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EFFECT OF CONDITION TRAINING IN THAI SOCCER PLAYERS ON GLUTATHIONE ANTIOXIDANT

Miss Sureeporn Satityanuruk

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สุรีพร สถิตยานุรักษ์: ผลการฝึกสมรรถภาพ ในนักกีฬาฟุตบอลไทย ต่อการเปลี่ยนแปลงของกลูตาไทโอนแอนติออกซิแดนท์ (Effects of Condition Training in Thai Soccer Players on Glutathione Antioxidant) อ. ที่ปรึกษา: ผศ.ดร.วิไล อโนมะศิริ,, 56 หน้า ISBN 974-17-0488-7

สมรรถภาพร่างกายที่ดีของนักกีฬาฟุตบอลมีความสำคัญสำหรับนักกีฬาในการแข่งขัน การออกกำลังกายมีผลต่อการเพิ่มขึ้นของอนุมูลอิสระ และ ลดการทำงานของตัวต้านอนุมูลอิสระภายในร่างกาย วัตถุประสงค์ของการศึกษาวิจัยนี้ คือ ศึกษาการเปลี่ยนแปลงของกลูตาไทโอนแอนติออกซิแดนท์ ในนักกีฬาฟุตบอลจำนวน 22 คน ที่ทำการฝึกซ้อมตามโปรแกรมการฝึก ของโรงเรียนอัสสัมชัญศรีราชา โดยทำการเก็บตัวอย่างเลือดก่อนการฝึกซ้อม หลังการฝึกซ้อมหนึ่งสัปดาห์ หลังฝึกซ้อมสองสัปดาห์ และ หลังการแข่งขันเรียบร้อยแล้ว ผลการศึกษาสมรรถภาพร่างกายของกลุ่มทดลองเปรียบเทียบกับกลุ่มอ้างอิงในเพศชาย ที่มีช่วงอายุเดียวกัน พบว่า ในกลุ่มนักกีฬาฟุตบอลมีค่าความแตกต่างจากกลุ่มอ้างอิง คือ อัตราการเต้นของหัวใจ (67.54±10.06 ครั้ง/นาที) อัตราการใช้ออกซิเจนภายในร่างกาย(50.03±12.10 มล./นน.ตัว/นาที) และ กำลังของกล้ามเนื้อขา (3.00±0.054 กก./กก. นน.ตัว) ที่ดีกว่ากลุ่มอ้างอิงที่มีอัตราการเต้นของหัวใจ (78±10.53 ครั้ง/นาที) อัตราการใช้ออกซิเจนภายในร่างกาย (45.60±9.98 มล./นน.ตัว/นาที) และ กำลังของกล้ามเนื้อขา (2.24±0.052 กก./กก. นน.ตัว) การเปลี่ยนแปลงปริมาณของรีดิวซ์กลูตาไทโอนในเลือดพบว่ามีค่าลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เมื่อเปรียบเทียบค่าก่อนการฝึกซ้อมกับหลังทำการฝึกซ้อมหนึ่งสัปดาห์ และหลังจากนั้นค่าจะค่อยๆกลับสู่ค่าใกล้เคียงค่าก่อนการฝึกซ้อม นอกจากนี้กลูตาไทโอนเปอร์ออกซิเดส พบมีการเปลี่ยนแปลงไปในทิศทางเดียวกับปริมาณของรีดิวซ์กลูตาไทโอน การฝึกซ้อมในโปรแกรมซึ่งทำการฝึกสองชั่วโมงต่อวัน ห้าวันต่อสัปดาห์ ประกอบด้วย อบอุ่นร่างกาย 15 นาที ฝึกพื้นฐาน 20 นาที ฝึกแข่งขันเกมเล็ก 20 นาที ฝึกทักษะ 20 นาที ฝึกแข่งขันเกมใหญ่ 30 นาที และ คลายกล้ามเนื้อ 15 นาที มีอัตราการเต้นของหัวใจเฉลี่ยในแต่ละช่วงการฝึกซ้อม ดังนี้ 118.37±11.18 ครั้ง/นาที 132.98±15.05 ครั้ง/นาที 130.48±10.41ครั้ง/นาที 141.24±22.7 ครั้ง/นาที 140.10±29.8 ครั้ง/นาที 148.60±27 ครั้ง/นาที ตามลำดับ และ อัตราการเต้นของหัวใจเฉลี่ยตลอดโปรแกรมมีค่า 134.37±8.86 ครั้ง/นาที ซึ่งคิดเป็นร้อยละ 66.5 ของอัตราการเต้นของหัวใจสูงสุด จึงนับว่า โปรแกรมการฝึกสมรรถภาพร่างกายที่ใช้ในการศึกษานี้ เป็นการออกกำลังแบบหนักปานกลาง จากการศึกษาพบว่า ผลการฝึกสมรรถภาพร่างกายทำให้ระดับของกลูตาไทโอนแอนติออกซิแดนท์นั้นลดลงในสัปดาห์แรกหลังการฝึกซ้อม ซึ่งแสดงถึงการมีอนุมูลอิสระเพิ่มขึ้น ดังนั้นโปรแกรมการฝึกซ้อมควรจะเป็นแบบค่อยเป็นค่อยไปในช่วงแรก เพื่อให้ร่างกายเกิดความเคยชิน และค่อยๆเพิ่มความหนักตามลำดับ เพื่อป้องกันการเกิดอันตรายเนื่องจากการเพิ่มขึ้นของอนุมูลอิสระในช่วงสัปดาห์แรก ตามที่แสดงผลในการศึกษานี้

หลักสูตร...เวชศาสตร์การกีฬา..... ลายมือชื่อนิสิต.....

สาขาวิชา...เวชศาสตร์การกีฬา..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2544..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....-.....

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KEY WORD: CONDITION TRAINING, GLUTATHIONE ANTIOXIDANT

SUREEPORN SATITYANURUK: EFFECT OF CONDITION TRAINING IN THAI SOCCER PLAYERS ON GLUTATHIONE ANTIOXIDANT. THESIS ADVISOR: ASSIST. PROF. WILAI ANOMASIRI, Ph.D .pp.56 ISBN 974-17-0488-7

Physical exercise is importance for soccer players in competition. However, physical exercise can induce free radicals and reduce endogenous antioxidants. The aim of this work is to determine the glutathione antioxidant status in 22 soccer players engaged in condition training program at Assumption College Sriracha. Blood samples were taken at pre-training, 1st week, 2nd week of training and post-competition. The results showed that physical characteristics of the subjects were compared with reference group the same age-range and sex. The anthropometric parameters that showed slightly difference were heart rate (67.54±10.06 beats /min), VO_2max (50.03±12.10ml/kg/min) and leg muscle strength (3.00±0.054kg/kg body weight) when compared to reference group's heart rate (78.32±10.53 beats /min), VO_2max (45.60±9.98ml/kg/min) and leg muscle strength (2.24±0.052kg/kg body weight). The results showed that blood reduced glutathione was significantly decrease after 1st week of training program and slowly restored to the pre-training level in the post-competition. Erythrocyte glutathione peroxidase in the subject's blood taken at the same time as blood reduced glutathione showed the same pattern of changes as reduced glutathione ($p<0.05$). The exercise intensity of program that was two hours training per day, five days per week including 15 min warm up, 20 min basic technique, 20 min small game, 20 min tactical technique, 30 min full game and 15 min cool down. The average heart rate of each activity were 118.37±11.18 beats /min, 132.98±15.05 beats /min, 130.48±10.41 beats /min, 141.24±22.7 beats /min, 140.10±29.81 beats /min and 148.60±27.0 beats /min respectively. The average heart rate of the entire program was 134.37±8.86 beats /min, which was 66.5% of maximum heart rate. Therefore, this program was considered to be the moderate intensity exercise according to the average heart rate. The glutathione antioxidant levels in this study demonstrated the significant decrease after the first week of training. In order to avoid this oxidative stress in young soccer players during the re-building period, coaches or trainers should consider to slowly increasing the training intensity at the beginning of the rebuilding period.

Program.....Sports Medicine..... Student's signature Sureeporn Satityanuruk

Field of study .....Sports Medicine... Advisor's signature Wilai Anomasiri

Academic year.....2001..... Co-advisor's signature .....

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LIST OF ABBREVIATION

ACSM	American College of Sports Medicine
BW	Body weight
dl	Deciliter
e.g.	Exempli gratia
Fig	Figure
GSH-Px	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
HR	Heart rate
HR max	Maximal heart rate
i.e.	Id est.
Kg	Kilogram
Km	Kilometer
L	Liter
LT	Lactate threshold
mg	Milligram
mm	Millimeter
mmol	Millimol
min	Minute
MPA	Metaphosphoric acid
ROS	Reactive oxygen species

SD	Standard deviation
SOD	Superoxide dismutase
µl	Microliter
VO_2	Oxygen uptake
VO_2 max	Maximum oxygen uptake
%	Percent

CHAPTER I

INTRODUCTION

Football (soccer) is one of the most widely play and complex sports in the world. The players need technical, tactical, and condition (physical skill) training to succeed. Exercise intensity in football ranges from standing and walking to sprinting, covering a mean distance closed to that observed during marathon running (70%-80% of the maximal oxygen uptake) (Helgerud J, Engen LC, Wisloff U and Hoff J, 2001 and Calbet JAL, Dorado C, Diaz-Herrera and Rodriguez-Rodriguez LP, 2001).

Physical fitness of football players is important at all levels of the game. Whilst being essential for top level players, it is beneficial for beginners who will improve both their effectiveness and enjoyment through good standards of fitness. The aim of fitness training in football is to enable a player to cope with physical demands of the game as well as to allow the efficient use of his various technical and tactical competencies throughout the match. Football players must be able to perform a prolonged intermittent exercise (endurance), high intensity, exercise sprint, and develop high level of power (force) when kicking and tackling. Good levels of agility and coordination are also necessary and distinguished between elite and average players (Wisloff U, Helgerud J and Hoff J, 1998).

Physical fitness, which includes power, muscular endurance, speed, flexibility, strength, and cardiorespiratory fitness can be acquired by training. The

physical training in soccer player is composed of several components (Fig.1.1), such as, aerobic training, anaerobic training, specific muscle training and coordination (Bangsbo J, 1994).

