing and finished the drink within 4 minutes. The test performance was Ball-sports Endurance and Sprint Test (BEAST) protocol. Test procedure was as follows:

- Blood sample was collected 5 minutes before received a drink.
- Subjects received a drink 45 minutes before warm up.
- Subjects performed a warm up for 10 minutes, and then they were asked to evaluate fatigue level before the test began.
- Subjects performed the test for 90 minutes period consisting of two 45 minute halves, with a half-time break of 15 minutes.
- After first half, subjects were measured fatigue level immediately and rest for 15 minutes.
- After second half, subjects were measured fatigue level and they were collected blood sample immediately.
- Subjects performed a cool down and stretching.
- 1 hour after second half, subjects were measured fatigue level and they were collected blood sample again.

Two weeks later, subjects performed the same activity of 1st to 5th day. In addition, they received another type of drink.

3.5.3 The Ball-sports Endurance and Sprint Test (BEAST) protocol

The BEAST (Figures 3.2 and 3.3) is a soccer-specific protocol designed to simulate the physical demands of a typical competitive soccer game (i.e. it is run continuously over a 90 minute period consisting of two 45 minute halves, with a half-time break of fifteen minutes). Only a single subject can run the BEAST at any given time.

The BEAST protocol consisted of two laps, which made up one circuit (Figures 3.3). Each circuit was repeated continuously for the duration of the half (45 minutes), followed by a half-time recovery (15 minutes); then repeated for a further 45 minutes (second half). One circuit has a distance of 380.4 m. Sprinting, backwards jogging, walking, jogging/decelerating and forwards running (75% at maximum effort), make up 8.4%, 8.4%, 9.7%, 24.5% and 39% of the total distance covered per circuit.

Figure 3.2 Simulated soccer field test using the BEAST protocol

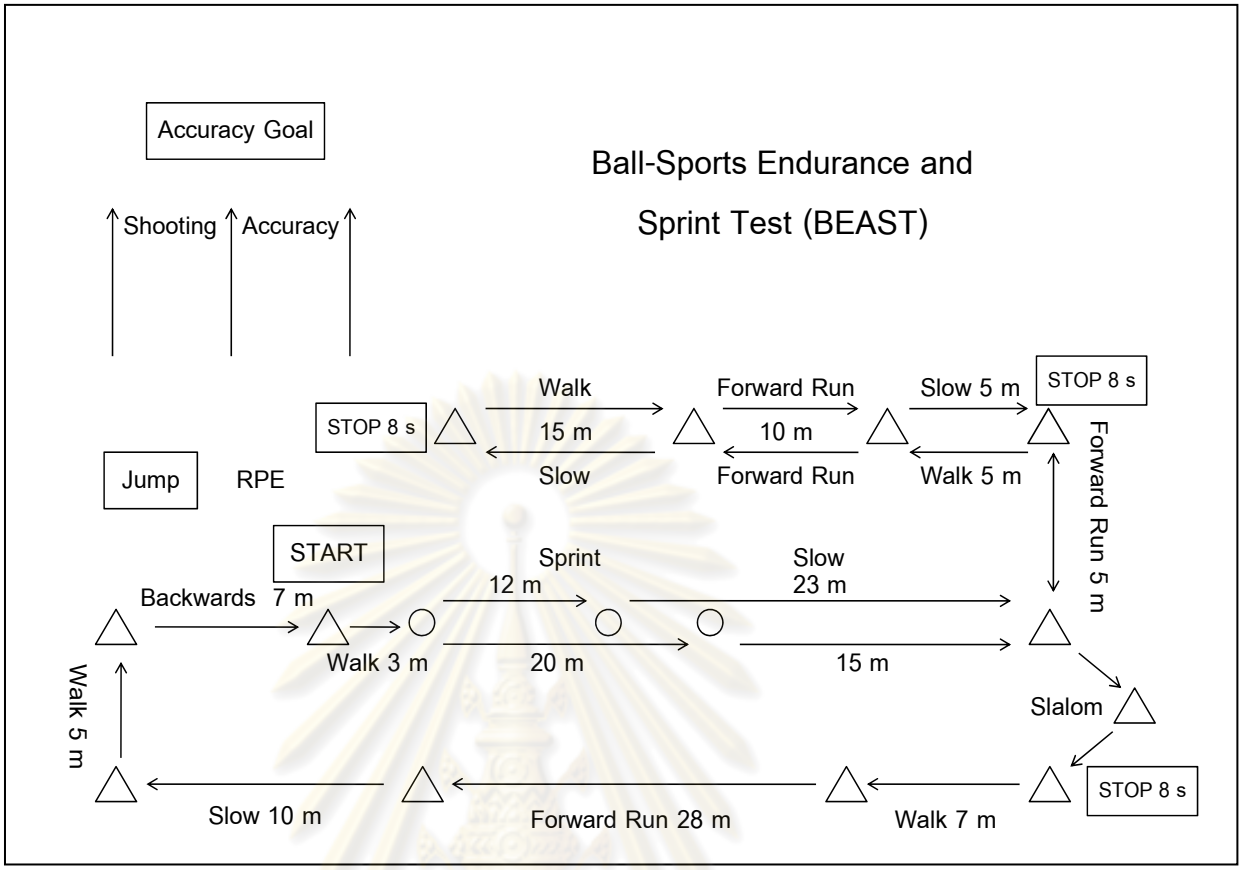

Figure 3.3 Schematic design of the BEAST protocol (Williams, Abt and Kilding, 2009)

3.5.3.1 Circuit Description

The first lap (of two) was completed as follows (Appendix B1):

The subject stood stationary at the start cone and when the signal was given by the circuit timer, the subject walked forward at a brisk pace for three meters. The subject then stopped at the sprint start line placed 50 cm behind the first photocell. Visible signs (i.e. sprint 12 m, run 5 m, walk 5 m etc) were placed at every station to remind the subject what movements they were to perform. On the researchers' signal (~2 s) the subject sprinted maximally through the 12 m photocell (the subject was instructed to sprint one meter past the 12 m gates to reduce a slow-down effect). The subject then decelerated in a straight line for 23 m to the next cone. He turned left at the cone and ran forwards five meters at 75% of maximum pace to the next cone. The circuit timer then instructed the subject to stop at this cone for a period of eight seconds (giving a verbal countdown of when to walk five meters to the following cone). After the

five meter walk the subject ran in a straight line ten meters at (75% of his maximum pace) to the next cone, decelerated 15 m and then stopped again for another eight seconds. On the circuit timer's signal, the subject briskly walked back the way he had come (15 m). He then ran ten meters (75% of his maximum pace) and decelerated five meters until he reached the next cone, where he rested again for another eight seconds. He then ran five meters at (75% of his maximum pace) and weaved in and out of the slalom cones for a distance of approximately 6.4 m, where he stopped briefly to rest for another eight seconds. The subject briskly walked to the next cone in a straight line (seven meters) then ran (75% of his maximum pace) for 28 m and decelerated for 10 m until he reached the next cone. He then briskly walked five meters to the next cone, then back-pedalled (backwards jog) seven meters to the start/ finish line. This was one lap (half a circuit).

