EFFECTS OF EXERCISE TRAINING ON LIPID PEROXIDATION, SCAVENGING ENZYMES AND MUSCLE DAMAGE IN YOUNG THAI ATHLETES

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(PHYSIOLOGY OF EXERCISE)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
1999

ISBN 974-662-500-4

Thesis

entitled

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was submitted to the Faculty of Graduate Studies, Mahidol University

for the degree of Master of Science (Physiology of Exercise)

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ACKNOWLEDGEMENT

I would like to express my deep gratitude and sincere appreciation to Asst. Prof. Buarong Lewchalermwongse, my advisor, for her valuable instruction, advice, helpful guidance, criticism and kindness which has enabled me to carry out the study successfully. I am greatly indebted to Assoc. Prof. Dr. Chaivat Toskulkao and Assoc. Prof. Dr. Chumpol Pholpramool, my co-advisors, for offering an opportunity to perform this research, as well as valuable suggestions for the study.

Sincere appreciation and gratitude are also expressed to Assoc. Prof. Pipat Cherdrungsi and Assoc. Prof. Dr. Pratoom Moungmee, the thesis examining committee, for their magnificent comments and the correction of this thesis.

I wish to thank the director and the staff of Sport School of Suphanburi, Kanasutsuksalai School and Choaprayayomaraj Hospital for their kindness and co-operation during this study. Special thanks are expressed to all volunteers for their helps and participations as subjects in this study.

I am very grateful to Sport Authority of Thailand for the financial support and also to Miss Chonlada Sapeeya for her excellent typing.

I would also like to extend my appreciation to my friends in both Physiology and Physiology of Exercise programs, and the staff of Physiology Department for their help and friendship.

Special thanks are given to Miss Kanjana Jittiporn, Miss Suchira Getkong, and especially Mr. Wannasith Suksom, my best friends, for their experimental assistance and encouragement throughout the study.

Finally, I would like to express my profound gratitude and appreciation to my dearest mother and my family for their encouragement, moral support and understanding throughout my life.

Daroonwan Chakraphan

3936398 SCEP/M : MAJOR : PHYSIOLOGY OF EXERCISE;
M.Sc. (PHYSIOLOGY OF EXERCISE)
KEY WORDS : EXERCISE TRAINING / LIPID PEROXIDATION / SCAVENGING ENZYMES / MUSCLE DAMAGE

DAROONWAN CHAKRAPHAN : EFFECTS OF EXERCISE TRAINING ON LIPID PEROXIDATION, SCAVENGING ENZYMES AND MUSCLE DAMAGE IN YOUNG THAI ATHLETES. THESIS ADVISORS : BUARONG LEWCHALERMWONGSE M.Sc., CHAIVAT TOSKULKAO, D.V.M., Ph.D., PIPAT CHERDRUNGSI, M.Sc., CHUMPOL PHOLPRAMOOL, Ph.D. 148 p. ISBN 974-662-500-4

This study was undertaken to investigate the effects of long-term regular exercise training on scavenging enzymes, namely, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in humans at rest and at one hour after endurance exercise at 70% maximum heart rate. Anthropometry, physical fitness, plasma lipid peroxide using malondialdehyde (MDA) as an indicator, and muscle damage, using lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) as indicators, were also studied. Thirty two boys, 10-14 years of age, were divided into 3 groups: sedentary, swim-trained, and football-trained subjects. After one year of regular exercise training (20 hours per week), besides the significant decrease in resting heart rate and the significant increases in physical fitness characteristics i.e. muscular strength, muscular endurance, and aerobic capacity-PWC_{170}, the trained subjects showed no increase in MDA either at rest or at one hour after endurance exercise. These results were in contrast with those found in sedentary subjects in which both resting and post-exercise MDA were found to be increased significantly after one year. For tissue damage indicators, there were no increases in either CPK or LDH in the trained subjects but a significant increase in LDH in sedentary subjects after exercise. Resting and exercise induced levels of scavenging enzymes in trained and sedentary subjects were also found to be different. After one year, SOD activities were significantly increased in football-trained subjects both before and after endurance exercise while no change was found in sedentary subjects. In addition, an increase in SOD level at 48 hours after exercise was also found in swim-trained subjects. Post exercise CAT was found to be decreased and post exercise GPX was found to be increased in both trained and untrained subjects after one year.

Our findings that long-term regular exercise training caused no increase in either resting or post exercise levels of MDA and that post exercise LDH was not increased in the trained subjects suggest that long-term exercise training has beneficial effects in preventing lipid peroxidation process and reducing tissue damage possibly by the increased induction of SOD scavenging enzymes found in the trained subjects in this study.

3936398 SCEP/M: สาขาวิชา : สรีรวิทยาของการออกกำลังกาย; วท.ม. (สรีรวิทยาของการออกกำลังกาย)

ดรุณวรรณ จักรพันธุ์ : ผลของการฝึกซ้อมกีฬา ต่อลิปิดเปอร์ออกซิเดชัน สคาเวนจิงเอนไซม์ และการทำลายเซลล์กล้ามเนื้อในนักกีฬาเยาวชนไทย (Effects of exercise training on lipid peroxidation, scavenging enzymes and muscle damage in young Thai athletes) คณะกรรมการควบคุมวิทยานิพนธ์: บัวรอง ลิ่วเฉลิมวงศ์, วท.ม., ชัยวัฒน์ ต่อสกุลแก้ว, สพ.บ., ปร.ด., พิพัฒน์ เจิดรังษี, วท.ม., ชุมพล ผลประมูล, Ph.D. 148 หน้า ISBN 974-662-500-4