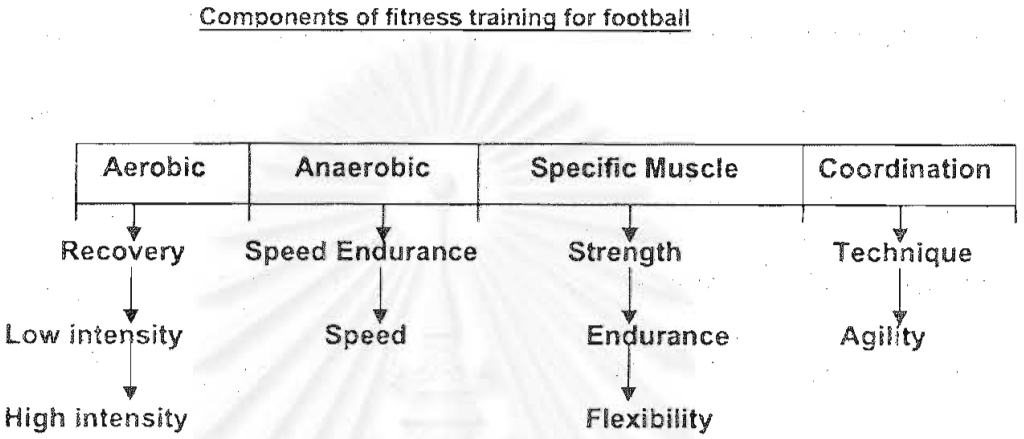


Figure 1.1 The major components involved in football specific training

In exercise physiology, the aerobic capacity of an individual is considered to be a widely accepted index for physical fitness. Thus, one of the primary goals of coaches, physical trainers, and athletes is to enhance the ability to consume atmospheric oxygen since oxidative metabolism is very energy cost efficient and avoids lactate formation during energy supply (Bangsbo J, 1994).

Physical exercise may increase skeletal muscle arteriovenous oxygen difference by three-fold and blood flow through the tissue by 30-fold. As a result, the players may have up to 100-fold increase in oxygen flux through the active skeletal muscle during exercise (Sen CK, 1995). An elevation of reactive oxygen

species (ROS) or free radicals accompanies the increase in oxygen uptake during exercise, which is considered to be oxidative stress.

A free radical is an atom molecule having an unpaired electron, which is very unstable, highly reactive. Under resting condition, the content in arterial and venous blood of the skeletal muscle tissue are 20 and 15 ml per 100 ml blood, respectively (Sen CK, 2001).

Catabolic and anabolic processes that occur in skeletal muscle during and after exercise are under the influence of various mediators in which oxygen free radical are major contributors. During exercise oxygen free radicals are released in muscle through mitochondria oxidative phosphorylation or from inflammatory cells, which may trigger and stimulate metabolic events that involve in the antioxidant defense system. Activated oxygen species appear to play a key role in exercise-induced injury to muscle membrane components and in exercise-induced the associated alteration of lysosomal and mitochondria enzyme activity. The magnitude of oxidative damage occurring after exercise is dependent on the rate of oxygen consumption and the dynamic balance of antioxidant and pro-oxidant cellular mechanism (Jacob RA and Burri BJ, 1996 and Mates JM et. al, 1999).

To prevent exercise induced oxidative stress, the organism is well equipped with an antioxidant defense system including enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), and non-enzymatic substances such as vitamins and reduced glutathione (GSH). Endurance

training can enhance the enzymatic antioxidant activity and the glutathione status (Margritis L, Tessier F, Richard MJ and Marconnet P, 1997).

Glutathione is well established as being important in the defense against cellular oxidative stress. In addition, it is a scavenger of hydroxyl radical and singlet oxygen. The reduced from of glutathione (GSH) is a substrate of glutathione peroxidase, which is involved in the decomposition of hydrogen and lipid peroxides. GSH is oxidized to be glutathione disulfide (GSSG) in response to oxidative stress (Dufaux B, Heine O, K othe A, Prinz U and Rost R, 1997). The ratio of reduced (GSH) vs oxidized glutathione (GSSG) is recognized as a sensitive measurement of oxidative stress. In humans, some conflicting results concerning the glutathione changes in blood after exercise have been published. Gohil et al. 1988 observed a 60% decrease of blood GSH in 8 subjects with a reciprocal rise in GSSG during a 90-min submaximal bicycle exercise bout. The essentially similar changes were obtained in the same group after a half marathon (21.1km). Duthie et al. (1990) reported a 36 % decrease of GSH in 7 well-trained subjects. The concentration of GSSG did not change significantly.

In 1999 Brites FD, investigated the lipoprotein profile and the plasma antioxidant status in soccer players engaged in a regular physical training consisted of 20 h of training and six soccer matches per week for at least 1 year. The results showed an improved plasma antioxidant status (significantly elevated levels of ascorbic acid and uric acid in the sportsmen) in comparison to sedentary controls.

Exercise training seems to reduce the incidence of oxidative stress in the body and increase a use of the antioxidant defense system. Antioxidant may not improve sporting performance but may be important in reducing damage during exhaustive exercise and during recovery from training, or injury. The most well known antioxidants are reduced glutathione, vitamin E and vitamin C (Inal M, Akyuz F, Turgut A and Getsfrid WM, 2001).

Since glutathione plays an important role in the maintenance of tissue antioxidant defense (Sen CK, 1999), this work was set to investigate whether the glutathione antioxidant system involved in the natural antioxidant defense system in soccer players who were trained by an exhaustive exercise during the re-building period and after the competition.

1.1 Research Question

How does condition training in soccer players effect on glutathione antioxidant?

1.2 Objective of this study

To study effect of condition training in soccer players on glutathione antioxidant

1.3 Assumptions

1. The recruited soccer players have continually experienced training 2 hours per day, 5 days per week and for at least one year.

2. Food consumption has no confounded effect on the studies.

1.4 Operational Definitions

Condition training: The soccer training program used at Assumption College Sriracha

1.5 Expected Benefits of the study

1. Evaluate the level of exercise intensity in soccer training program.

2. Assay the oxidative stress in soccer players according to the glutathione antioxidant

3. The result could be applied for the requirement of glutathione supplements.

CHAPTER II

LITERATURE REVIEW

Football (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. It is estimated that the mean distance covered during the game closes to 11 km at an average intensity comparable to that was observed during marathon running (70% -80% of the maximal oxygen uptake). This closes to the anaerobic threshold or the lactate threshold (LT) which is equivalent to 80% – 90 % of maximum heart rate (Helgerud J, 2001). During a 90 min 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.

In order to be able to maintain a high level of intensity throughout the whole game, a soccer player should has or high levels of physical fitness. The aim of fitness training in football is to enable a player to cope with the physical demands of the game as well as to allowing the efficient use of his various technical and tactical. Fitness training in football can be divided into a number of components as mentioned by Bangsbo J, (1994).

Aerobic training in soccer training program

Aerobic power has been well recognized as an important physical contributor to soccer player performance and can be improved by aerobic training. The training will help soccer players as follows: (Bangsbo J, 1994)

1. Supplying a larger percentage of the energy required for exercise
2. Improving endurance, which allows a player to exercise at a higher intensity throughout a game.
3. Requiring less time to recover after a period of high – intensity exercise before being able to perform maximally in a subsequent match activity

An aerobic training can be divided into three main areas. The recovery training aims at helping a player return to pre – exercise status as quick as possible after a match or a hard training session. An aerobic low intensity enables the players to work with relatively high exercise intensity throughout a match, and aerobic at high intensity enhances the ability to repeatedly exercise at a high – intensity during a match.

An aerobic training should mainly be performed with a ball. The definition of the three categories of aerobic training takes into account that the heart rate of a player will alternate continuously during training. Table 2.1 illustrates the principles behind the various categories of aerobic training. It misleads to quantify training by the total exercise time. Any activity, whether it lasts for 15 or 90 minutes, can have a favorable effect on the player's aerobic work capacity.

Table 2.1 Target heart rate of aerobic training in soccer players at various level of training

	Heart Rate			
	% of HR max			
	Mean	Range	Mean*	Range*
			Beats/min	
Recovery training	65%	40-80%	130	80-160
Low-intensity training	80%	65-90%	160	130-180
High- intensity training	90%	80-100%	180	160-200

* If HR max is 200 beats /min

Anaerobic training in soccer training program

An anaerobic training consists of speed training and endurance training, of which the latter can be divided into production training and maintenance training. In soccer, speed is not only merely dependent on physical capacity, but also involves rapid decision making which must then be translated into quick movements. Therefore, the aim of speed training is also to improve the player's ability to perceive, evaluate, and act quickly in match situation where speed is essential. In order to obtain this effect, speed training should mainly be performed with a ball.

Speed endurance training increases the muscle's ability to rapidly produce force and improves the capacity of muscle to maintain a high power output. This type of training enables a player to exercise at a high intensity more frequently and

for longer periods of time. This ability is especially important for top–class players because of the following reasons:

1. Improving performances of intense match activities, such as accelerating, sprinting, tackling, and shooting.
2. Elevating ability to perform a prolonged high – intensity exercise during a game.
3. Be able to perform a high – intensity exercise more frequently during a game.

When planning fitness training the phases of a soccer year should be taken into account. Nevertheless can be divided into a pre–season, a season, and a mid– season break. However, a coach should prepare to change or adjust a planned training session at anytime.

Pre – season

Pre season can be subdivided into a maintenance period and a re – building period. The maintenance period is from the last match of the previous season to the resumption of team training. Traditionally, the maintenance period has used for mental recovery with very little physical training. This means that the players will have a good basic fitness level for the re – building period. During the last month before the re–building period the training frequency should be increased to at least two sessions per week. Then, the maintenance period can

consist of other ball games, as to help the player to relax mentally in the maintenance period.

The re–building period is forming the resumption of team training until the first match of the next season. The re–building period has focused mainly on fitness training with an emphasis on a long distance running and muscle endurance training. During the re – building period, an aerobic high – intensity exercise, and various type of anaerobic training should be performed. As the start of the season approaches, the number of training sessions should be gradually increased. In some countries the playing surface is change during the re –building period which can cause problems for the player as their muscles are stressed in a different way and decrease the risk of injury. The transition between playing surfaces should be gradual. During the re-building period training matches are a good and appreciate form of fitness training, but they should not be applied before the players are prepared physically for the demands of a full match. (Bangsbo J, 1994)

The pre-season covers the period between maintenance period and a re-building period. During the maintenance period, an aerobic with low intensity training should be performed to ensure a good physical foundation before the start of the re-building period. During the re-building period, it is important to play match regularly at a high competition level.

Season

During the season, the level of fitness achieved during the re-building period should be maintained and perhaps even improved.

An aerobic with high intensity training should give a high priority during a season. Speed training for top-class player, and speed endurance training should also be performed regularly. The endurance capacity may be maintained by frequently prolonged training sessions with only short rest periods. (Bangsbo J, 1994).

Free radicals and oxidative stress

During the re-building training, which is an exhaustive exercise, there is evident of damage to cell by free radicals. This is due to increase oxygen consumption during exercise, an increase in lactic acid production and increases in inflammatory response during injury and exhaustion. Free radicals are produced during the normal process of metabolism, this frequently happens to reduce to be water in the mitochondria. However, small fractions (2 to 5%) of the oxygen intermediates are produced and leak out of the electron transport chain. Free radicals are highly reactive atoms or molecules that have an unpaired electron in their orbits. Indeed, an intense or prolonged muscle exercise can result in oxidative injury to lipids, proteins, within myocytes skeletal muscle (Scott PK and Christiaan L, 1999).