The second lap (Appendix B2) was identical to the first lap except this time the subject sprinted 20 m (instead of 12 m) after the initial three meter walk. At the end of the second lap, the circuit time was recorded and the subject was fed six individually rolled soccer balls (the ramp was four meters away from the shooting zone, 1.5 m long, and placed 115 cm high from floor) onto his dominant foot (e.g. left foot is fed balls from the right side and vis a versa) and had to shoot at a soccer goal (indoor soccer goal measured 4.5 m long x 3.0 m high). Points were scored by shooting at either side of a centre target (inside each goal-post there were two leather straps 20 cm wide, dangling down the cross-bar 90 cm in ward from the side post to replicate a goal keeper). After each ball was rolled, the subject briskly jogged from the start line to the shooting line (four meters), took his shot and back-pedalled to the start line ready for his next shot. The ball was rolled as soon as the subject back-pedalled and touched the start line with his foot. After the shooting station, the subject walked briskly to the jump place and performed three vertical jumps. The subject then verbally called out or pointed to a number on the rate of perceived exertion scale (RPE) shown to him by a research assistant.

The subject continued to complete the circuits until 45 minutes had elapsed then the subject had a fifteen minute half-time break.

Procedure

Day 1-4	Day 5							
Supplementation and training	Preparation			Soccer Match				
	Time (min)							
	-60	-55	-10	45	60	105	110	165
	Blood Sample	Supplementation	Warm up and Measured fatigue level	BEAST (simulated soccer match)	Measured fatigue level and Rest	BEAST (simulated soccer match)	Measured fatigue level and Blood Sample	Measured fatigue level and Blood Sample

Data analysis

The results were reported as mean and standard deviation (SD) calculated by conventional procedures. All data were analyzed using the Statistical Package for the Social (SPSS Version17.0). Paired t-test was used to test for significant differences of CBC, ammonia, uric acid, urea, lactate and %fatigue level in pre, post and 1 h post test between glutamine supplementation and placebo group. Whereas One – way ANOVA with Repeated Measures was used to test for significant differences of CBC, ammonia, uric acid, urea, lactate and %fatigue level in pre, post and 1 h post test within glutamine supplementation and placebo group. The differences at significance level of $p < 0.05$ were considered to be significant.

CHAPTER IV
RESULTS

4.1 Characteristics of subjects

This study was a cross-over design with a total of 12 subjects from Surasakmontree School. Subjects in the glutamine supplementation group received 100 mg per kilogram body weight of glutamine in 500 ml drinking water whereas subjects in placebo group received only 500 ml drinking water. Table 4.1 was the demographic data of all subjects participated in this study. They gave the written informed consent before enrollment. This study was approved by the human ethical committee for research of faculty of medicine, Chulalongkorn University.

Table 4.1 Characteristics of subjects (N = 12). Data are expressed as Mean (SD) and range.

Characteristics of subjects	Subjects (n = 12)	
	Mean (SD)	Range
Ages (yr)	16.0 (0.4)	15.0 – 17.0
Weight (kg)	57.8 (4.9)	48.3 – 64.6
Height (cm)	168.8 (3.7)	164.0 – 176.0
BMI (kg/m^2)	20.3 (1.5)	17.7 – 22.9
Predicted VO_{2max} (ml/kg/min)	56.09 (6.42)	50.03 – 69.25

4.2 Physical performance using the BEAST protocol

4.2.1 Comparison of physical performance according to the BEAST protocol between placebo group and glutamine supplementation group

Each subject performed the simulated soccer match according to the BEAST protocol. This protocol consisted of two 45 minutes halves with 15 minutes half time break. The simulated soccer protocol was composed of two laps per one circuit. Subjects performed as many circuits as they could within 45 minutes period. Each circuit included several stations for different physical activities such as 12 meters sprint, vertical jump, running, walking and jogging. Their physical performance according to this simulated soccer program were summarized in Table 4.2. All comparative parameters were statistical significant at the level of $p < 0.05$. The observed data showed significant difference both at 1st half and 2nd half in 20 m sprint time and vertical jump. However, RPE was significant difference between groups at 2nd half.

Table 4.2 Physical performance of subjects according to the BEAST protocol

Measure	Interval	Mean (SD)		P - value
		Placebo	Glutamine supplementation	
Circuit Time (min)	1st half	4.27 (0.90)	4.08 (1.22)	0.53
	2nd half	4.43 (0.85)	4.49 (0.77)	0.48
12 m Sprint Time (s)	1st half	2.32 (0.16)	2.28 (0.10)	0.35
	2nd half	2.42 (0.07)	2.37 (0.04)	0.11
20 m Sprint Time (s)	1st half	3.45 (0.09)	3.35 (0.12)	0.01[d]
	2nd half	3.66 (0.15)	3.49 (0.13)	0.00[d]
Vertical Jump (cm)	1st half	49.92 (0.69)	48.84 (0.49)	0.00[d]
	2nd half	49.52 (1.05)	47.09 (1.39)	0.00[d]
Shooting Accuracy	1st half	4.14 (0.46)	4.10 (0.34)	0.29
	2nd half	4.26 (0.35)	3.97 (0.29)	0.13

Table 4.2 (Cont.)

Measure	Interval	Mean (SD)		P - value
		Placebo	Glutamine supplementation	
Heart Rate (bpm)	1st half	172.75 (14.17) (84.68% HR_{max})	172.92 (14.79) (84.76% HR_{max})	0.97
	2nd half	168.92 (17.07) (82.80% HR_{max})	168.17 (16.33) (82.44% HR_{max})	0.87
RPE*	1st half	13	11	0.08
	2nd half	15	13	0.01[d]
Fatigue level (%)	1st half	67.79 (30.65)	51.08 (21.54)	0.05
	2nd half	67.00 (27.30)	66.75 (30.27)	0.98

[d] = Significant difference between groups ($p < 0.05$)

* = Data are expressed as Mode and Wilcoxson Signed Ranks Test were used to test for significant difference ($p < 0.05$)

4.2.2 Fatigue at 1st half and 2nd half of the BEAST protocol

Fatigue parameters included heart rate (HR), rate of perceived exertion (RPE) and % fatigue level. Fatigue at 1st half and 2nd half of the BEAST protocol were showed in Figure 4.1 – 4.3.