การวิจัยนี้มีจุดประสงค์เพื่อศึกษาผลของการฝึกซ้อมกีฬาระยะยาว (1 ปี) ต่อระดับของเอนไซม์ที่ช่วยทำลายอนุมูลอิสระ (สคาเวนจิงเอนไซม์ : SOD, CAT, GPX) ทั้งในภาวะพักและในภาวะหลังการกระตุ้นด้วยการออกกำลังกาย (เป็นเวลา 1 ชั่วโมงที่ความหนัก 70% ของอัตราชีพจรสูงสุด) นอกจากนี้ยังศึกษาถึงผลของการฝึกซ้อมกีฬาต่อสัดส่วนและองค์ประกอบของร่างกาย สมรรถภาพร่างกายทั่วไป ระดับของการเกิดลิปิดเปอร์ออกซิเดชันโดยใช้มาลอนไดอัลดีไฮด์ (MDA) เป็นตัวบ่งชี้ และระดับการบาดเจ็บของกล้ามเนื้อโดยใช้เอนไซม์แลคเตทดีไฮโดรจีเนส (LDH) และครีเอตินฟอสโฟไคเนส (CPK) ในพลาสมาเป็นตัวบ่งชี้ ผู้ถูกทดสอบเป็นเด็กชายจำนวน 32 คน อายุประมาณ 10-14 ปี แบ่งเป็น 3 กลุ่มคือ 1) กลุ่มที่ไม่ได้รับการฝึกกีฬา, 2) กลุ่มฝึกกีฬาว่ายน้ำ และ 3) กลุ่มฝึกกีฬาฟุตบอล หลังจากการฝึกซ้อมกีฬาอย่างสม่ำเสมอ (20 ชั่วโมงต่อสัปดาห์) เป็นระยะเวลา 1 ปี นอกจากจะพบว่ากลุ่มฝึกกีฬา (ทั้งว่ายน้ำและฟุตบอล) มีอัตราการเต้นของหัวใจในภาวะพักลดลง และสมรรถภาพทางกายดีขึ้นอย่างมีนัยสำคัญทางสถิติแล้ว ยังพบว่าระดับ MDA ซึ่งเป็นตัวบ่งชี้ของการเกิดลิปิดเปอร์ออกซิเดชันไม่เพิ่มขึ้นในกลุ่มฝึกกีฬาทั้งในภาวะพักและภาวะหลังการออกกำลังกาย ผลนี้แตกต่างจากกลุ่มที่ไม่ได้รับการฝึกกีฬาซึ่งพบว่าระดับ MDA เพิ่มสูงขึ้นตามเวลา 1 ปีที่ผ่านไป และสูงขึ้นอีกอย่างมีนัยสำคัญทางสถิติหลังการออกกำลังกาย สำหรับตัวบ่งชี้ของการเกิดการบาดเจ็บของกล้ามเนื้อ ภายหลังระยะเวลา 1 ปี พบว่าหลังการออกกำลังกายทั้ง CPK และ LDH ไม่สูงขึ้นในกลุ่มฝึกกีฬา แต่พบ LDH สูงขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่มที่ไม่ได้รับการฝึก นอกจากนี้ยังพบความแตกต่างของสคาเวนจิงเอนไซม์ในกลุ่มที่ได้รับและไม่ได้รับการฝึกกีฬาด้วย โดยพบว่า ระดับของ SOD เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่มฝึกกีฬาฟุตบอลทั้งก่อนและหลังการออกกำลังกาย, ไม่มีการเปลี่ยนแปลงในกลุ่มที่ไม่ได้รับการฝึก และสูงขึ้นเฉพาะที่ 48 ชั่วโมงหลังการออกกำลังกายในกลุ่มฝึกกีฬาว่ายน้ำ สำหรับ CAT และ GPX พบว่าระดับ CAT หลังการออกกำลังกายลดลง และระดับ GPX หลังการออกกำลังกายเพิ่มขึ้นภายหลังระยะเวลา 1 ปีในทุกกลุ่มของผู้ถูกทดสอบ

จากผลการทดลองนี้ซึ่งพบว่าการฝึกกีฬาอย่างสม่ำเสมอเป็นระยะเวลานาน 1 ปี ไม่ทำให้เกิดการเพิ่มขึ้นของ MDA ทั้งในภาวะพักและในภาวะหลังการกระตุ้นด้วยการออกกำลังกาย และระดับของ LDH หลังการออกกำลังกายไม่เพิ่มขึ้นในกลุ่มฝึกกีฬานั้น ชี้ให้เห็นว่า การฝึกกีฬาระยะยาวมีผลดีในการป้องกันการเกิดกระบวนการลิปิดเปอร์ออกซิเดชัน และมีส่วนในการลดการทำลายเซลล์กล้ามเนื้อ โดยกลไกอาจจะเนื่องมาจากการฝึกกีฬาอย่างสม่ำเสมอเป็นเวลานานสามารถกระตุ้นการสร้างสคาเวนจิงเอนไซม์ SOD ให้เกิดมากขึ้น ดังที่พบในกลุ่มที่ได้รับการฝึกกีฬาทั้ง 2 ประเภท

CONTENTS

LIST OF TABLES

Table **Page**

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

ADP	adenosine 5′-diphosphate
ANOVA	analysis of variance
ATP	adenosine 5′-triphosphate
BD	body density
BW	body weight
Ca^{2+}	calcium ion
CAT	catalase
cm	centimeter
CO	cardiac output
CP	creatine phosphate
CPK	creatine phosphokinase
Cu	copper
°C	degree Celcius
DNA	deoxyribonucleic acid
DHER	dehydroepiandrosterone
EDTA	ethylene diaminetetraacetic acid
et al	and colleagues
% BF	percent body fat
Fe^{2+}	ferrous ion
Fe^{3+}	ferric ion

g	gram
g%	gram percent
g/dl	gram per decilitre
GPX	glutathione peroxidase
GSH	glutathione
GSSG	glutathione disulfide
H^+	hydrogen ion
Hb	hemoglobin
Hct	hematocrit
H_2O_2	hydrogen peroxide
HR	heart rate
HRmax	maximum heart rate
hr	hour
kg	kilogram
LBM	lean body mass
LDH	lactate dehydrogenase
$L^{\bullet}$	lipid radical
LH	lipid
$LO^{\bullet}_2$	peroxyl radical
LOOH	lipid hydroperoxide
M	molar
MDA	malondialdehyde
mg	milligram