Exercise is also postulated to generate free radicals by other means, including 1) increase in epinephrine and other catecholamines that can produce

oxygen radicals when they are metabolically inactivated, 2) production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl), and 3) inflammatory responses to secondary muscle damage incurred with overexertion (Clarkson PK and Thompson HS, 2000).

Free radicals are molecules that have an unpaired electron. This makes them be electrically charged, highly unstable and very reactive. Free radicals strip electrons from other molecules in order to restore their own balance. This, in turn, creates another free radical, which participates in a chain reaction of electron stripping and free radical perpetuation. The body tissue involved in this process becomes physically altered. Thousands of free radical reactions can occur within seconds, leaving behind a wake of damaged tissue (Debe JA, 1998).

When the body natural defense system against free radical are overwhelmed by the excess formation of reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH), and nitric oxide (NO), lead to lipid peroxidation or specific oxidation of some enzymes, or and protein oxidation degradation (Table 2.2).

Table 2.2 Reactive oxygen species (Marks DB, Marks AD and Smith CM. Basic medical biochemistry a clinical approach)

Superoxide anion (O_2^-)	Produced by the electron transport chain and at other sites. Generates other reactive oxygen species, but cannot diffuse far from the site of origin.
Hydrogen peroxide (H_2O_2)	Not free radical, but can generate free radicals by reaction with a transition metal (e.g. Fe^{2+}). Can diffuse into and through cell membranes.
Hydroxyl radical (OH°)	The most reactive species in attacking biological molecules. Produced by H_2O_2 in the presence of Fe^{2+}
Organic radicals (R°)	An organic free radical produced from RH by OH° attack. RH can be the carbon of a double bond in fatty acid (resulting in $-C^\circ=C-$) or RSH (resulting in $R-S^\circ$).
Organic peroxide radicals ($RCOO^\circ$)	An organic peroxide radicals, such as occurs during lipid degradation.
Hypochlorous acid (HOCl)	Produced in bateria during the respiratory burst to destroy invading organisms.
Singlet oxygen ($O^2 \uparrow\downarrow$)	Oxygen with antiparallel spins. Produced at high oxygen tensions from the absorption of energy. Decays with the release of light.

Reactive oxygen species are generated continually as by products of aerobic metabolism, UV light exposure, hypoxia, pollution and other stress (Chao WH et. al, 1999). Oxidative stress may increase during strenuous physical activity, due to a 10 to 15 fold increase in oxygen consumption to meet energy demands, coupled with a small amount (1%-2%) of "electron leakage" from the electron transport chain with subsequent direct reduction of molecular oxygen to the superoxide anion (Chao WH et. al, 1999). This oxidative of cellular components (oxidative stress) can occur when an imbalance between oxidants and antioxidants. Oxidative stress occurs under conditions when local antitoxins are depleted because of oxidants or when the rate constants of the radical reactions are greater than the constants of the antioxidant defense mechanisms (Scott KP and Christiann L, 1999).

Antioxidant defense mechanism

It is now widely accepted that free radical generation is enhanced during strenuous exercise. This undoubtedly can cause alterations in cellular antioxidant status (Inal M et al, 2000). The human body has an elaborate antioxidant defense system. Two major classes of endogenous protective mechanisms work together to prevent harmful effects of oxidants in the cell: 1) enzymatic defenses (e.g superoxide dismutase, glutathione peroxidase and catalase), and 2) nonenzymatic antioxidants (e.g. glutathione) and exogenous antioxidant e.g. ascorbic acid (vitamin C), α- tocopherol (vitamin E), β-carotene and vitamin A (Table 2.3(Sies H, 1991)).

Table 2.3 Antioxidant defense in biological systems

System	Remarks
Enzymatic	
Superoxide dismutases	CuZn enzyme, Mn enzyme
GSH peroxidases	Selenoenzyme; non-Se enzyme: some GSH S-transferases
Catalase	Heme enzyme; predominantly in peroxisomal matrix
Nonenzymatic	
α-tocopherol (Vitamin E)	Membrane bound; receptor, regeneration from chromanoxyl radical
Ascorbic acid (Vitamin C)	Water soluble
Glutathione (GSH)	Plant antioxidants, food additives and thiol
Flavonoids	compounds (GSH precursors)
Chemical	
β-carotene	Singlet oxygen quencher
Urate	Singlet oxygen quencher and radical scavenger

Antioxidants can act by: 1.) removing oxygen or decreasing local concentrations, 2.) removing catalytic metal ions, 3.) removing key reactive oxygen species such as superoxide and hydrogen peroxide, 4.) scavenging initiating free radicals such as hydroxyl, alkoxyl, and peroxyl species, 5.) breaking the chain of an initiated sequence and 6.) quenching or scavenging singlet oxygen (Gutteridge JMC, 1995).

The antioxidant scavenging enzyme removes superoxide and hydrogen peroxide. Vitamin E, vitamin C, and carotenoids, generally referred to as the antioxidant vitamins can terminate the free radicals chain reactions. The defense mechanism of compartmentation refers to separation of species and sites involved in ROS generation from the rest of cell (Fig.2.1) (Marks DB, Marks AD and Smith CM. Basic medical biochemistry a clinical approach).

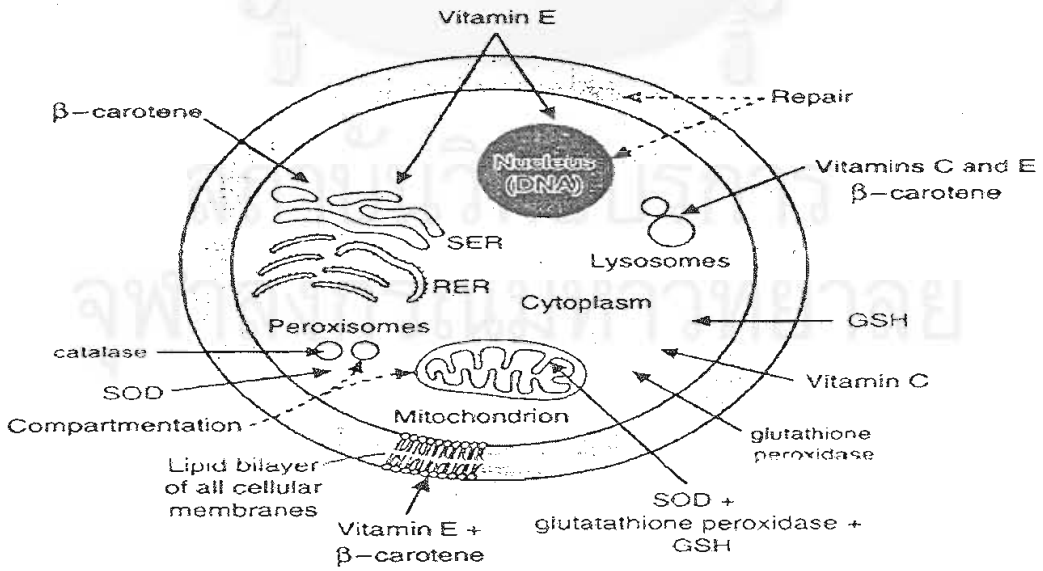


Figure 2.1 Cellular defense mechanisms against ROS

Glutathione antioxidant system

Glutathione is an important water phase antioxidant and an essential cofactor antioxidant as well as an essential cofactor for antioxidant enzyme. It provides a protection for the mitochondria against endogenous oxygen radicals (Kidd PM, 2000).

Glutathione is a tripeptide composed of glutamate, cysteine and glycine that has numerous important functions within cell. Glutathione exists in two forms; the antioxidant "reduced glutathione" is conventionally called glutathione and abbreviated as GSH; the oxidized form is a sulfur-sulfur-linked compound, known as glutathione disulfide or GSSG (Fig.2.2).

The GSH/GSSG ratio may be a sensitive indicator of oxidative stress. Glutathione occurs predominantly intracellular in concentrations that ranges from about 0.5 mM to about 10 mM; more than 95% of intracellular glutathione is in the from of GSH (Anderson ME and Meistor A, 1980)