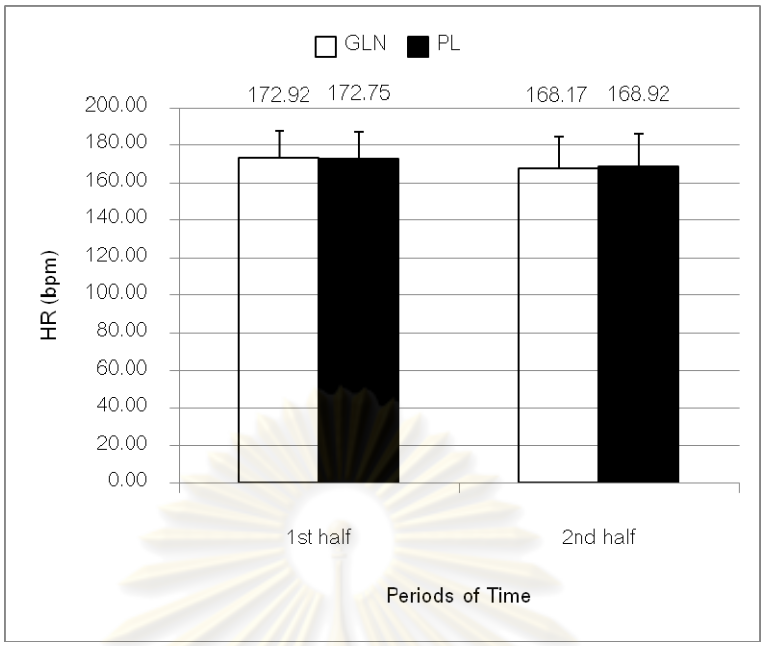

Figure 4.1 Heart rate at 1st half and 2nd half between groups of the BEAST protocol

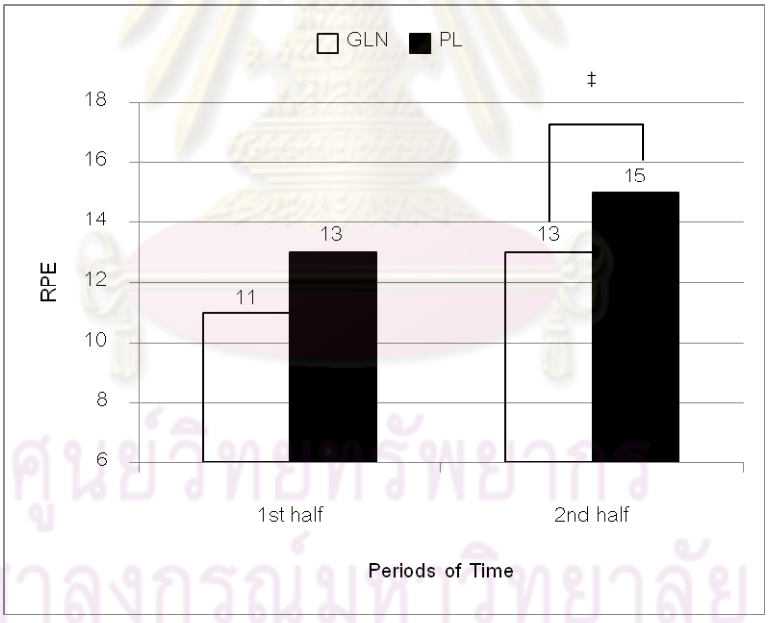

Figure 4.2 Rate of perceived exertion at 1st half and 2nd half between groups of the BEAST protocol

‡ = Significant difference between groups ($p < 0.05$)

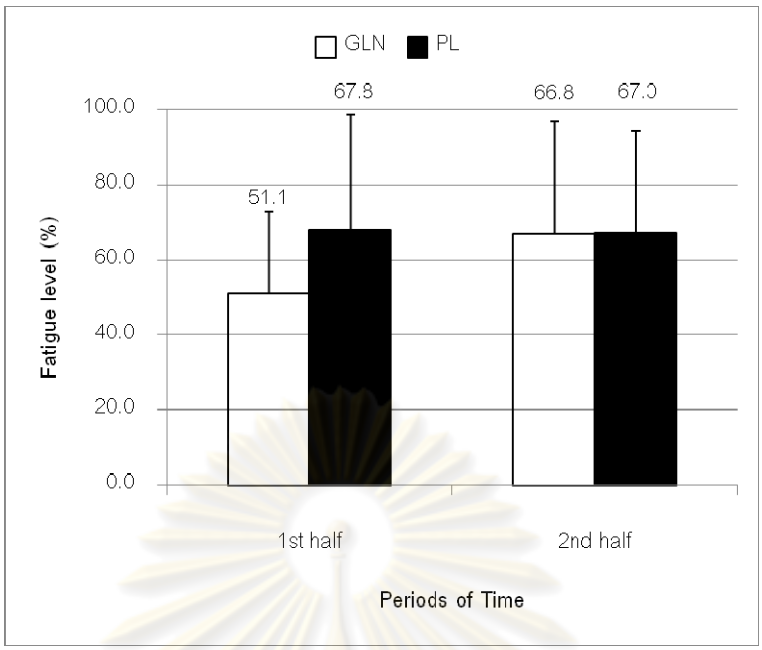

Figure 4.3 % fatigue level at 1st half and 2nd half between groups of the BEAST protocol

4.3 Effects of glutamine supplementation on fatigue after simulated soccer matches

During the test, HR, RPE and % fatigue level were recorded at pre, after 1st half and after 2nd half of simulated soccer matches. The comparisons between and within group at pre, after 1st half and after 2nd half of simulated soccer matches were showed in Table 4.3.

4.3.1 Heart rate

Subjects' heart rate at pre, after 1st half and after 2nd half of simulated soccer matches compared between placebo and glutamine supplementation were not significant different. However, subjects heart rate in both placebo and glutamine supplementation were significant increased after 1st half and after 2nd half compared to pre-simulated soccer matches ($p < 0.05$).

4.3.2 RPE

Subjects' RPE at after 2nd half of simulated soccer matches were significant different when compared between placebo and glutamine supplementation. Furthermore, subjects' RPE in both placebo and glutamine supplementation significantly

increased after 1st half and after 2nd half when compared to pre-simulated soccer matches ($p < 0.05$). In addition, significantly increase of RPE after 2nd half when compared to after 1st half of simulated soccer matches were observed in both placebo and glutamine supplementation ($p < 0.05$).

4.3.3 % Fatigue level

% Fatigue level at pre, after 1st half and after 2nd half of simulated soccer matches between placebo and glutamine supplementation were not significant different. However, % fatigue level in both placebo and glutamine supplementation were significant increase after 1st half and after 2nd half compared to pre-simulated soccer matches ($p < 0.05$).

Table 4.3 Fatigue parameters compared between and within groups at pre-, after 1st half and after 2nd half of soccer simulation protocol

Fatigue	Pre	1st half	2nd half	P - value
HR				
- Placebo	91.08 (15.92)	172.75 (14.78)[b]	168.92 (17.04)[b]	0.00[a]
- Glutamine supplementation	89.92 (9.15)	172.92 (14.79)[b]	168.17 (16.33)[b]	0.00[a]
P - value	0.83	0.97	0.87	
RPE				
- Placebo	6	13[b]	15[b, c]	0.00[a]
- Glutamine supplementation	6	11[b]	13[b, c]	0.00[a]
P - value	1.00	0.07	0.01[d]	
% fatigue level				
- Placebo	11.29 (12.05)	67.79 (30.65)[b]	67.00 (27.30)[b]	0.00[a]
- Glutamine supplementation	5.88 (8.30)	51.08 (21.54)[b]	66.75 (30.27)[b]	0.00[a]
P - value	0.17	0.05	0.98	

[a] = Significant difference within groups ($p < 0.05$)

[b] = Significant difference within groups compared to pre-match ($p < 0.05$)

[c] = Significant difference within groups compared to 1st half of the match ($p < 0.05$)

[d] = Significant difference between groups ($p < 0.05$)

4.4 Glutamine supplementation on blood ammonia, uric acid, urea, lactate and glucose

During the test, subjects' blood sample were collected at pre-, post and 1 h post of simulated soccer matches for analysis of ammonia, uric acid, urea and lactate whereas glucose was analyzed only at pre-match. The comparisons between and within groups of blood chemistry in placebo and glutamine supplementation at pre, post and 1 h post were shown in Table 4.4 and Figure 4.4 (a – d).