mg%	milligram percent
min	minute
ml	millilitre
mM	millimolar
mmHg	mercury millimeter
µl	microlitre
µmole	micromole
n	number
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced form
NADP	nicotinamide adenine dinucleotide, 3'-phosphate
NADPH	nicotinamide adenine dinucleotide, 3'-phosphate, reduced form
nmole	nanomole
O_2	oxygen molecule
$O_2^{\bullet}$	superoxide anion or superoxide radical
O.D.	optical density
OH^-	hydroxide ion
$OH^{\bullet}$	hydroxyl radical
pH	log concentration of $(H^+)^{-1}$
RBC	red blood cell
RNA	ribonucleic acid
ROS	reactive oxygen species
rpm	round per minute

sec	second
SEM	standard error of means
SDS	sodium dodecyl sulfate
SOD	superoxide dismutase
s.v.	stroke volume
TBA	Thiobarbituric acid reactive substance
TBARS	Thiobarbituric acid reactive substance
TMP	1,1,3,3 tetramethoxypropane
$\dot{V}O_2max$	Maximum oxygen consumption
U/gHb	Unit per gram of hemoglobin
U/L	one micromole of NADPH per minute per litre
wk	week
yr	year
Zn	zinc

CHAPTER I

INTRODUCTION

In recent years, exercise has expanded in scope from competitive sports to disease prevention and health promotion (1). Although physical exercise is known to have many beneficial effects, there is also some evidence that exercise increases the free radical production (2-4). Strenuous physical exercise and sports are associated with a dramatic increase in oxygen uptake both at the whole body level and in the skeletal muscle (5). Most of the oxygen consumed is used in the mitochondria for oxidative phosphorylation and is reduced to water (6, 7). However, a small fraction of oxygen (approximately 2% to 5%) is converted into several oxygen intermediates, i.e., superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) (5, 8, 9). Collectively, they are classified as "reactive oxygen species" (10, 11). In the past decades, increasing evidence has accumulated indicating that the production of reactive oxygen species may be involved in numerous biochemical and physiological events leading to cell and tissue injury (5). They are potentially deleterious to the cell because they are able to abstract hydrogen atoms from polyunsaturated fatty acids of cellular membranes which leads to the subsequent chain reaction process called lipid peroxidation (12-15). The degree of lipid peroxidation occurred can be estimated by the assessment of its main product, malondialdehyde or MDA (16, 17). Lipid peroxidation of cell membrane causes loss of fluid property and membrane flexibility due to the alteration in the fluid-mosaic bilayer, which perturbs cell integrity (18-20).

The leakage of intracellular enzymes across the abnormally permeable membrane into the plasma, provides important clinical parameters of cell injury (21). The enzymes most commonly used as cell injury indicators are creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) (22, 23).

However, during evolution living organisms have developed a sophisticated antioxidant system to cope with the deleterious byproducts of oxygen reduction. These systems include chemical compounds capable of "scavenging" reactive oxygen species as well as a series of enzymes specializing in reducing free radicals to more stable species (24). The three most important antioxidant enzymes in our body are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (25-27). It is believed that the antioxidative defense system is in a dynamic equilibrium, so that a decrease in one of the factors will, to a certain extent, be compensated through an increased capacity of the other antioxidants. This implies that evaluating the capacity of the antioxidative defense system requires determination of the whole antioxidant status at the time (28).

Despite the exercise-induced free radical changes as mentioned above, there is a positive side of oxidative stress associated with regular exercise (3). The enzymatic defense system has been reported to be increased in trained tissue of rats, following regular exercise (26, 29-31). For human, Toskulkao et al. (32) have reported that exercise training programme resulted in the higher activities of scavenging enzymes (SOD, CAT, and GPX) in either short or long distance runners when compared to those in sedentary subjects. However, a study on levels of antioxidant enzymes in highly trained subjects by Marzatico et al. (33) showed a significant reduction in CAT

after training. Ohno et al. (34) who also studied the effect of physical training in human reported no change in SOD activity after training. Therefore, the information about the effects of exercise on lipid peroxidation and scavenging enzymes in human are still controversial and somewhat limited. Moreover, there is still no longitudinal study in human comparing the level of lipid peroxidation and scavenging enzymes after exercise in the same group of subjects after long-term exercise training. This study was thus performed to investigate the effects of long-term exercise training on lipid peroxidation, tissue damage, and scavenging enzymes in human. Besides the effects of long-term exercise training, the effect of single bout endurance exercise and types of exercise (weight bearing and non-weight bearing) on lipid peroxidation, tissue damage and scavenging enzyme responses in trained and untrained subjects were also investigated.

The objectives of this study were as follow:

1. To study the effect of endurance exercise on lipid peroxidation (plasma MDA level), tissue damage (plasma CPK, LDH) and erythrocyte scavenging enzymes (SOD, CAT, GPX) in trained and untrained boys.

2. To study the effect of long-term (one year) exercise training on resting levels and also on changes after endurance exercise of MDA, LDH, CPK and scavenging enzymes (SOD, CAT, GPX).

3. To study whether different types of exercise training (weight bearing as football and non-weight bearing as swimming) lead to different responses in lipid peroxidation, tissue damage and scavenging enzymes after endurance exercise.

CHAPTER II

LITERATURE REVIEW

I. Free radicals and lipid peroxidation

Free radicals

Free radicals may be broadly defined as molecules or ions that capable of existing independently and containing one or more unpaired electrons in their outer orbitals (11, 12, 14, 18, 35, 36). The conventional radical dot (•) designates the presence of one or more of the unpaired electrons (35, 36). The unpaired electron causes the free radical to be highly reactive and unstable (13, 21, 38, 39). To regain the stability, the free radicals will pull electrons from adjacent molecules. If free radical generation is intense and uncontrolled, the outcomes are general molecular chaos and loss of cell functions (32, 40).