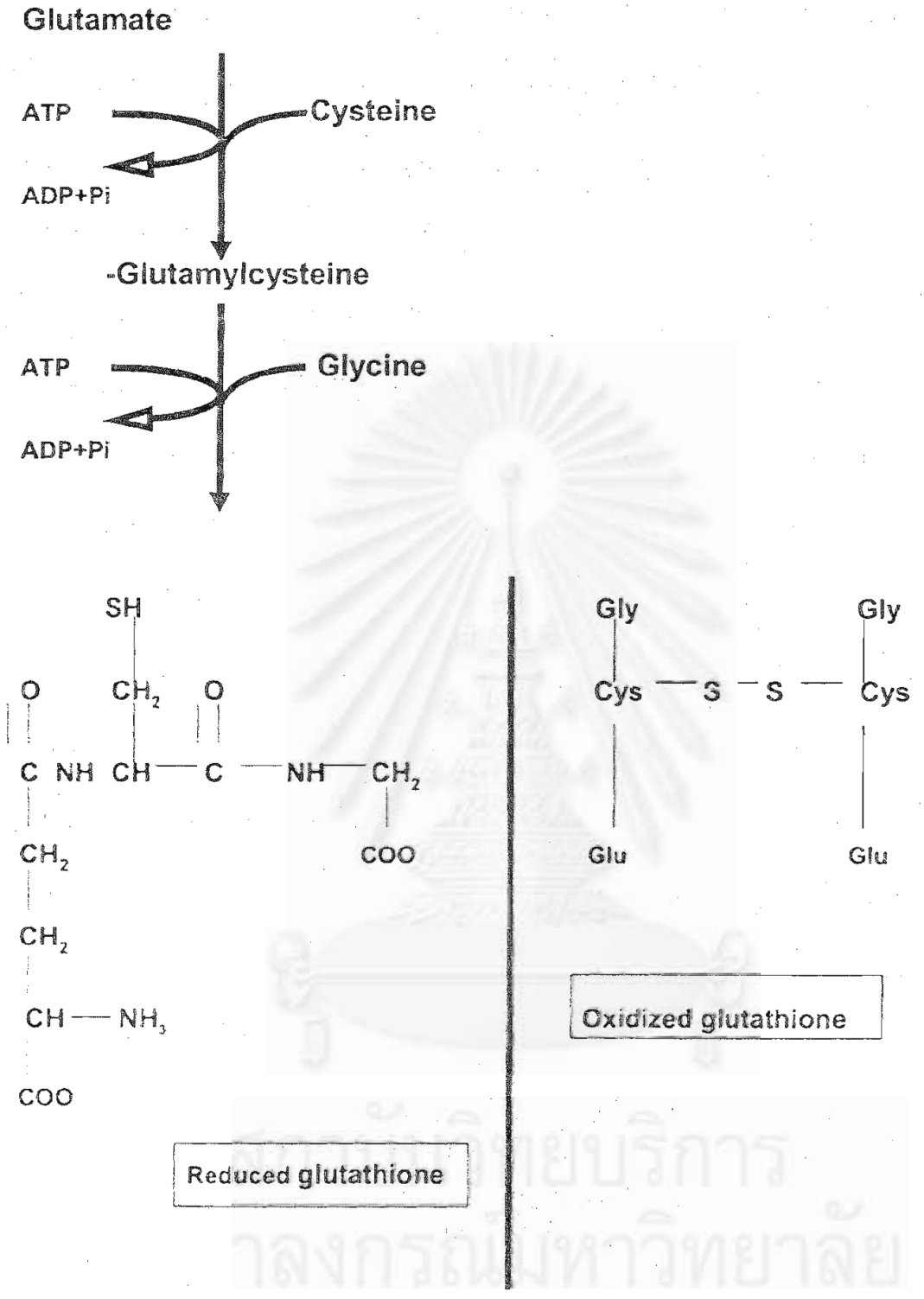
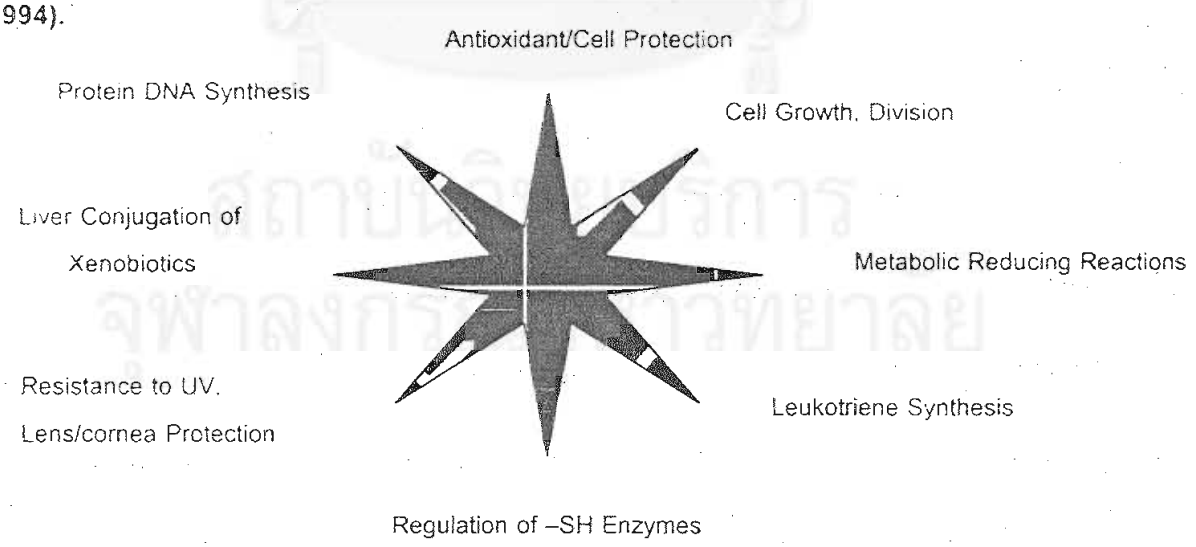


Figure 2.2 The glutathione synthesis and structure of GSH and GSSG

Glutathione (GSH) is the most important antioxidant of GSH is function to remove hydrogen peroxide and organic peroxides (e.g. lipid peroxide) and is catalyzed by the selenium-dependent enzyme forming in water (Scott KP and Christiann L, 1999).

Glutathione status is homeostatically controlled, being continually self-adjusting with respect to the balance between GSH synthesis (by GSH synthase enzymes), it recycled from GSSG (by GSH reductase), and its utilization (by peroxidases, transferases, transhydrogenases, and transpeptidases).

That GSH profound importance for cellular homeostasis diverse cellular functions was essential. It is evident that glutathione reducing power is used in conjunction with ascorbic acid and other antioxidants to protect the entire spectrum of biomolecules, to help regulate their function, and to facilitate the survival and optimal performance of the cell as a living unit (Fig 2.3)(Meister A, 1994).



Figures 2.3 GSH Reducing Power

Life style choices can be fateful, because negative lifestyle factors (smoking, alcohol consumption, legal or illegal drug use, emotional stress) can converge with environmental stress to attack the body through related oxidative pathways (Fig 2.4)(Kidd PM, 1991).

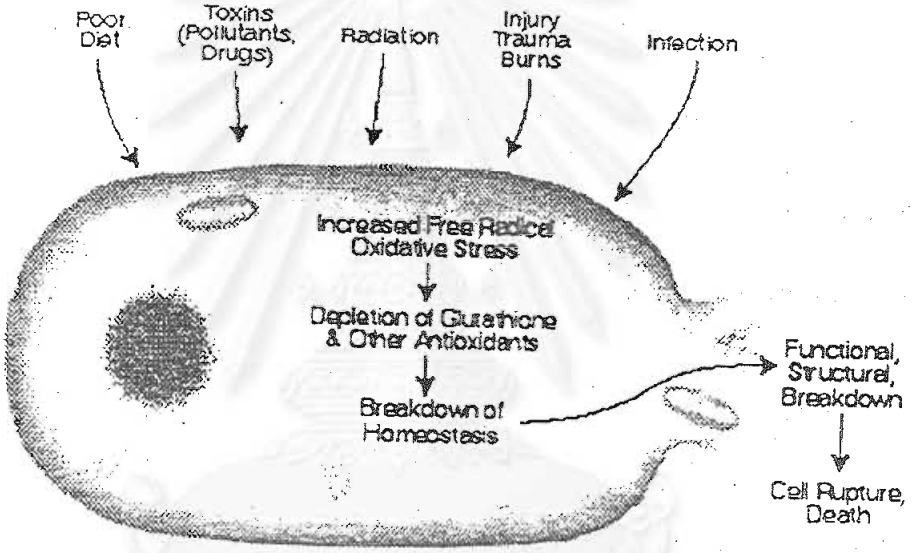


Figure 2.4 Cell breakdown related to depletion of GSH

Strenuous aerobic exercise can deplete antioxidant from the skeletal muscle, and sometimes also from the other organs. Exercise increases the body's oxidative burden by calling on the tissue to generate more energy. Making more ATP requires using more oxygen and this in turn results in greater production of oxygen free radicals. Studies in humans and animals indicated that GSH was

depleted by exercise. The data support that strenuous and chronic exercise increased antioxidant defense. Evelo CTA et.al, 1992 reported increased blood GSH in the first 20 week of training, but these values returned to initial concentrations in the next 20 week. In 1992, Sastre J. et. al observed changes in glutathione redox status in the blood after exercise. They found that trained men who were exercise to exhaustion on a treadmill had increased blood amounts of GSSG immediately after exercise, but the elevated values returned to rest within 1 h. On the contrary the amounts of blood GSH did not change significantly. Tessier F et al, 1995, reported that blood GSSG increased in response to a maximal aerobic capacity test, thereby reducing the ratio of GSH to GSSG. In contrast to the previous studies, Duthie GG et. al, 1990 found that erythrocyte GSH decreased and GSSG was unchanged after a half-marathon. Erythrocytes contain glutathione as an antioxidant to prevent the oxidation of hemoglobin to methemoglobin.

This favorable characteristic makes glutathione peroxidase (GPX) an important cellular protectant against reactivity species mediated damage to membrane lipid, proteins, and nucleic acids (Scott PK and Christiaan L, 1999).

Working together of glutathione as a substrate or cofactor and glutathione peroxidase as an enzyme in antioxidant defense mechanism should provide some informative values on the antioxidant status in soccer during the trained at Assumption College Sriracha during the re-building period and after the competition. Whether these players required any supplement during the exhaustive training was remained unknown.

CHAPTER III

MATERIALS AND METHODS

3.1 Research Design

The design of this study is an observational study with a longitudinal method. The healthy young soccer player gave their written informed consent for this experiment, which had been approved by the Faculty of Medicine, Chulalongkron University Committee for Ethics in Human experimentation.

3.2 Population and sample

3.2.1.Population

The target population were young Thai male soccer players who were 16-18 years old.

Sample population

The sample population were Thai male soccer players at Assumption College Sriracha, who were at their senior high school.

3.2.2.Eligibility criteria

Inclusion criteria

1. All participants are the soccer players at Assumption College Sriracha, who have been training for 2 hours per day, 5 days per week, for the past year.
2. Do not use drug or chemical during the study period.

Exclusion criteria

1. Subjects have injury in the training session.

2. Subjects have fever or acute illness during the training session.

3. Do not complete the training program

3.3 Materials

1. A weighting scale (Yamato DP-6100GP)
2. A wall-mounted height measuring board
3. A wet spirometer
4. A scale for flexibility (Will sit and reach)
5. Sphygmomanometer
6. Hand-grip dynamometer
7. Heart rate telemetric set (Polar accurex plus, Polar eletro, Finland) and Software Analysis (Polar Precision Performance 2.0, Italy)
8. UV-visible Spectrophotometer
9. Bicycle ergometry (CORIVA 400)
10. Oxygen and carbon dioxide gas analyzer; Quinton Metabolic Cart: (QMC, USA)
11. ELISA Reader
12. Reagent kit for glutathione peroxidase (GPx-340™, BIOXYTECH®.)
13. Standard glutathione (Glutathione oxidoreductase, EC 1.6.4.2, type IV;Sigma Chemical Co.,St.Louis,MO)
14. DTNB (5,5'-dithiobis-(2-nitro-benzoic acid,)

15. MPA (Metaphosphoric acid,)

16. NADPH (nicotinamide adenine dinucleotide phosphate)

3.4 Measurement

3.4.1. Data collection

Twenty-two soccer players participated in condition training program. The data collections were as follows:

Day 1: -Subjects arrived at Exercise Physiology Lab, Faculty of Medicine, Chulalongkorn University at 9.00 a.m.

-Subjects were given instructions and signed informed consent.

-Five ml of blood sample were taken from each subject. (1st blood colletion)

-Physical fitness test were performed.

Day 2: Every subjects participated in the soccer training program until post-competition

Day 8: 2nd blood collection

Day 15: 3rd blood collection

Day 16 – 31: Subjects were entering the soccer game competition at Ubonrachatani Province.

Day 35: 4th blood collection

3.4.2. Body weight and height

Body weight (kg) was measured without shoes to the nearest 0.02 kilogram on a digital platform scale (Yamato DP-6100GP).

Height (cm) was measured to the nearest 0.5 centimeter by the standardized wall-mounted height board.