4.4.1 Ammonia

Blood ammonia concentration at pre, post and 1 h post of simulated soccer matches compared between placebo and glutamine supplementation were not significant different. However, immediate post-match ammonia concentration significantly increased compared to pre-simulated soccer matches. Then, ammonia concentration in both placebo and glutamine supplementation decreased 1 h post compared to post of simulated soccer matches ($p < 0.05$).

4.4.2 Uric acid

Uric acid concentration at pre, post and 1 h post of simulated soccer matches in both placebo and glutamine supplementation groups were not significant different. However, uric acid concentration in both placebo and glutamine supplementation significantly increased at post and 1 h post compared to pre-simulated soccer matches ($p < 0.05$).

4.4.3 Urea

Urea concentration at post and 1 h post of simulated soccer matches in placebo and glutamine supplementation groups were significant different. However, urea concentration at post and 1 h post match significantly increased from pre-simulated soccer match in both placebo and glutamine supplementation ($p < 0.05$).

4.4.4 Lactate

There were no significant different on lactate concentration at pre, post and 1 h post of simulated soccer matches between placebo and glutamine supplementation

groups. However, lactate concentration at post-match significantly increased from pre-simulated soccer matches. Then, lactate concentration in both placebo and glutamine supplementation at 1 h post match decreased from immediate-post-simulated soccer matches ($p < 0.05$).

4.4.5 Glucose

Blood glucose concentrations at pre-simulated soccer matches in both placebo and glutamine supplementation groups were not significant different.

Table 4.4 Blood chemistry compared between and within groups at pre, post and 1 h post simulated protocol

Blood chemistry	Pre	Post	1 h post	P - value
Ammonia (μg/dL)				
- Placebo	78.68 (17.33)	112.38 (30.45)[b]	80.77 (18.92)[c]	0.00[a]
- Glutamine supplementation	78.13 (12.47)	106.59 (21.23)[b]	82.21 (19.22)[c]	0.00[a]
P - value	0.92	0.54	0.82	
Uric acid (mg/dL)				
- Placebo	6.65 (1.34)	7.31 (1.37)[b]	7.36 (1.34)[b]	0.00[a]
- Glutamine supplementation	6.49 (1.14)	7.25 (1.19)[b]	7.21 (1.20)[b]	0.00[a]
P - value	0.54	0.77	0.41	
Urea (mg/dL)				
- Placebo	29.25 (4.34)	33.48 (4.80)[b]	34.03 (4.66)[b]	0.00[a]
- Glutamine supplementation	30.96 (5.24)	40.48 (6.83)[b]	40.70 (7.37)[b]	0.00[a]
P - value	0.31	0.01[d]	0.02[d]	
Lactate (mmol/L)				
- Placebo	2.20 (0.53)	3.47 (0.99)[b]	2.13 (0.53)[c]	0.00[a]
- Glutamine supplementation	2.25 (0.68)	2.81 (0.69)[b]	2.27 (0.27)[c]	0.00[a]
P - value	0.79	0.12	0.45	

Table 4.4 (Cont.)

Blood chemistry	Pre	Post	1 h post	P - value
Glucose (mg/dL)				
- Placebo	95.00 (16.32)	-	-	-
- Glutamine supplementation	87.33 (7.75)			
P - value	0.12			

[a] = Significant difference within groups ($p < 0.05$)

[b] = Significant difference within groups compared to pre-match ($p < 0.05$)

[c] = Significant difference within groups compared to immediate post-match ($p < 0.05$)

[d] = Significant difference between groups ($p < 0.05$)

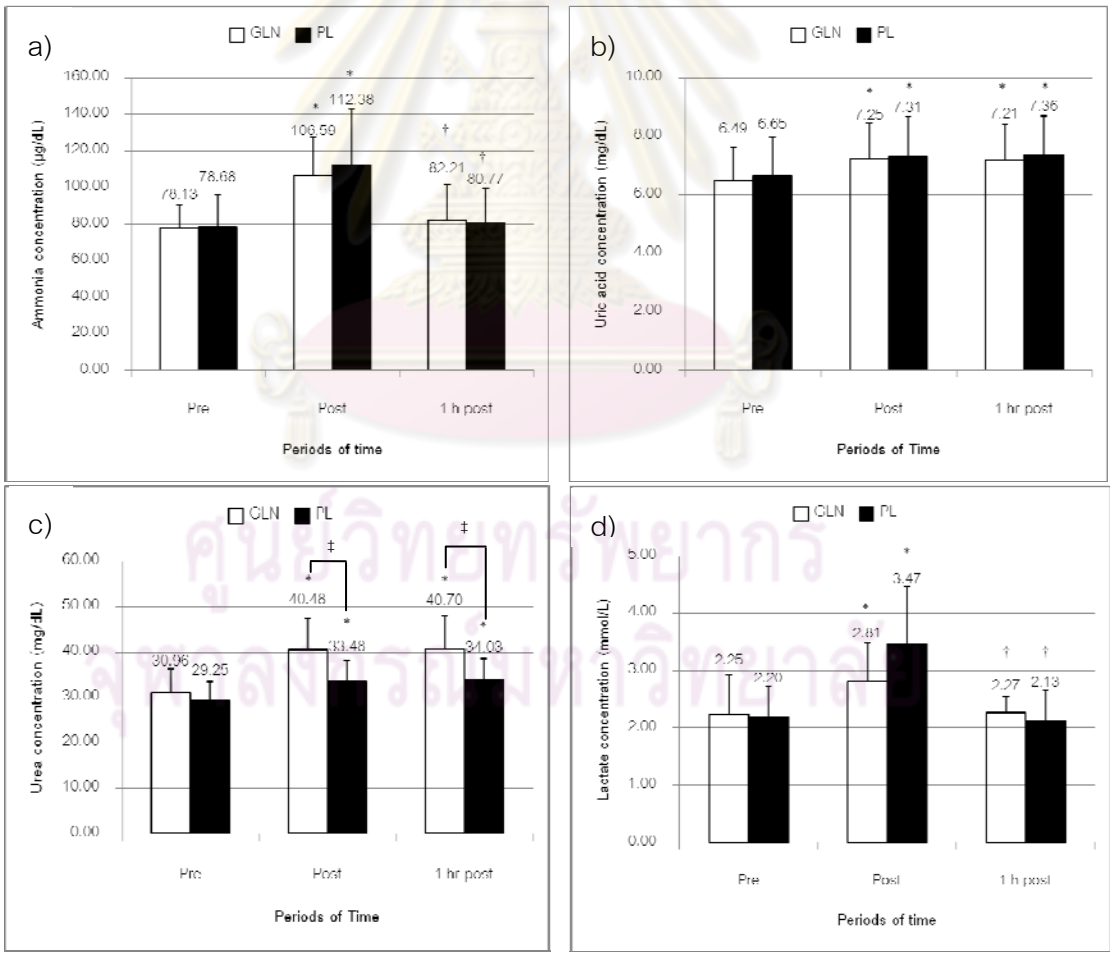

Figure 4.4 Blood chemistry compared between and within group at pre, post and 1 h post