In general, human cell is aerobic and consumes molecular oxygen in energy producing process. The oxygen free radical intermediates partly yielded from the electron transferred process in respiratory chain are harmful for aerobic cells. The three most important oxygen derived free radicals are superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) (5, 21, 27, 30). They are all occurred through the reduction process of a molecular oxygen. The first addition of one electron forms superoxide ($O_2^{\bullet}$), the second converts this to hydrogen peroxide (H_2O_2) and the third produces hydroxyl radical ($OH^{\bullet}$) (18). (Figure 1)

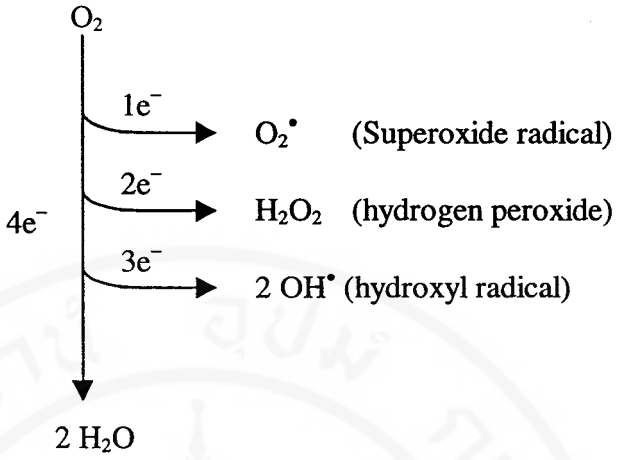


Figure 1. Univalent pathway for the reduction of molecular oxygen. By a series of single-electron transfers molecular oxygen is reduced first to the superoxide radical ($O_2^{\bullet}$) and from superoxide, with the addition of two electrons, to hydrogen peroxide (H_2O_2). Hydrogen peroxide is itself then univalently reduced, with the addition of another proton, to water and the hydroxyl radical ($OH^{\bullet}$). A final univalent reduction and the addition of another proton convert the hydroxyl radical to water. [Adapted from Bulkley Gb. (18)].

These three reactive oxygen species (ROS) can cause a wide spectrum of cell damage including inactivation of enzymes, alteration in intracellular oxidation-reduction state, damage of DNA and lipid peroxidation (37, 41, 42).

Lipid peroxidation

Lipid peroxidation is a process that any free radical ($R^{\bullet}$) in the presence of molecular oxygen (O_2), with sufficient energy, abstracts a hydrogen atom from a methylene group ($-CH_2$) of unsaturated fatty acid (LH) (11,15, 16, 19). (Figure 2)

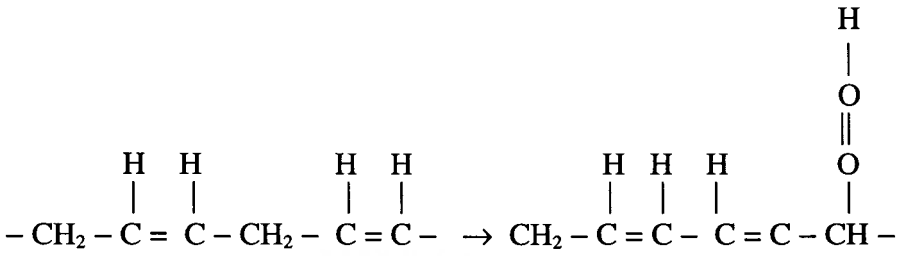


Figure 2. Change in the structure of lipid due to hydrogen abstraction by free radicals (36).

The process of lipid peroxidation can be divided into three distinct steps as followed (Figure 3)

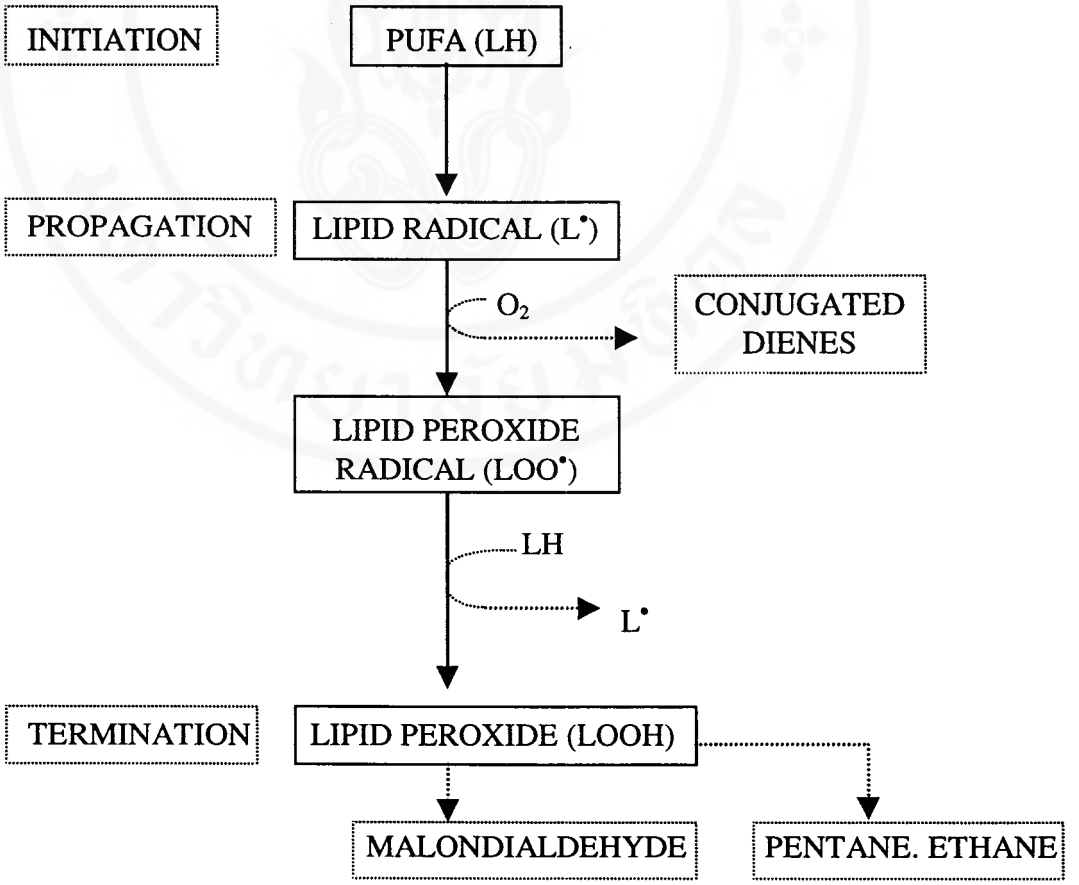


Figure 3. Three steps of lipid peroxidation : initiation, propagation and termination (4).