3.4.3. Vital capacity

The vital capacity tests were performed when the subject was in the standing position. He would be asked to fully inhale before wearing the nose clip. Then he would totally exhale to a wet spirometer. The observed reading represents the pulmonary capacity as milliliters

3.4.4. Flexibility

The Sit-and Reach Test was used for the measurement of flexibility primarily for the lower back, buttocks, and calf muscle. The subject is asked to sit on the floor with legs extended. Subjects slowly reached forward in an attempt to project his fingertips as close to the toes, or as further as possible. A ruler measured the distance between fingertips and toes. The best of two attempts is scored. If the reference point is considered as "zero" and a meter stick is used as a ruler, scores of –30 cm. (fingers not reaching the toes) to +15 cm. (fingers beyond the toes) are typical.

3.4.5. Handgrip strength test

Handgrip strength is a main function of the forearm muscles in addition to muscles of hand. The subjects should be in the standing position, his wrist and forearm was at the midprone position on the handgrip. Then he was asked to try his best to grip the handgrip as hard as possible.

3.4.6. Leg strength test

The subject was asked to stand in the position that his back was against the wall while his knees were bent at 130-140 degrees. Then he had to pull the handle bar, which was between his thighs without using his back muscle. The pulling must be slow and vigorous enough to have the maximum load. The observed peak was recorded as kilograms.

3.4.7. Measurement of oxygen consumption (VO_2 max)

The actual measurement of VO_2 is typically performed in laboratory or clinical settings using a procedure called open-circuit spirometry. The open-circuit spirometry involves the subject inspiring room air and expiring into a gas collection and measurement system. Aside from a breathing valve with mouthpiece (which provides unidirectional flow), a nose clip (which ensures that all gas exchange take place at the mouth), and tubing involved in such a system, there are three basis pieces of equipment that are necessary.

- A device that measures the volume of expired air (V_E) or inspired air (V_1) over a fixed period of time, such as a dry gas meter. This volume must then be corrected to standard temperature and pressure, dry (STPD) conditions, that is, the volume which would be present if the prevailing ambient conditions was 0°C, 760 mmHg barometric pressure, with no water vapor pressure
- An oxygen analyzer, which measures the fraction of O_2 in the expired air ($F_E O_2$)

- A CO_2 analyzer, which measures the fraction of CO_2 in the expired air ($F_E CO_2$)

These individual pieces of equipment, or similar devices, are often integrated and interfaced with a computer in commercially available metabolic units. The fractions of O_2 and CO_2 in the inspired air are constant and known ($F_1 O_2$= 0.2093 (20.93%) and $F_1 CO_2$= 0.0003 (0.03 %). VO_2 (in the same units as V_E) (ACSM's Guidelines, 1995).

3.4.8. Measurement of exercise intensity (ACSM's Guidelines, 1995 and McArdle WD,

Katch FI and Katch VL, 2000)

Intensity and duration of exercise determine the total caloric expenditure during a training season, and are integrally related. Intensity reflects the activity's energy requirements per unit time and the specific energy systems activated. One can express exercise intensity in several ways:

- As a percentage of VO_2 max
- As a particular heart rate or percentage of maximum heart rate
- As calories expended per unit time
- As a particular exercise level or power output
- As a level of exercise below, at or above the lactate threshold
- As multiple of resting metabolic rate (METs)

3.4.9. Measurement of heart rate during the condition training program by electrocardiograph (ECG) recorder

The ECG of each subject was recorded at 1 minute interval during training using Polar Accurex Plus (Polar electro, Finland). The equipment consists of electrode belt with a transmitter, which records ECG and send signals to a receiving watch incorporated with a microprocessor. The data stored in the watch transferred to the computer in order to be processed by the computer interface. The average heart rate at different training interval was used to calculate the exercise intensity.

3.4.10. Blood total glutathione assay

Five milliliters of whole blood sample were collected via puncture from the antecubital vein and immediately transferred to chilled heparinized glass tubes. Immediately after collected, a sample of 300 µl was deproteininziation with four volumes of ice-cold 50 g/L metaphosphoric acid (MPA). After 10 to 20 min, acid extracts were obtained by centrifugation at 13000 g for 5 min. The MPA extract was aliquoted into cryovials and strored at –20°C. The control samples were stable for at least 1 year (Richie JP, Skowronski L, Abraham P and Leutzinger Y, 1996).

The assay buffer (Richie JP, Skowronski L, Abraham P and Leutzinger Y, 1996) consists of 100 mmol/L NaH_2PO_4 and 5 mmol/L EDTA adjusted to pH7.5 with NaOH. The solutions of 1.26 mmol/L 5,5'-dithiobis-(2-nitro-benzoic acid)(DTNB), 0.72 mmol/L NADPH, and 2.5 kU/L glutathione oxidoreductase were prepared in assay buffer on the day of use. The DTNB solution was maintained at room temperature and the NADPH and glutathione reductase solutions were kept at 0-4°C.

Just before analysis, the MPA extracts were diluted 20 fold in assay buffer. Then, 50 µl of each working calibrator or diluted sample extract was added to each well of a flat-bottomed 96 well microtiter plate. Both calibrator and sample were analyzed in duplicate in adjacent columns of wells. Using an eight-channel pipettor, then added 130 µl each of assay buffer and adding 20 µl of DTNB solution to each well plate. The rate of change in absorbance at 405 nm was monitored with 96 well plate reader.

GSSG represents only a small percentage of total acid-soluble (free) glutathione. The results were presented as GSH+GSSG (tGSH) and expressed in units of GSH equivalents.

3.4.11. Erythrocyte glutathione peroxidase assay

Erythrocytes were promptly separate from the plasma by centrifugation at 5000 g and the buffy coat was aspirated. The erythrocytes were washed three times with isotonic saline, and hemolyzed by diluting four folds with water. The samples were freezed at -70°C before analyse (Pleban PA, Munyani A and Beachum J, 1982).

The reagent kit of glutathione peroxidase assay (GPx-340™) is the product of BIOXYTECH®. It composes of one bottle of 120 ml assay buffer; 0.05 M Tris-HCL and 5 mM EDTA, pH 7.5. NADPH was restored in assay buffer to the desire volume on the day of use and should be discarded at the end of the day. Tert-butyl hydroperoxide substrate was diluted 1/10,000 in deionized water and set in a dark container on ice. It should be made fresh each day.

Determination of glutathione peroxidase activity;

The frozen hemolysate was brought to 4°C on ice and diluted the sample by assay buffer, typically 1/10 in assay buffer. Using pipette to add the following reagent into a 1.5ml cuvette; 350 µl assay buffer, 350 µl NADPH, 70 µl sample and 350 µl of tert-butyl hydroperoxide. The solution was gently mixed by pipetting up and down twice. Turn on spectrophotometer set to measure absorbance at 340 nm and set the assay temperature recommended 23-25 °C. The changes in absorbance at 340 nm were recorded interval for three minutes in order to calculate the enzyme reaction rate. The first 15 seconds of the reaction (after adding substrate) should be exclude from data analysis as the rate may not be representative of the enzyme activity due to sample mixing.

4. Data Analysis

The results are reported as mean and standard deviation (SD) calculated by conventional procedures. All data were analyzed using the Statistical Package for the Social (SPSS Version10.0). When data did not follow the normal distribution, the Friedman test was used to data significant and Wilcoxson test was used to compare the differences. The differences at significance level of $p < 0.05$ were considered to be significant.

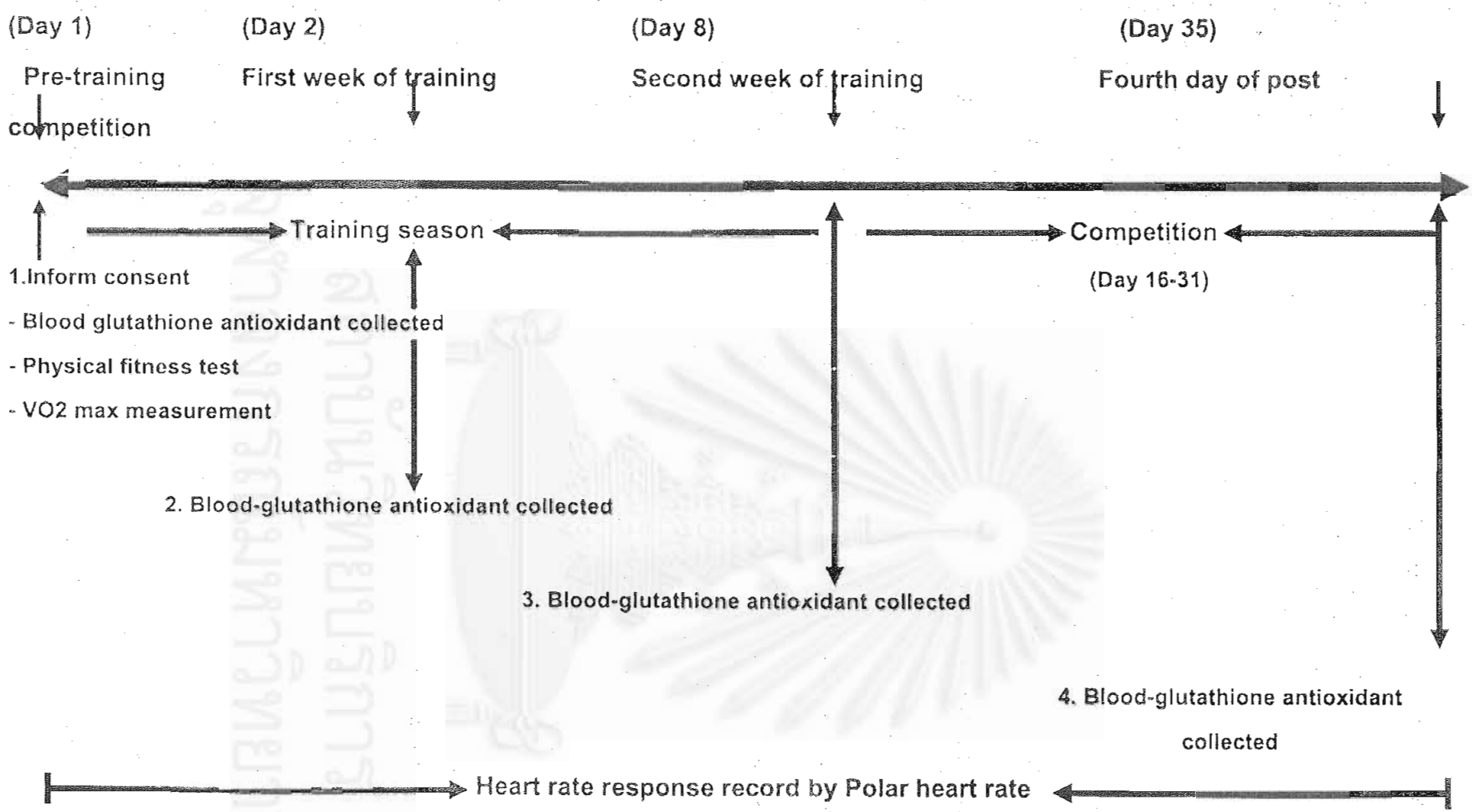


Figure 3.1. Diagram of data collection

CHAPTER IV

RESULTS

4.1Characteristics of subjects

The physical characteristics of subjects in this study group were compared with the reference group reported by Sport Authority of Thailand, 2000 at the same age and sex. As listed in Table 4.1. They were the same weight (61±5.83 kg) and height (168±4.19 cm). Average heart rate at rest was 67.54±10.06 beats/min. The means and standard deviations of other characteristics were as follows: systolic blood pressure was 110±10.23 mmHg, diastolic blood pressure was 68.18±5.83 mmHg, vital capacity was 57.16±5.61 ml/kg body weight, leg strength was 3±0.05 kg/ kg body weight, handgrip strength was 0.68±0.01 kg/ kg body weight, flexibility was 13.77±7.08 cm and maximum oxygen uptake (VO_2 max) was 50.03±12.10 ml/kg/min.