* = Significant difference within groups compared to pre-match ($p < 0.05$)

† = Significant difference within groups compared to immediate post-match ($p < 0.05$)

‡ = Significant difference between groups ($p < 0.05$)

4.5 Effects of glutamine supplementation on leucocytes number after simulated soccer matches

During the test, subjects' blood sample at pre-, immediate-post and 1 h post-simulated soccer matches were collected for assay of CBC, included white blood cells, neutrophils, monocytes, lymphocyte, eosinophils, basophils, Hct and MCV. The comparisons between and within groups of CBC at pre-, immediate-post and 1 h post-match were shown in Table 4.5 and Figure 4.5 (a – d).

4.5.1 White blood cells count assay

The WBC of pre, immediate-post and 1 h post of simulated soccer matches in both placebo and glutamine supplementation groups were not significant different. However, significant increases of WBC in both placebo and glutamine supplementation at immediate-post and 1 h post-match from pre-simulated soccer matches were observed.

4.5.2 Neutrophils assay

Neutrophils number at pre, immediate-post and 1 h post of simulated soccer matches compared between placebo and glutamine supplementation were not significant different. However, neutrophils number significantly increased at immediate-post and 1 h post compared to pre of simulated soccer matches in both placebo and glutamine supplementation. Furthermore, neutrophils number was still significant increase at 1 h post-match from immediate-post of simulated soccer matches in placebo group.

4.5.3 Monocyte assay

Monocytes number at pre, post and 1 h post of simulated soccer matches compared between placebo and glutamine supplementation were not significant

different. However, monocytes number in both placebo and glutamine supplementation at immediate post and 1 h post-match significantly increased from pre-simulated soccer matches.

4.5.4 Lymphocytes assay

Lymphocytes number at pre, post and 1 h post of simulated soccer matches compared between placebo and glutamine supplementation were not significant different. Subjects in placebo group had significant increase of lymphocytes number at immediate post and 1 hr post match from pre-match. Subjects in glutamine supplementation group showed significant decrease of lymphocytes number at 1 h post compared to pre and post of simulated soccer matches.

4.5.5 Eosinophils assay

Eosinophils number at pre, immediate post and 1 h post of simulated soccer matches in both placebo and glutamine supplementation groups were not significant different. However, eosinophils number at 1 h post match in both groups significantly decreased from pre and post of simulated soccer matches.

4.5.6 Basophils assay

Basophils number at pre, immediate post and 1 h post of simulated soccer matches in both placebo and glutamine supplementation were not significant different. However, basophils significantly increased post when compared to pre of simulated soccer matches both placebo and glutamine supplementation. Moreover, basophils decreased 1 h post when compared post of simulated soccer matches both placebo and glutamine supplementation ($p < 0.05$).

4.5.7 Hct

Hct at 1 h post of simulated soccer matches compared between placebo and glutamine supplementation were significant different. However, Hct at post-match significantly increased from pre-simulated soccer matches in both groups. Then, Hct at

1 h post match significantly decreased compared to post of simulated soccer matches ($p < 0.05$).

4.5.8 MCV

MCV at pre, post and 1 h post of simulated soccer matches in placebo and glutamine supplementation were not significant different. However, MCV at post and 1 h post match significantly decreased from pre-simulated soccer matches in both placebo and glutamine supplementation ($p < 0.05$).

Table 4.5 Comparison of complete blood count (CBC) between and within group at pre, post and 1 h post simulated soccer match

CBC	Pre	Post	1 h post	P - value
WBC (10^3/μL)				
- Placebo	6.02 (0.82)	9.89 (1.81)[b]	9.91 (2.54)[b]	0.00[a]
- Glutamine supplementation	6.38 (1.12)	10.17 (1.75)[b]	10.19 (2.67)[b]	0.00[a]
P – value	0.28	0.69	0.76	
Neutrophils (10^3/μL)				
- Placebo	3.34 (0.68)	6.08 (1.53)[b]	7.21 (2.47)[b, c]	0.00[a]
- Glutamine supplementation	3.48 (0.77)	6.67 (2.08)[b]	7.69 (2.58)[b]	0.00[a]
P – value	0.48	0.43	0.59	
Monocytes (10^3/μL)				
- Placebo	0.30 (0.07)	0.49 (0.10)[b]	0.46 (0.12)[b]	0.00[a]
- Glutamine supplementation	0.34 (0.08)	0.52 (0.11)[b]	0.48 (0.12)[b]	0.00[a]
P – value	0.17	0.38	0.51	
Lymphocyte (10^3/μL)				
- Placebo	1.97 (0.36)	2.85 (0.62)[b]	1.89 (0.48)[c]	0.00[a]
- Glutamine supplementation	2.19 (0.37)	2.63 (0.79)	1.75 (0.52)[b, c]	0.00[a]
P – value	0.11	0.11	0.27	

Table 4.5 (cont.)

CBC	Pre	Post	1 h post	P - value
Eosinophils (10^3/μL)				
- Placebo	0.37 (0.35)	0.40 (0.43)	0.31 (0.34)[b, c]	0.01[a]
- Glutamine supplementation	0.33 (0.28)	0.30 (0.27)	0.23 (0.23)[b, c]	0.00[a]
P – value	0.34	0.11	0.10	
Basophils (10^3/μL)				
- Placebo	0.04 (0.02)	0.07 (0.02)[b]	0.04 (0.02)[c]	0.00[a]
- Glutamine supplementation	0.05 (0.03)	0.06 (0.03)[b]	0.05 (0.02)[c]	0.00[a]
P – value	0.42	0.36	0.41	
Hct (%)				
- Placebo	43.38 (2.26)	44.74 (1.73)[b]	42.79 (1.64)[c]	0.00[a]
- Glutamine supplementation	44.21 (2.49)	45.28 (2.24)[b]	43.68 (2.12)[c]	0.00[a]
P – value	0.10	0.17	0.02[d]	
MCV (fL)				
- Placebo	83.32 (4.72)	82.68 (4.70)[b]	82.68 (4.64)[b]	0.00[a]
- Glutamine supplementation	83.60 (4.62)	82.98 (4.70)[b]	83.04 (4.86)[b]	0.00[a]
P – value	0.11	0.11	0.21	

[a] = Significant difference within groups ($p < 0.05$)

[b] = Significant difference within groups compared to pre-match ($p < 0.05$)

[c] = Significant difference within groups compared to immediate post-match ($p < 0.05$)

[d] = Significant difference between groups ($p < 0.05$)