1. Initiation Step

The first step is the initiation process by which a free radical ($R^{\bullet}$) removes a H atom from a lipid molecule (LH), thus generating a lipid radical ($L^{\bullet}$); as in the reaction below:

$$LH + R^{\bullet} \longrightarrow L^{\bullet} + RH$$

2. Propagation Step

The second steps are series of propagation reactions in which the lipid radical ($L^{\bullet}$) quickly reacts with molecular oxygen and forms another radical, the peroxy radical ($LOO^{\bullet}$) which in turn removes another H atom from an unsaturated fatty acid, producing a lipid hydroperoxide (LOOH) as well as another $L^{\bullet}$. As long as a carbon centered $L^{\bullet}$ continues to be produced in the presence of oxygen, the generation of $LOO^{\bullet}$ and $L^{\bullet}$ will continue as will the demise of the unsaturated fatty acid (36). The propagation reactions are shown below:

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}$$

3. Termination Step

Propagation of the lipid peroxidation process will continue until the substrate is depleted or the process is interfered by antioxidant (AH). The antioxidant will react with the free radicals and interrupt the chain reactions process, thereby terminate the reaction (32, 43, 44).

$$LOO^{\bullet} (\text{or } L^{\bullet}) + AH \longrightarrow LOOH \text{ (or LH)} + A$$

$$LOO^{\bullet} (\text{or } L^{\bullet}) + L^{\bullet} \longrightarrow LOOL \text{ or } L_2$$

Normally, biomembranes contain relatively large amounts of polyunsaturated fatty acid (PUFAs). The greater the number of double bonds in the fatty acid side chain the easier the hydrogen atom can be removed. So, PUFAs are major sites of lipid peroxidation (11, 16, 36). Oxidation of PUFAs or lipid peroxidation leads to the disintegration of fatty acid and formation of hydrocarbon gasses (e.g., pentane) and aldehydic compounds, in particular the volatile low molecular weight aldehyde, malondiadehyde (MDA) (5, 45). The deleterious health outcomes associated with accumulation of large amounts of lipid peroxidation byproducts such as MDA, include an increase in tumor frequency, incidence of atherosclerosis and cancer (36).

Lipid peroxidation induced cellular damage.

Free radicals damage cell membranes by catalyzing amino acid oxidation, protein-protein cross-linking, protein strand scission, and attacking polyunsaturated fatty acid in cell membrane leading to lipid peroxidation and MDA production. (Figure 4). MDA can induce polymerization of membrane components and variety of cross-linking reactions such as lipid-lipid cross-linking and lipid-protein cross-linking (18). These effects will result in deteriorative changes in cellular membranes such as a decrease in membrane fluidity and an inability to maintain ionic gradients. Cellular swelling, a loss of cell integrity and cell inflammation will follow (3, 46).

Free radicals may also disrupt subcellular membrane of important organelles such as mitochondria, microsome and lysosome (18, 21, 47). Lipid peroxidation of the mitochondrial membrane leads to swelling, lysis, and disintegration of the mitochondria. Microsomal membrane damage results in disaggregation of