4.2 Effects of condition training on glutathione antioxidant

4.2.1 Reduced glutathione (GSH) assay

Blood reduced glutathione (GSH) concentration of each subject over the entire study period is shown in Table 4.2. The GSH level significantly decreased after first week of training when compared to pre-training. However this went back to be the same level as the pre-training in the second week of training session and 4 days after the competition ($p< 0.05$) (Fig 4.1).

4.2.2. Erythrocyte glutathione peroxidase assay

Glutathione peroxidase (GSH-Px) concentrations are shown in Table 4.3. The data reported were the GSH-Px values per gram of hemoglobin (Hb). The GSH-Px activities also decreased after the first week of training when compared to those in the pre-training. However, this went back to be the same level as the pre-training in the second week of training and post - competition ($p<0.05$) (Fig 4.2).

4.3 Classification of condition training program by heart rate

The exercise intensity of each physical activity in soccer game was continuously varying. The exercise intensity, which was expressed as percentage of maximum heart rate, was used to determine how hard a player did as well as evaluate the achievement of the aim of training program. During the condition training activity, heart rates were monitored by a cardiotachmeter (Polar Accurex Plus monitor) for measurement of the 1 minute interval of heart rate in drills. The heart rate data were used for analyzing intensity of exercise throughout the program. The data are demonstrated in Figure 4.3 and 4.4. The condition training program included follows; 15 min warms up, 20 min basic technical, 20 min small size game, 20 min tactical, 30 min 11:11 game and 15 min cool down. The average heart rate of the condition training period was 134.44± 8.86 beats /minute, which included warm up (118.37±11.18 beats /minute), basic technical (132.98±15.05 beats /minute), small size game (130.48±10.41 beats /minute), tactical

(141.14±22.75 beats /minute), 11:11 game (140.10±29.81 beats /minute) and cool down (148.60± 27.16 beats / minute).

The condition training program was classified as the moderate exercise intensity since the average heart rate was 134.44±8.86 beats /minute which was 66.5% of maximum heart rate

Table 4.1 The physical characterictics of subjects compared to the reference group which was reported by Sports Authority of Thailand*

Characterictics	Subjects (n=22)	Reference (n=194)
Ages (years)	17-18	17-19
Sex	Male	Male
Weight (kg)	61(5.83)	58.44(11.36)
Height (cm)	168(4.19)	168(6.71)
Heart rate at rest (beats/min)	67.54(10.06)	78.32(10.53)
Systolic blood pressure (mmHg)	110(10.23)	115.52(12.03)
Diastolic blood pressure (mmHg)	68.18(5.83)	73.49(9.13)
Vital capacity (ml/kg)	57.16(5.61)	53.43(8.50)
Leg strength (kg / kg body weight)	3(0.054)	2.24(0.052)
Hand grip strength (kg / kg body weigh	0.68(0.007)	0.73(0.12)
Flexibility (cm)	13.77(7.08)	12.54(7.54)
Maximum oxygen uptake	50.03(12.10)	45.60(9.98)
VO_2max (ml/kg/min)		

Data are expressed as mean (standard deviation)

* = Sports Authority of Thailand

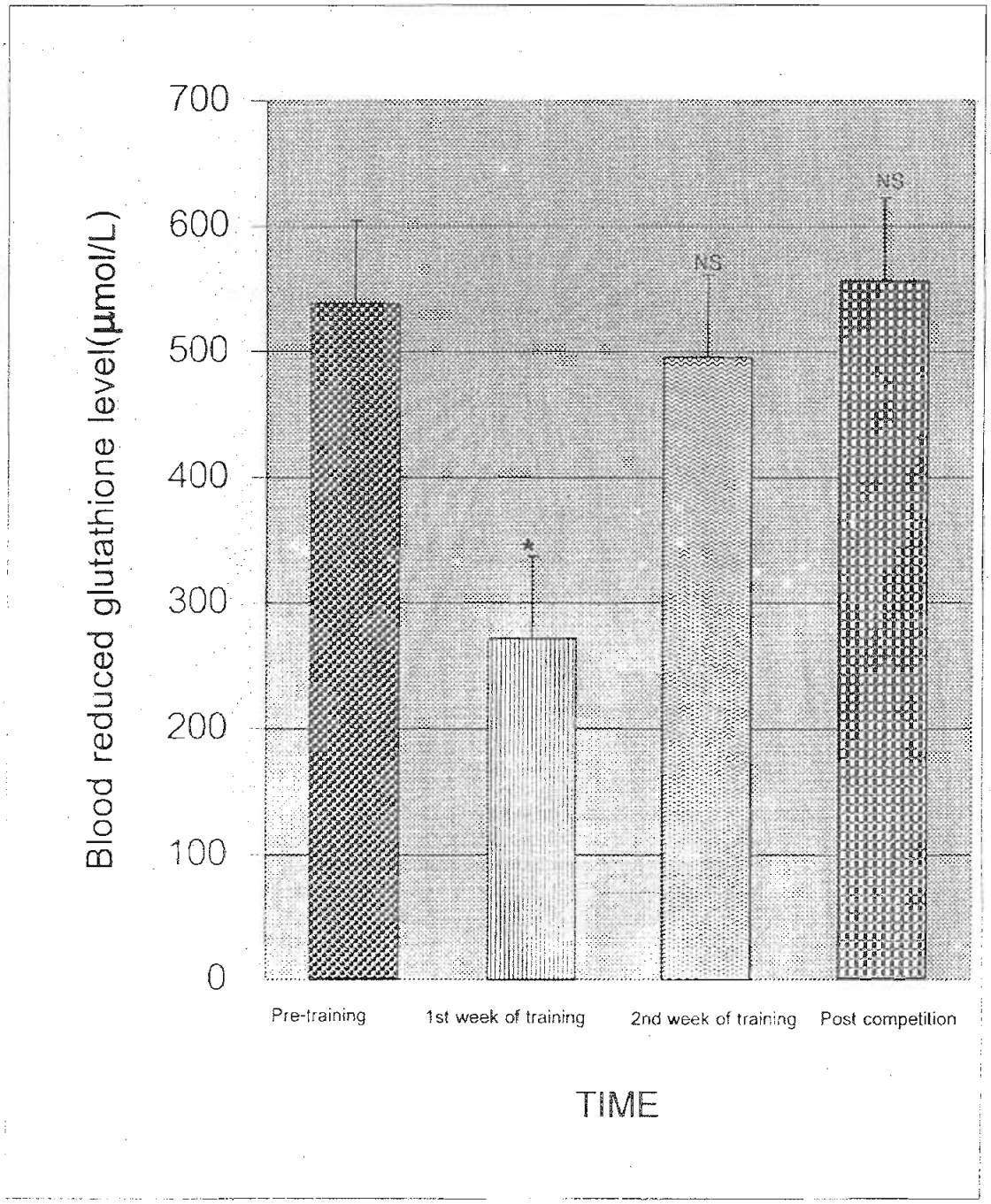
Table 4.2 Blood reduced glutathione level (µmole/L) in subjects taken at pre- training, 1st week of training, 2nd week of training and post-competititon

NO	Pre- training	First week of training	Second week of training	Post-competition
1	568.5	368.5	574.5	304.5
2	632.5	362.5	461	969.5
3	710	70.5	465	775.5
4	783	105.5	461	950
5	21	77	461	305
6	816.5	71	461	111
7	265	180.5	608	608
8	410	101	461	461
9	612.5	367	461	408
10	822.5	213.5	461	898.5
11	690.5	114.5	509	770.5
12	745	77	637	486.5
13	679.5	164	288	910.5
14	146.5	93	544	664.5
15	635.5	301.5	564	180
16	425.5	77	502	358
17	76.5	318.5	367	821
18	114.5	568.5	611	272.5
19	439	632.5	526	537
20	699.5	710	482	603
21	815	783	401	535
22	733	210	596	317
Mean	538.25	271.2	495.52	556.65
SD	260.6	222.17	84.09	260.87

Table 4.3 Erythrocyte glutathione peroxidase activity per gm hemoglobin at pre-training, 1st week of training, 2nd week of training and post-competition

NO.	Pre-training	First week of training	second week of training	Post-competition
1	22.52	1.75	43	23.32
2	33.52	15.79	35.65	10.62
3	27.61	38.91	31.54	21.64
4	39.04	23.8	35.65	21.86
5	35.99	23.07	35.65	32.62
6	39.98	20.32	35.65	41.85
7	55.91	54.22	19.45	33.29
8	19.19	26.72	35.65	15.74
9	26.23	13.55	35.65	18.14
10	33.28	18.8	35.65	40.23
11	44.97	26.23	20.92	21.86
12	35.44	27.79	29.15	32.14
13	55.39	29.26	22.59	40.7
14	40.81	40.32	36.32	27.76
15	41.23	24.27	49.85	21.86
16	49.35	29.26	42.27	48.1
17	57.57	50.56	53.05	72.15
18	41.23	36.07	30.61	37.44
19	24.05	23.77	28.06	56.6
20	41.71	12.68	30.12	32.06
21	26.96	19.19	33.3	27.69
22	76.09	87.45	64.58	54.8
Mean	39.46	29.26	35.65	33.29
SD	13.21	18.66	12.72	15.28