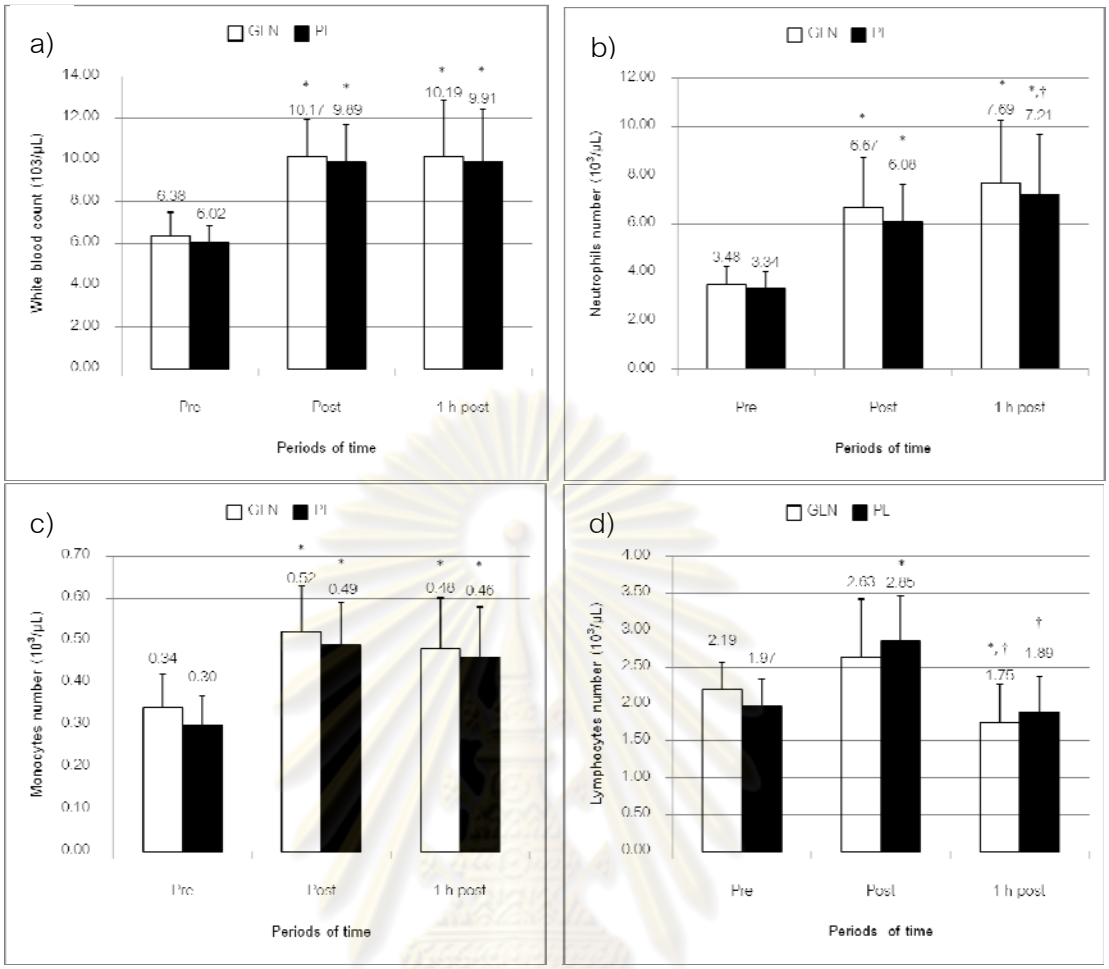

Figure 4.5 Complete blood count (CBC) compared between and within group at pre, post and 1 h post

* = Significant difference within groups compared to pre-match ($p < 0.05$)

† = Significant difference within groups compared to immediate post-match ($p < 0.05$)

CHAPTER V
DISCUSSION AND CONCLUSION

The purpose of this study was to investigate the effects of glutamine supplementation on leucocytes number and fatigue after simulated soccer matches in Thai youth soccer players. Twelve male soccer players volunteered to participate in this study with written informed consents. The characteristics of the subjects in table 4.1 showed that the maximal oxygen consumption was high. This indicated that the subjects represented those who had high cardiovascular fitness.

Physical performance

The physical performance according to the BEAST protocol (simulated soccer matches) included circuit time, 12 m sprint time, 20 m sprint time and sport specific skill (vertical jump and shooting accuracy). The results demonstrated subjects in glutamine supplementation group had more tolerance to fatigue than subjects in placebo group. Glutamine supplementation significant augmented 20 m sprint performance during 1^{st} half and 2^{nd} half. In addition, RPE of glutamine supplementation subjects was significant lower at the end of 2^{nd} half.

During soccer, it is common to observe a gradual decline in physical performance. Specifically, Abt et al. (2003) reported 8% drop in sprint performance after a 90 minute non-motorized treadmill simulation protocol. The results in this study also showed decrease of 20 m sprint time after the BEAST at 6.09% in placebo group and 4.18% in glutamine supplementation group. Thus glutamine supplementation exhibited a better sprint performance than placebo.

Williams et al. (2009) reported 4.33% decrease of vertical jump after 90 minute soccer performance test ($BEAST_{90}$). A decrease of 3.58% vertical jump in glutamine supplementation and 0.80% in placebo after the BEAST were observed suggesting that the BEAST induced fatigue. However, it was unclear whether glutamine supplementation has effect on vertical jump performance.

Effects of glutamine supplementation on fatigue after simulated soccer protocol

Fatigue parameters including heart rate, RPE and %fatigue level were found significant difference within groups compared to pre-exercise. Moreover, mode of RPE level in glutamine supplementation at 2nd half (the end of simulated soccer match) was significant lower than placebo. The lower RPE represented a lower psychological fatigue. Similar results of %fatigue level were also observed.

Following competitive soccer match play, an average intensity was closed to the anaerobic threshold, being 80%-90% of maximal heart rate (HR_{max}) (Helgerud, 2001; Reilly, 1994; Van Gool, 1988). Heart rate at the end of 1st half and 2nd half in both groups were approximately 85% and 83% of HR_{max} respectively demonstrated intensity of simulated soccer match in this study was close to anaerobic threshold.

Metabolism of glutamine on ammonia, uric acid, urea, lactate and glucose

Ammonia has been used as an indicator of metabolic activity during exercise because it is associated with the requirement and production of ATP. Previous study showed that high ammonia levels can be toxic to both muscles and the central nervous system (CNS), and induce peripheral and central fatigue (Banister and Cameron, 1990; Castell and Newsholme, 1997; Eriksson et al., 1985; Graham et al., 1997; Sahlin et al., 1990). This study showed that ammonia concentration at pre, immediate post and 1 h-post of simulated soccer matches were not significant different between placebo and glutamine supplementation groups. Due to decrease the toxic effect caused by increased ammonia, the CNS enhances glutamine (Gln) synthesis to buffer free ammonia. The concentrations of plasma Gln decrease as a function of exercise intensity because of the increase in gluconeogenesis and urea synthesis (Cameron et al., 2007). It was consistent with this study that blood urea concentration raised from baseline at the end of the games and continue rising at 1 h-post soccer game. The significant increase of urea concentration in glutamine supplementation group confirmed the increase metabolism of glutamine in this group.