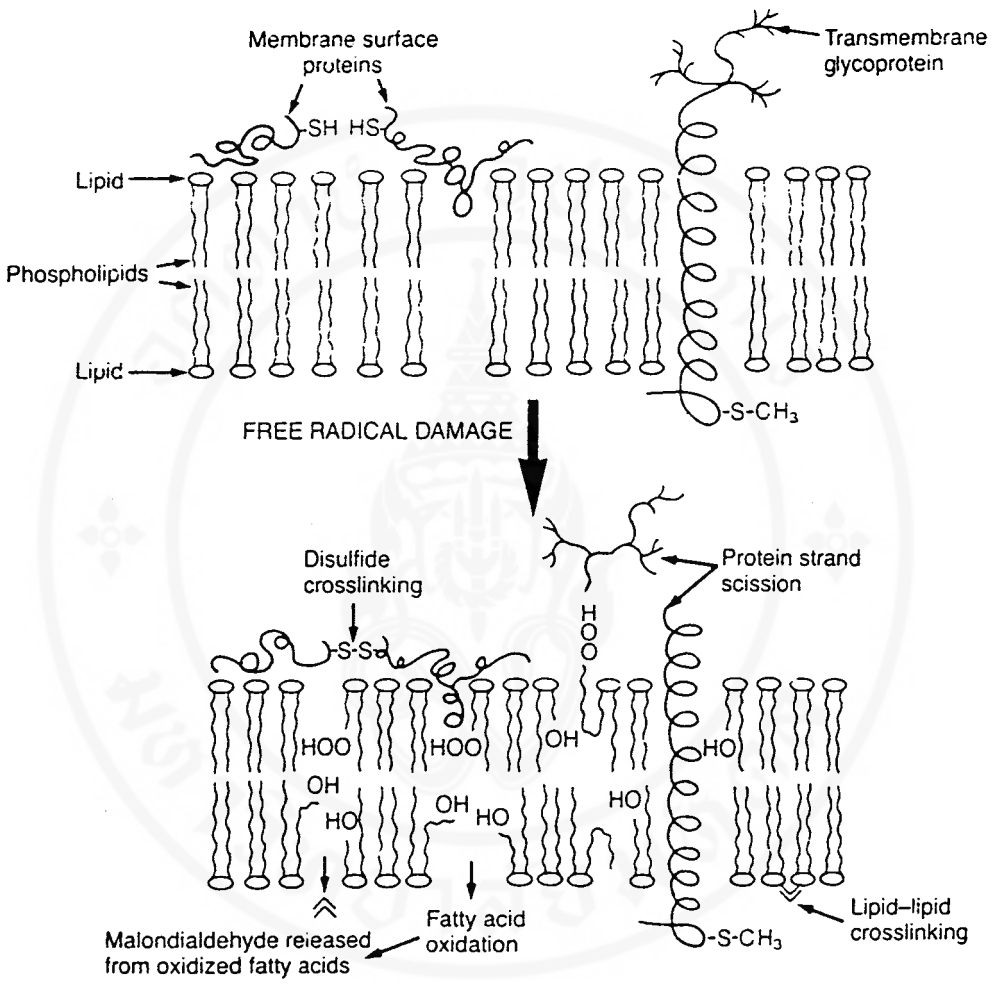


Figure 4. Free radical damage of membranes. Free radicals can affect lipids by initiating peroxidation, which leads to short chain fatty acyl derivatives and the byproduct malondialdehyde. Variety of cross-linking reactions can be mediated malondialdehyde reactions. Free radicals can also catalyze amino acid oxidation, protein-protein cross-linking, and protein strand scission.

polyribosomes and inhibition of protein synthesis. For the lysosomes, since they contain hydrolytic enzymes, the lysosomal membrane damage will be followed by the enzymatic digestion of other cell components (11).

II. Scavenging enzymes

Our body is equipped with a sophisticated antioxidant system to deal with the production of reactive oxygen species (ROS). The system includes antioxidant enzymes and antioxidant vitamins. Antioxidant enzymes or "scavenging enzymes" provide the first line of defense against ROS by converting them to more reduced and more stable species. The three major scavenging enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (5, 25-27, 48) (Figure 5). A second line of defense is provided by exogenous antioxidants primarily obtained as nutrients or nutritional supplements such as vitamins, carotenoids and reduced glutathione (11, 17).

The possible mechanisms by which antioxidants may protect against oxygen toxicity are as follows: (11, 35)

1. decreasing localized oxygen concentration and prevention at ROS formation.

2. chain-breaking mechanism whereby intermediate radicals (e.g., $O_2^{\bullet}$) are scavenged to prevent continuation of more deleterious forms (e.g., $OH^{\bullet}$, H_2O_2).

3. interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistance of sensitive biological targets to ROS attack.

4. facilitating the repair of damage caused by ROS and triggering the expression of genes that encode antioxidant proteins.

5. providing a favorable environment for the effective functioning of other antioxidants (e.g., as a cofactor or by acting to maintain a suitable redox status).

Superoxide dismutase (SOD)

SOD is the enzyme involves in cellular defense against uncontrolled oxidative processes. It catalyzes the dismutation of the superoxide radical anion and hence diminishes toxic effects due to this radical or to other free radicals derived from secondary reactions (36). Thus, the principle function of SOD is to catalyze the conversion of superoxide ($O_2^{\bullet}$) to hydrogen peroxide (H_2O_2) as in the reaction below (5, 11).

$$2O_2^{\bullet} + 2H^{+} \xrightarrow{SOD} O_2 + H_2O_2$$

Catalase (CAT)

Catalase is an enzyme present in nearly all animal and plant cells (49). In mammalian cells, catalase is mostly localized in the peroxisomes (microbodies) of liver and kidney, and in much smaller aggregates (micro-peroxisomes) found in other tissues (36). For human, catalase, in soluble state, is also found in red blood cells (32). It is suggested to play a dual roles (32, 36, 49) which are:

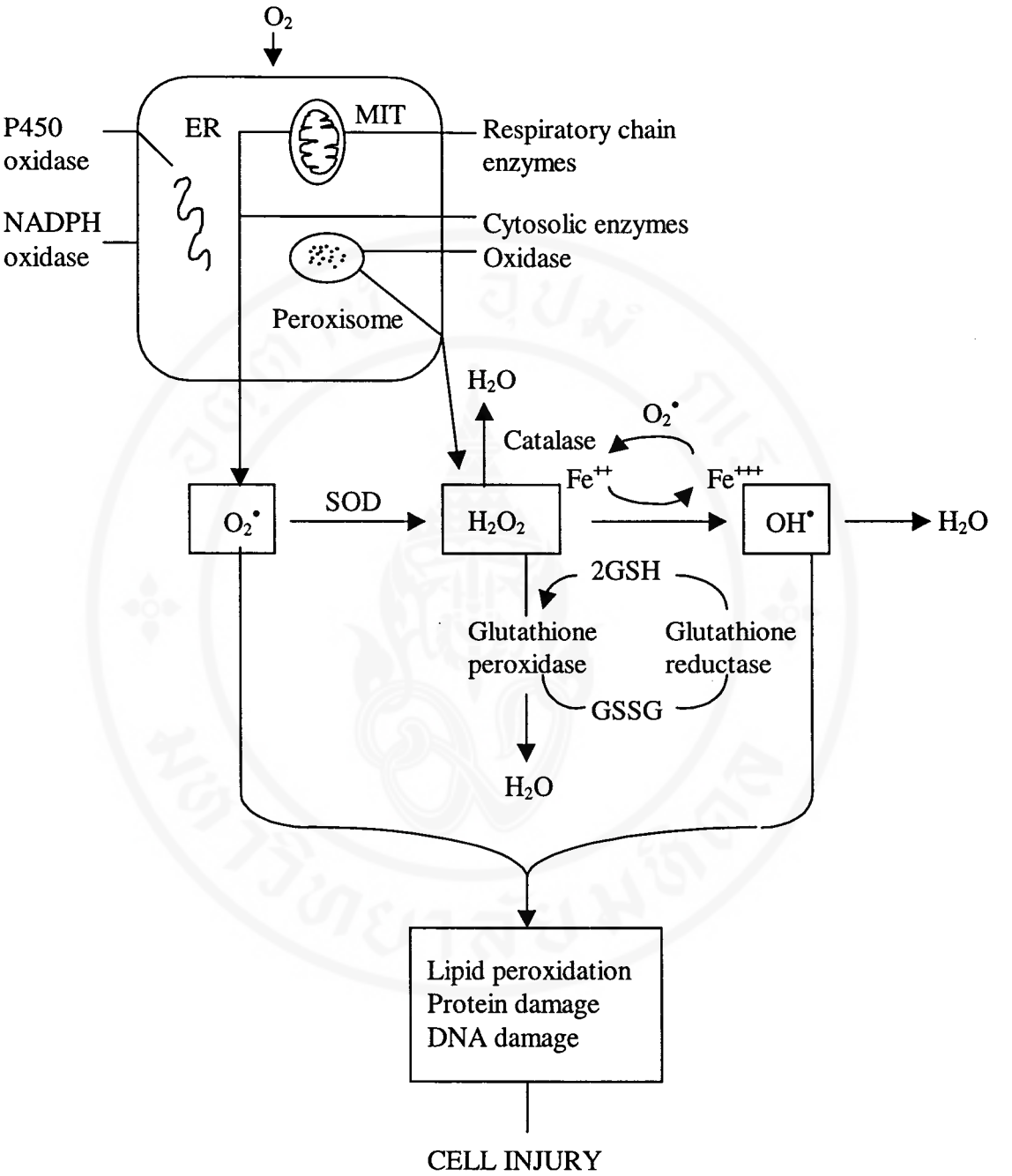


Figure 5. Formation of reactive oxygen species and anti-oxidant mechanisms in biological systems. O_2 is converted to superoxide ($O_2^{\bullet}$) by oxidative enzymes in the ER, mitochondria, plasma membrane, peroxisomes, and cytosol. $O_2^{\bullet}$ is converted to H_2O_2 by dismutation and thence to $OH^{\bullet}$ by the Cu^{++}/Fe^{++} catalyzed Fenton reaction. H_2O_2 is also derived directly from oxidases in peroxisomes. Not shown is another potentially injurious radical, singlet oxygen. Resultant free radical damage to lipid (peroxidation), proteins, and DNA leads to various forms of cell injury. Note that superoxide catalyzes the reduction of Fe^{+++} to Fe^{++}, thus enhancing $OH^{\bullet}$ generation by the Fenton reaction. The major antioxidant enzymes are superoxide dismutase, catalase, and glutathione peroxidase.

1. to decompose hydrogen peroxide (H_2O_2) as in the reaction below.

$$2H_2O_2 \xrightarrow{CAT} H_2O_2 + O_2$$

2. to catalyse a peroxidation reaction in which the hydroperoxide (ROOH) is utilized to oxidize a range of H donors (AH_2) such as methanol, ethanol and formal dehyde, as in the reaction below.

$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

In each case an active enzyme-H_2O_2 complex is firstly formed followed by an exceedingly rapid second stage in which a second molecule of H_2O_2 serves as a H-donor for the enzyme-H_2O_2 complex.

Glutathione peroxidase (GPX)

GPX is found in animal's liver and lung and human's erythrocytes. It consists of four apparently identical subunits, each of which contains one atom of selenium (Se). Selenium in native glutathione peroxidase is in the reduced form which is not bound to oxygen. It has long been known that Se is an essential nutrient. Se-deficient animals have markedly decreased GSH peroxidase activity (30, 50).

GPX catalyzes the following reactions:

1. $2GSH + H_2O_2 \xrightarrow{GPX} GSSG + H_2O_2$

GPX has been shown to have high specificity to the detoxification of hydrogen peroxide (H_2O_2) by the oxidation of reduced glutathione (GSH) (51).

$$2.\quad 2GSH + ROOH \xrightarrow{GPX} ROOH + GSSG$$

GPX catalyzes the reduction of hydroperoxides (ROOH) with glutathione (GSH) as the reductant to generate oxidized glutathione (GSSG) (52).

III. Effects of exercise and exercise training

Effects of exercise

Exercise is an important lifestyle change which may bring about significant benefits in health and quality of life (53). It is referred to as physical activity or work of a fairly vigorous nature leading to a number of important bodily changes. Many of these changes influence physiological as well as psychological functions in very striking ways. The changes in function of the body are brought about by both single and repeated bouts of exercise.

The functional changes that occur when one exercises a single time are called responses to exercise. These functional changes are sudden, temporary and disappear shortly after the exercise period is finished. They are, for example, the increase in heart rate, the rise in blood pressure, the increase in breathing, the increase in blood flow to the working muscles and the decrease in blood flow to the stomach and the kidney. Each of these responses is no longer present a few minutes after the exercise is over (54, 55).

However, there is some evidence that acute exercise may be harmful. The increase in oxygen uptake during exercise is accompanied by an elevation of reactive oxygen species (ROS), which is considered to be oxidative stress (17).

Exercise induced lipid peroxidation and muscle damage

Physical exercise and sports are associated with a dramatic increase in oxygen uptake both at the whole-body level and in the active skeletal muscle (5). The rate of oxygen uptake by the body during exercise may increase by 10–15 folds (11). Most of the oxygen consumed is used in the mitochondria for oxidative phosphorylation and is reduced to water. However as has been mentioned before, a small fraction of oxygen consumed is converted into several oxygen free radicals such as superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). Electron spin resonance and paramagnetic resonance spectrometry are two methods that are used to detect free radicals in both *in vitro* and *in vivo* models (3). Devies et al. (2) measured electroparamagnetic resonance of tissue homogenates from animals at rest compared with exercise and reported a two to three folds increase in paramagnetic signal peaks of muscle and liver tissues following exhaustive exercise. Jackson et al. (56) examined the effects of 30 min of excessive muscle contractile activity on the amplitude of a major electron spin resonance signal. They reported a 70% increase in amplitude of the major signal in active intact muscle compared with rest. These two studies showed that free radicals are increasingly produced during exercise. These deleterious free radicals when react with components of cell such as polyunsaturated fatty acid will lead to lipid peroxidation and cell damage (4, 8).