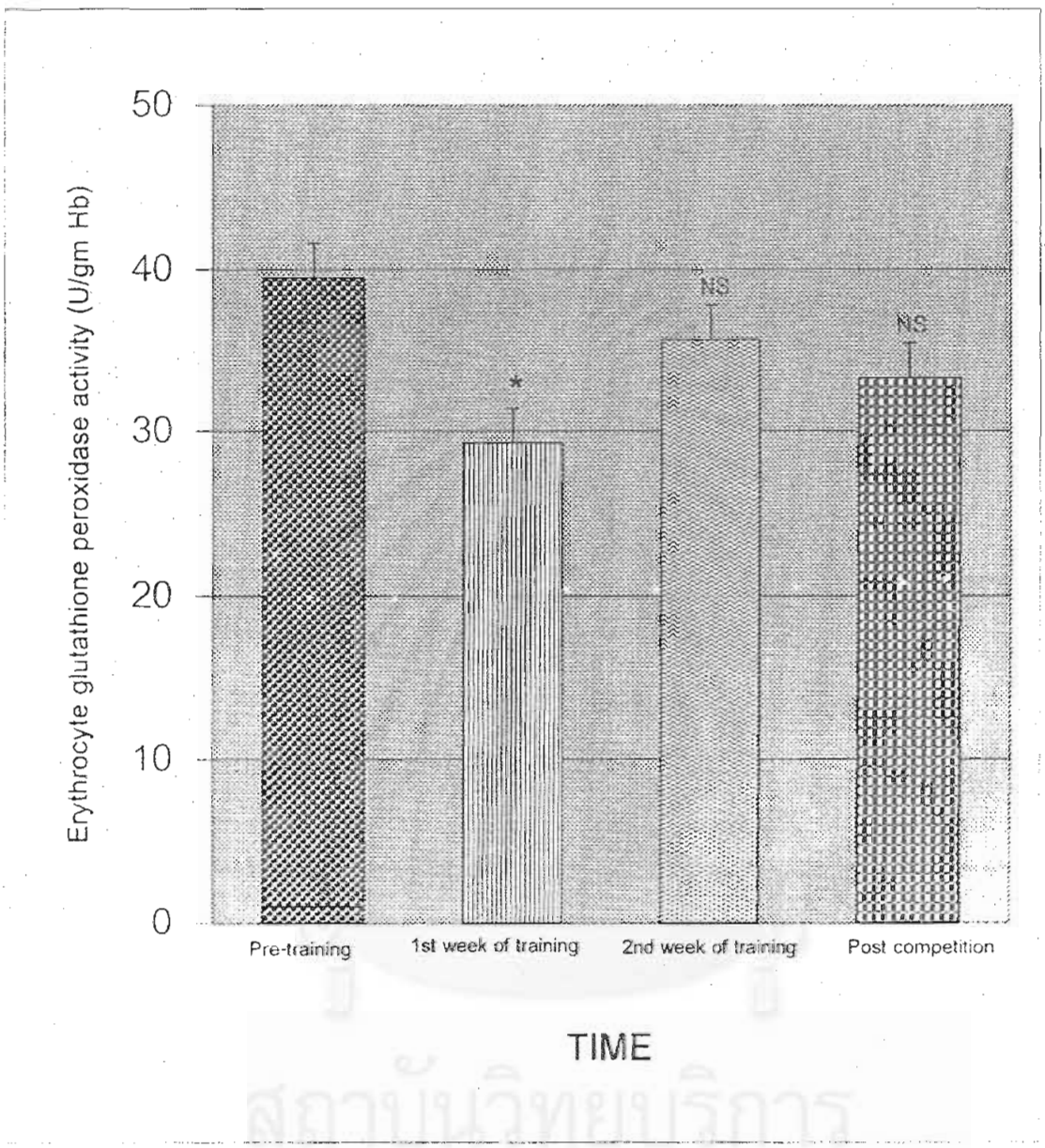
Figure 4.1. Reduced glutathione level (µmol/L) in subjects at pre-training, 1st week of trainin 2nd week of training and post competition



* =Significant difference $p < 0.05$

NS = No significant difference

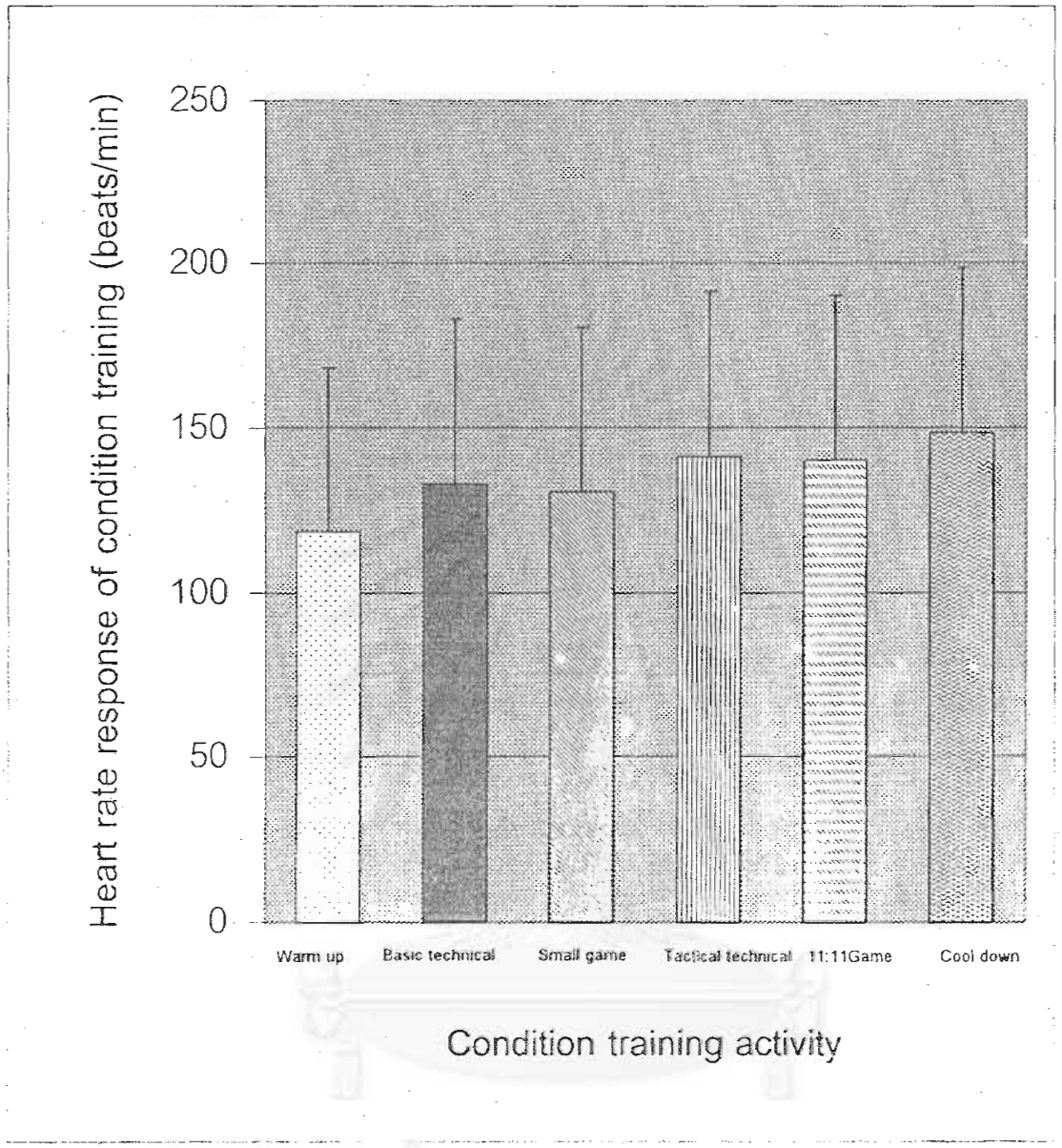
Figure 4.2. Erythrocyte glutathione peroxidase activity per gram hemoglobin at pre-training, 1st week of training 2nd week of training and post competition