Boyum et al. (2002) showed that uric acid and urea increase during high-intensity exercise in response to the IMP and ammonia clearance demand. The result of this study showed that uric acid concentration in pre, immediate post and 1 h post of

simulated soccer matches were not significant different between groups. Moreover, urea concentration of immediate post and 1 h post of simulated soccer matches compared between placebo and glutamine supplementation were significant difference. However, uric acid and urea concentration significantly increased at immediate post and 1 h post compared to pre-simulated soccer matches both placebo and glutamine supplementation ($p < 0.05$). Glutamine supplementation had a lower uric acid concentration than placebo at immediate post and 1 h post-match. On the other hand, glutamine supplementation had significantly higher urea concentration than placebo at immediate post and 1 h post-match.

The intense exercise periods during a soccer game lead to high anaerobic-energy turnover with an associated accumulation of lactate and lowering of pH in the exercised muscles. In addition, after 90 minutes of standard training was significantly increased blood lactate (Karakoc et al., 2005). In this study showed that lactate concentration of pre, post and 1 h post of simulated soccer matches when compared between placebo and glutamine supplementation were not significant difference. However, lactate concentration significantly increased post when compared to pre of simulated soccer matches. At post-match, lactate concentration in glutamine supplementation was lower than placebo. Moreover, Karakoc et al. (2004) showed that the increase in plasma glucose concentration after training may be due to raised blood lactate concentration. On the other hand, lactate concentration decreased 1 h post when compared to post of simulated soccer matches both placebo and glutamine supplementation ($p < 0.05$). After exercise, excess lactate is converted back into glucose in the liver. This newly made glucose can then be used to resynthesise the glycogen depleted during exercise (Abernethy, 1997).

In this study showed that glucose concentration at pre of simulated soccer matches when compared between placebo and glutamine supplementation were not significant different. Mean and standard deviation of placebo and glutamine supplementation groups were 95.00 (16.32) mg/dL and 87.33 (7.75) mg/dL respectively which was in normal range (< 110 mg.dL) (Ensminger et al., 1994).This was to confirm that muscle cells may use glutamine as energy fuel in the supplementation. Following catabolism of glutamine as energy fuel, production of ammonia as waste product would

be increase. At the same time that glucose was not utilized as the only fuel, amount of lactic acid production would be decrease.

Blood cells

White blood cell counts increase after both laboratory and field exercise and at the end of a football training session compared with baseline values (Perez et al., 2001; Rebelo, 1998). This study showed significantly increased of white blood cell numbers at immediate post and 1 h-post compared to pre-simulated soccer matches in both placebo and glutamine supplementation. Glutamine supplementation had increased higher than placebo due to glutamine is utilized at high rates by leukocytes (particularly lymphocytes) to provide energy and optimal conditions for nucleotide biosynthesis and hence, cell proliferation (Ardawi and Newsholme, 1983). The increase of white blood cell numbers in response to exercise in these subjects may be attributed to a demargination process caused by exercise or adrenaline (epinephrine) administration. Normally about half of the blood leucocytes are in a marginal pool – that is, loosely adherent to the vascular endothelium or trapped in the microcirculation. With exercise or adrenaline administration, these cells are released into the circulating pool and the leucocyte count rises, a process called demargination (Bunn, 1991).

Neutrophil numbers had significantly increased at the end of a football training session compared with baseline values (Rebelo, 1998). Another study showed numbers of circulating neutrophils had returned to baseline in the glutamine group compared to the placebo group, in whom they were still slightly elevated (Castell and Newsholme, 1998). In this study showed that neutrophil numbers had significantly increased at immediate post and 1 h-post compared to pre-simulated soccer matches in both placebo and glutamine supplementation. In addition, neutrophil numbers significantly increased at 1 h-post match compared to immediate post- simulated soccer matches in placebo. When compared between placebo and glutamine supplementation in pre, post and 1 h post was not significant difference. Glutamine supplementation had increased higher than placebo due to free oxygen radicals and prostaglandin released by the elevated number of neutrophils (Pedersen and Hoffman-Goetz, 2000).

The circulating monocyte numbers have been found to increase or not change during and after acute exercise and may be decreased during prolonged periods of intense endurance training (Mackinnon, 1999; Woods et al., 2000). In this study showed that monocyte numbers had significantly increased at post and 1 h post compared to pre simulated soccer matches both placebo and glutamine supplementation. When compared between placebo and glutamine supplementation in pre, post and 1 h post was not significant difference. Glutamine supplementation had increased higher than placebo due to monocytes may influence the function of lymphocytes and contribute to the impaired function of the later cells (Pedersen and Hoffman-Goetz, 2000).

Lymphocytes usually increase in circulation during exercise and return to baseline levels or below within a few hours after exercise (Malm, 2004). In same, exercise increases the concentration of lymphocytes in peripheral blood; however, 1 – 2 hours into the recovery, the lymphocytes count decreases to, or even to below, the pre-exercise level (Castell, 1997; Nielsen, 2003). In this study showed that when subjects received placebo, lymphocyte numbers had significantly increased at post compared pre of simulated soccer matches. Moreover, lymphocytes number significantly decreased 1 h post when compared to pre of simulated soccer matches. While subjects received glutamine, lymphocytes number significantly decreased 1 h post when compared to pre and post of simulated soccer matches. When compared between placebo and glutamine supplementation in pre, post and 1 h post was not significant difference. Glutamine supplementation had increased and decreased lower than placebo due to glutamine is used at a high rate by lymphocytes and macrophages. It is oxidized as a fuel and for the synthesis of DNA and RNA (Venkatraman and Pendergast, 2002).

Keen et al., 1995 showed that during a major multi-stage race in the evening, individual eosinophil numbers was inside the normal range both in the morning and in the evening. In the evening, eosinophil numbers was not significantly decreased 16%, it is likely that the samples were taken as the count was still falling in the morning, and rising again in the evening, leaving the observed values little different. However, in this study showed that eosinophil numbers had significantly decreased 1 h post compared pre and post of simulated soccer matches both placebo and glutamine

supplementation. When compared between placebo and glutamine supplementation in pre, post and 1 h post was not significant difference.

During a major multi-stage race in the evening, those of individual basophils was inside the normal range both in the morning and in the evening. In the evening, basophil numbers increased 28% higher than morning counts (Keen et al., 1995). However, in this study showed that basophils significantly increased post when compared to pre of simulated soccer matches both placebo and glutamine supplementation. In addition, basophils decreased to be the same level as the pre of simulated soccer matches ($p < 0.05$). When compared between placebo and glutamine supplementation in pre, post and 1 h post was not significant difference.

The result of this study showed that Hct of 1 h post of simulated soccer matches when compared between placebo and glutamine supplementation were significant difference. On the other hand, MCV of pre, post and 1 h post of simulated soccer matches compared between placebo and glutamine supplementation were not significant difference. Bassini-Cameron et al. (2007) showed that Hct have been significantly increased after 90 minutes of soccer training. In this study, Hct significantly increased post when compared to pre of simulated soccer matches both placebo and glutamine supplementation. Glutamine supplementation had a higher Hct than placebo at immediate post and 1 h post-match. Moreover, Hct significantly decreased 1 h post when compared to post of simulated soccer matches both placebo and glutamine supplementation. However, MCV have been significantly decreased after 90 minutes of soccer training (Karakoc et al., 2005). In this study, also MCV significantly decreased post and 1 h post when compared to pre of simulated soccer matches both placebo and glutamine supplementation ($p < 0.05$). Glutamine supplementation had a higher MCV than placebo at immediate post and 1 h post-match.