* = Significant difference $p < 0.05$

NS = No significant difference

Figure 4.3. Mean (SD) of heart rate responses of condition training program



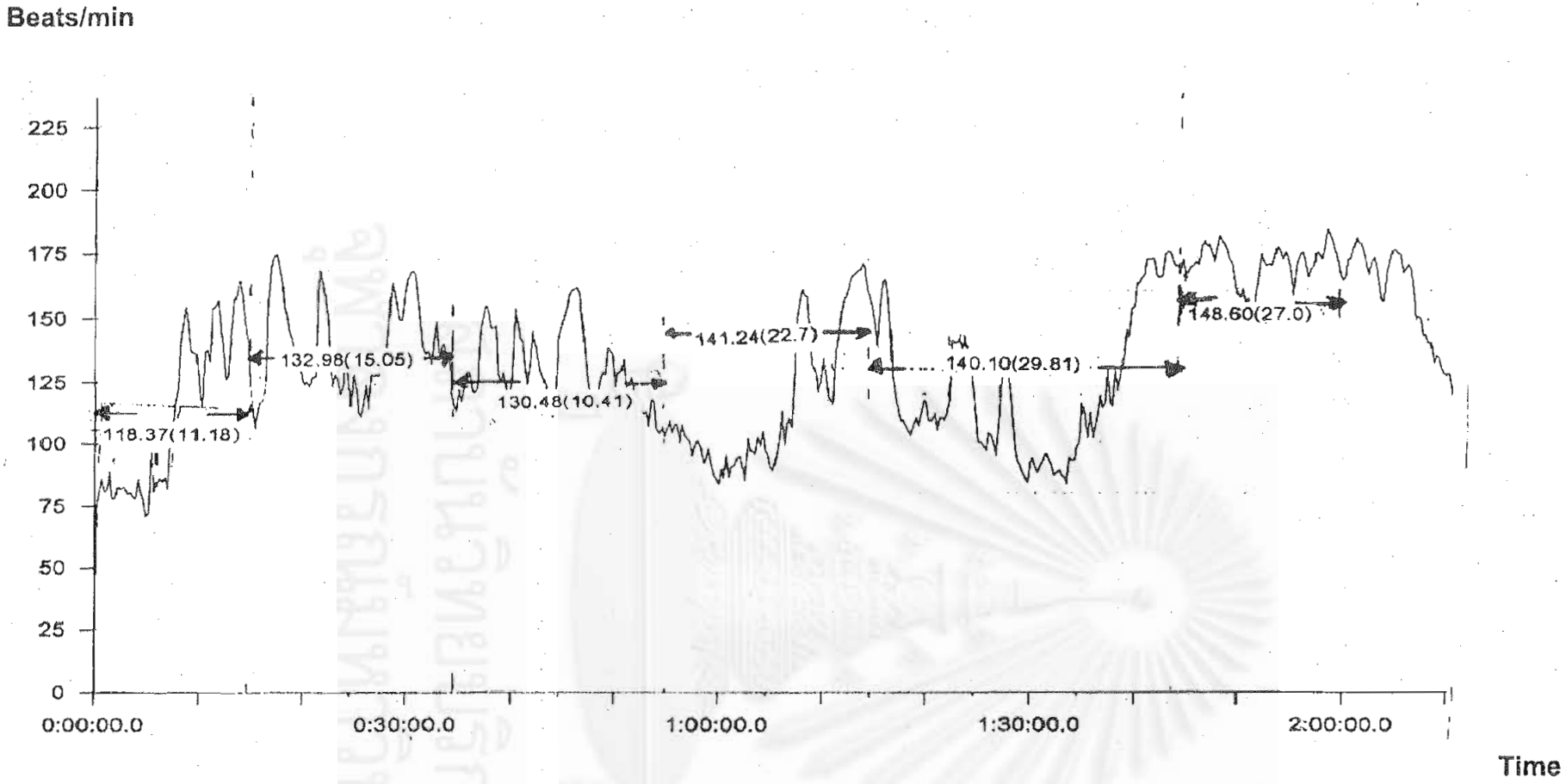


Figure 4.4 Mean (SD) of heart rate response of condition training program

CHAPTER V

DISCUSSION

The characteristics of 22 soccer players of Assumption College Sriracha with average age of 17-18 years old were compared to the reference group (Sport Authority of Thailand, 2000) with the same age range and sex. Most of the studied characteristics were comparable such as weight, height, handgrip strength, blood pressure, vital capacity, and flexibility. The anthropometric parameters that showed slightly different were heart rate, VO_2max, and leg muscle strength (Table 4.1). The studied group heart rate (67.54±10.06 beats/min) was lower than the reference group (78.32±10.53 beats/min) because of different life style. In addition, the maximum oxygen consumption (VO_2max) of the subjects (50.03±12.10ml/kg/min) was higher than those of the reference group's (45.60±9.98 ml/kg/min). The aerobic training for endurance in the soccer athletes enhanced the function of respiratory - cardiovascular system in order to increase more oxygen transport to muscle cell for use during work or exercise. Helgerud J et al reported the increase of VO_2 max from 58.1±4.5 ml/kg/min to 64.3 ±3.9 ml/kg/min in18 years old soccer players. They were trained at 90-95% VO_2max for 4 min and running at 50-60% VO_2max for 3 min whereas there was no observed significant differences in the control group who were trained twice a week. The observed leg muscle strength in soccer players in our studies (3.00±0.054 kg/kg body weight) was higher than those of the reference group's leg muscle strength (2.24 ± 0.052 kg/kg body weight). Therefore, the

continuing condition training with the appropriate exercise intensity was the factor that affects the athletes' physical fitness.

The increase in oxygen consumption which involved in the exhaustive physical exercise causes changes in the cellular redox in muscle, liver, and blood of rats (Lew H, Pyke S, and Quintanilha ,1985). Aerobic cells, especially muscle cells, are subjected to oxidative stress which is defined as a disturbance in the prooxidant and antioxidant balance in favor of the former. A major intracellular antioxidant system that prevents cell damage from oxidative stress is the redox cycle of the glutathione. Reduced glutathione (GSH) protects cells against oxidative damage caused by free radicals (Vina J, 1990).

The aim of this work is to determine whether the exhaustive physical exercise in re-building soccer training program as well as the season activity causes oxidative stress. Blood samples were taken at pre-training, 1st week and 2nd week of training and post-competition. The results showed that blood reduced glutathione was significantly decreased after the first week of training program and restored to the pre-training level in the post competition. Previously, Gohil K et al, 1988 reported the effect of O_2 utilization on the glutathione antioxidant system in blood. They demonstrated that a prolonged submaximal exercise decreased blood reduced glutathione levels from 0.4 mM at rest to 0.15 mM during the first 15 min of exercise, which was accompanied by a corresponding increase in GSSG levels. These changes are confined to the blood cells, most likely in the erythrocytes. Plasma GSH and GSSG were not detectable at any time point. This can be

explained by the very low concentration in plasma in humans (<0.01 mmol/L). This result is consistent with the observation of Gohil et al. and Dufaux B et al. in 1996 that studied the changes of blood glutathione status of 12 moderately trained subjects after a 2.5 h run. The data showed that the blood glutathione returned to pre-running baseline levels after one day of post-exercise. Sastre et al 1992 founded that blood GSH levels decreased within 30 and 60 min after an exercise bout. They also speculated that the decrease in GSH levels after the exercise may be due to an inhibition of GSH synthesis attributed to adrenergic stimulation of stress. The depletion of this antioxidant system after the exhaustive exercise may help to explain the secondary or delayed onset injury to cells caused by free radicals after physical exercise (Davies KJA, Quintaniha AT, Brooks GA, and Pecker L., 1982).

The reduced form of glutathione is a substrate of glutathione peroxidase, which is involved in the decomposition of hydrogen and lipid peroxides. In this study, the changes of erythrocyte glutathione peroxidase activity in subjects' blood taken at the same time as blood reduced glutathione showed the same pattern of changes as reduced glutathione levels. There was a significant decrease of glutathione peroxidase level in the first week of training and slowly restored to the base line. Mena P et al (1991) compared the erythrocyte glutathione peroxidase in sedentary subjects, amateur bicycle racers and professional bicycle racers. The study showed that the activity of glutathione peroxidase in professional cyclist under resting condition was significantly higher ($p<0.01$) than those in both

sedentary subjects and amateur cyclists. The enzyme activities were not modified significantly after a bout of exercise of 22 km in 5 hr. Therefore, the aerobic endurance training at the professional level produces an increase in the erythrocyte activity of the free radicals scavenger enzymes, glutathione peroxidase. The increase in this enzyme activity was also founded in mice submitted to a 21-week swim training program. On the contrary, Ohno H et al (1986) reported the finding that there was a slightly reduced of erythrocyte glutathione peroxidase activity in sedentary students after a brief (30 min) physical exercise. This is consistent with this study that demonstrated the slightly decrease of glutathione peroxidase activity in the amateur soccer players at Assumption College Sriracha after one week of training in the re-building program.

The exercise intensity of the soccer condition training program used in this study group was also analyzed. The program, which was two hours training per day, five days per week including 15 min warm up, 20 min basic technique, 20 min small game, 30 min tactical technique, 20 min full game and 15 min cool down. The average heart rate of the entire training program was 134.44±8.86 beats/min which was 66.5 % of the maximum heart rate. Therefore, this program was considered to be the moderate intensity exercise. The young soccer players who performed a prolonged moderate exercise would have an increase in oxygen consumption and a consequent oxidative stress, which was a rise in the production of reactive oxygen species. Brites FD et al (1999) demonstrated that a group of young soccer players engaged in a regular physical training program showed increased total

antioxidant capacity values, ascorbic acid, uric acid and alpha-tocopherol levels and superoxide dismutase. The training program in this study also caused the production of oxidative stress which was responded by the changes of glutathione antioxidant status. The blood reduced glutathione level and erythrocyte glutathione peroxidase activity were decreased after the first week of training according to the overloaded training program. In order to avoid this oxidative stress in young soccer players during the re-building period, coaches or trainers should consider to slowly increasing the training intensity at the beginning of the re-building period. After the first week of training when players can adjust themselves to cope with the oxidative stress, then the training could be increased to higher intensity without any potential harmful effect.

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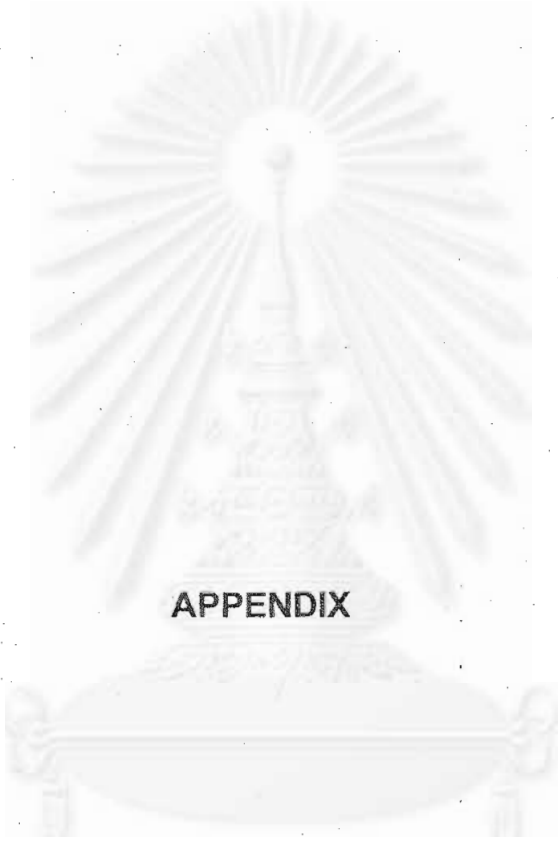

APPENDIX

APPENDIX A

คำรับรองของผู้รับผิดชอบโครงการ

ข้าพเจ้านางสาวสุรีพร สถิตยานุรักษ์ ได้อธิบายชี้แจงรายละเอียดและข้อมูลต่าง ๆ ที่เกี่ยวข้องกับโครงการวิจัยในครั้งนี้แก่ผู้เข้าร่วมโครงการวิจัยและผู้ปกครอง จนเป็นที่เรียบร้อยแล้ว และผู้เข้าร่วมโครงการวิจัยสามารถถอนตัวจากการวิจัยครั้งนี้ได้โดยไม่มีเงื่อนไขใด ๆทั้งสิ้น

คำยินยอมของผู้เข้าร่วมโครงการวิจัย

ข้าพเจ้า.....ได้ทราบถึงรายละเอียดในโครงการวิจัยนี้จนเป็นที่เข้าใจดีแล้ว ข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยนี้ด้วยความสมัครใจและยินดีจะปฏิบัติตามข้อตกลงในการวิจัยนี้รวมทั้งยินยอมให้เก็บตัวอย่างเลือด

ลงชื่อ.....วันที่.....
(ผู้เข้าร่วมโครงการวิจัย)

ลงชื่อ.....วันที่.....
(ผู้ปกครอง)

ลงชื่อ.....วันที่.....
(พยาน)

APPENDIX B

แบบบันทึกข้อมูลผู้เข้าร่วมการวิจัย

ลำดับที่.....

ข้อมูลส่วนบุคคล

ชื่อ-นามสกุล.....เพศ.....อายุ.....ปี

โรคประจำตัว ○ ไม่มี ○ มี ระบุโรค.....

การบาดเจ็บของร่างกาย○ ไม่มี ○ มี ระบุตำแหน่ง.....

น้ำหนัก..... กิโลกรัม ส่วนสูง..... เซนติเมตร (BMI =.....kg/m^2)

อัตราการเต้นของหัวใจขณะพัก..... ครั้ง/นาที

ความดันโลหิต..... มิลลิเมตรปรอท

ความจุปอด..... มิลลิลิตร

ความแข็งแรงของกล้ามเนื้อขา..... กิโลกรัม

แรงบีบมือ..... กิโลกรัม

ความอ่อนตัว..... เซนติเมตร

อัตราการใช้ออกซิเจนสูงสุดของร่างกาย..... มิลลิลิตร/น้ำหนักตัวกิโลกรัม/นาที

BIOGRAPHY

Miss Sureeporn Satityanuruk was born on June 14, 1975 in Kanchanaburi province, Thailand. She received Bachelor Degree in Nursing Science from the Faculty of Ramathibodi Hospital, Mahidol University in 1997. She is working at Ramathibodi Hospital.