Conclusion

This study demonstrated that glutamine supplementation tended to increase higher than placebo in WBC, neutrophils and monocytes of the innate immune system which was responsible for inflammation process from exercise induced injury. In addition, there was lower blood lactate in glutamine supplementation and significant

higher blood urea in glutamine supplementation group, suggesting less fatigue and faster ammonia clearance after simulated soccer matches. Thus glutamine supplementation may improve immune function.

Prior to this study, glutamine supplementation was always deployed after the exercise to prevent the upper respiratory infection and improve immune function of the athletes. However, this study demonstrated the trend that glutamine supplementation at the dose of 100 mg/kg body weight for five days before the soccer games not only improved immune function but also helped slow down the fatigue.

Further study should be conducted to confirm this finding by increasing number of volunteers, developing the more efficient protocol for lactate analysis, and assay for blood glutamine level to support the relationship of metabolism of glutamine and lactic acid production.

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APPENDICES

Appendix A

Test for reliability of cross over research design

Cross over research design was used in this study. The data might be influenced by not enough time for washout period (period effect) or the first drink had effect on the second drink (treatment effect). In order to demonstrate that two weeks washout period was enough to overcome the period effect and the treatment effect, statistical analysis were performed as follows.

– **Period effect**: Mean difference of post and pre-simulated soccer game blood ammonia concentration was compared between subjects in program A and subjects in program B as shown in **Table A.1**. There was no significant different between two groups (p=0.43) demonstrated that two weeks washout period was long enough to avoid the carry over effect.

– **Treatment period interaction**: Mean difference of post and pre-simulated soccer game blood ammonia concentration was compared within each subjects at in program A and subjects in program B as shown in **Table A.2**. Comparison of means of ($\triangle$G + $\triangle$P)/2 between program A and program B using the independent sample t-test showed p=0.46. Therefore, no treatment period interaction in this study was observed.

The operational definition of terms used in Table A.1 and A.2 are as follows.

Program A* = Subjects received glutamine supplementation followed by 2 weeks washout and receiving placebo drink (N = 7)

Program B** = Subjects received placebo drinks followed by 2 weeks washout and receiving glutamine supplementation drink (N = 5)

$\triangle$G = Post and pre-game blood ammonia difference when received glutamine supplementation

$\triangle$P = Post and pre-game blood ammonia difference when received placebo drink

*** = Significant difference between program ($p < 0.05$) using independent sample t-test.

Table A.1 Comparison of blood ammonia mean difference between pre- and post-simulated soccer games in subjects who participated in program A*/ program B** in order to investigate 2 weeks washout period effect (period effect) (N = 7 and 5 respectively).

Subject no	Ammonia (µg/dL)		
	△G	△P	△G - △P
Program A*			
1	22.3	4.0	18.3
2	34.7	100.1	-65.4
3	83.8	8.4	75.4
5	7.5	38.0	-30.5
6	53.6	34.5	19.1
8	15.9	7.1	8.8
9	32.8	42.1	-9.3
Mean	35.8	33.5	2.3
SD	25.9	33.5	44.2
Program B**			
4	33.7	-18.2	-51.9
7	53.2	43.8	-9.4
10	41.6	32.2	-9.4
11	22.6	-1.3	-23.9
12	19.0	34.4	15.4
Mean	34.0	18.2	-15.8
SD	14.0	26.5	24.6
P value*** of mean(SD) of mean difference of program A and B =0.43			

Table A.2 Comparison of blood ammonia mean difference between pre- and post-simulated soccer games in subjects who participated in program A*/ program B** in order to test for treatment period interaction of the study (N = 7 and 5 respectively).

Subject no	Ammonia (μg/dL)		
	△G	△P	(△G + △P)/2
Program A*			
1	22.3	4.0	13.2
2	34.7	100.1	67.4
3	83.8	8.4	46.1
5	7.5	38.0	22.8
6	53.6	34.5	44.1
8	15.9	7.1	11.5
9	32.8	42.1	37.5
Mean	35.8	33.5	34.6
SD	25.9	33.5	20.2
Program B**			
4	33.7	-18.2	7.8
7	53.2	43.8	48.5
10	41.6	32.2	36.9
11	22.6	-1.3	10.7
12	19.0	34.4	26.7
Mean	34.0	18.2	26.1
SD	14.0	26.5	17.3
P value*** of mean(SD) of (△G + △P)/2 of program A and B =0.46			

Appendix B

Performance tasks at each station of the BEAST

1) Lap 1 (half circuit)

Station	Task	Task Description	Performance measure
1	3 m walk	Walk to next station	
2	Sprint 12 m	12 m Straight line sprint, slow 23 m	Time
3	Run	75% run 5 m	
4	Stop	Stop 8 sec	
5	Walk	Walk 5 m	
6	Run	75% run 10 m	
7	Decelerate	Decelerate 15 m	
8	Stop	Stop 8 sec	
9	Walk	Walk 15 m	
10	Run	75% run 10 m	
11	Decelerate	Decelerate 5 m	
12	Stop	Stop 8 sec	
13	Run	75% run 5 m	
14	Slalom	Weave through 3 cones 100%	
15	Stop	Stop 8 sec	
16	Walk	Walk 7 m	
17	Run	75% run 28 m	
18	Decelerate	Decelerate 10 m	
19	Walk	Walk 5 m	
20	Backpedal	Jog backwards 7 m	

2) Lap 2 (half circuit)

Station	Task	Task Description	Performance measure
1	3 m walk	Walk to next station	
2	Sprint 20 m	20 m straight line sprint, slow 15 m	Time
3	Run	75% run 5 m	
4	Stop	Stop 8 sec	
5	Walk	Walk 5 m	
6	Run	75% run 10 m	
7	Decelerate	Decelerate 15 m	
8	Stop	Stop 8 sec	
9	Walk	Walk 15 m	
10	Run	75% run 10 m	
11	Decelerate	Decelerate 5 m	
12	Stop	Stop 8 sec	
13	Run	75% run 5 m	
14	Slalom	Weave through 3 cones 100%	
15	Stop	Stop 8 sec	
16	Walk	Walk 7 m	
17	Run	75% run 28 m	
18	Decelerate	Decelerate 10 m	
19	Walk	Walk 5 m	
20	Backpedal	Jog backwards 7 m	
21	Accuracy	Shoot at target x 6 balls	Number of goals
22	Jump	X 3 Vertical jumps	Jump height
23	RPE	Rate of perceived exertion	RPE Scale 6 - 20

BIOGRAPHY

Miss Natkrita Imkrajang was born on November 04, 1981 in Phitsanulok province, Thailand. She received Bachelor Degree in Science from the Faculty of Physical Education, Srinakharinwirot University in 2006.