Markers for lipid peroxidation and muscle damage

Significant increases in free radical production during exercise with subsequently induction of muscle damage can be indirectly measured by the presence of several lipid peroxidation byproducts. Conjugated dienes appear at the onset of

lipid peroxidation and seem to be linked to several steps of lipid peroxide degradation so they are considered to be one of reliable measurements of lipid peroxidation (57). However, dienes do not indicate which specific lipid peroxidation products are being measured. In fact, only 30-55% of lipid peroxidation is actually detected by diene measurement (57-59). Another biomarker of lipid peroxidation, lipid hydroperoxide, is formed when hydroperoxides are reduced by the peroxidase activity of hemoglobin. Because hemoglobin has broad substrate specificity, including nonperoxides, the sensitivity of this method to measure lipid peroxidation is low in unpure system (60). Furthermore when exercising at intensities below maximum effort, an increase in lipid hydroperoxide was not always evident.

TBARS have been widely used to measure lipid peroxidation in cell membranes and fatty acids. This thiobarbituric acid technique has been shown to be sensitive to malondiadehyde, a good general index of oxidative stress in biological systems (61). Kanter et al. (62) reported a 77% increase above the resting concentration of TBARS in both plasma and serum from highly trained humans following exhaustive running exercise. Davies et al. (2) reported a similar percent increase in malondialdehyde (81%) above rest following exhaustive running exercise in untrained rats. Regardless of training status exhaustive exercise appeared to have a similar effect on TBARS and malondialdehyde in both human and rat models.

Plasma MDA measurements during exercise have also been correlated with creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), markers for muscle damage (22, 63-65). These enzymes are the efflux of intramuscular proteins from the damaged muscle fibers into the extracellular space (21, 22, 66-68). The use of CPK

and LDH as the indicators for muscle damage is on the basis of the assumption that the release of the enzymes, reflected by the increased plasma enzyme activities, is related to the muscle enzymes content as well as to the degree of exercise-induced muscle damage (22).

Malondialdehyde (MDA)

MDA, a lipid peroxidation byproduct, is a toxic compound capable of reacting toward sulfhydryl and amino groups of proteins. It is a bifunctional cross-linking agent since it can produce both intramolecular and intermolecular linkages which can lead to inactivation and polymerization of enzymes such as ribonuclease. Its reactivity toward amino groups can result in interactions with the endogenous base of DNA and inhibition of DNA, RNA and protein synthesis thereby affecting their biological functions (47, 69, 70).

Malondialdehyde levels have been found to increase during exercise in a variety of tissues and the levels also seem to depend on the intensity of exercise or work load. It is the most common parameter that has been studied with respect to exercise. In most cases, the thiobarbituric acid (TBA) reactivity approach has been used for MDA assay (3, 11, 45, 71, 72). Eventhough the reliability of using malondialdehyde as an indication for oxidative stress has been questioned recently, it remains a useful marker for gross oxidative damage if a multifaceted approach is adopted and the limitations are recognized (5).

Creatine phosphokinase (CPK)

Creatine phosphokinase is a dimeric enzyme in the muscle cells that catalyzes the reversible phosphorylation of ADP by creatine phosphate to form ATP and free creatine. The reaction is : phosphocreatine + ADP $\overset{CPK}{\rightleftharpoons}$ Creatine + ATP. CPK is a large molecule (80,000 Da) and therefore does not enter the blood stream directly. The disruption of the muscle cell membrane can cause CPK to leak from the muscle tissue into the interstitial fluid passing into the lymphatic system and finally emptying into the general blood circulation (73).

The two dimers of CPK, M (muscle) and B (brain), combine to form three isozymes : CK-MM, CK-MB, and CK-BB. Each isoenzyme carries out the identical reaction in different tissues of the body : CK-MB in the cardiac tissue, CK-BB in the brain and CK-MM in the muscle (73).

The M subunit can combine to form three possible CK-MM isoforms CK-MM1 is the pure gene product and is released from the muscle tissue into the circulation and converted into CK-MM2 and then CK-MM3. CK-MM2 and CK-MM3 are formed by a carboxpeptidase-catalyzed hydrolysis of c-terminal lysine residues (74).

Lactate dehydrogenase (LDH)

Lactate dehydrogenase is also commonly used as a marker for muscle tissue damage (22). The formation and the turnover rate of lactate during heavy muscular exercise is catalyzed and regulated by the enzyme LDH (75). This enzyme catalyzes the interconversion between lactate and pyruvate in glycolytic pathway.

The reaction is :

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

LDH is composed of four subunits of either M (muscle) or H (heart) type, which consequently may combined in 5 different ways and result in 5 isozymes. LDH-3 is the predominant fraction found in the lung and spleen. The heart specific isozymes (LDH-1 and LDH-2) are bound to mitochondria and favor the oxidation of lactate to pyruvate during aerobic metabolism, while the muscle specific isozymes (LDH-4 and LDH-5) seem to be bound to the sarcoplasmic reticulum and reduce pyruvate under anaerobic condition (75-77).

Effects of exercise training

A single bout of exercise is sometimes called "acute exercise" whereas repeated bouts of exercise over several days or months is called "chronic exercise" or "exercise training" (54). The major objective of training is to facilitate biologic adaptations that improve performance in specific tasks. An "adaptation" is a more or less persistent change in structure or function following training that apparently enables the body to respond more easily to subsequent exercise bouts. Ordinarily, adaptations are not seen until several weeks of training have passed, but some occur after only four or five days of training. One example of an adaptation to training is a reduction in heart rate for a submaximal exercise load that nearly always follows several weeks of training. This reduction in exercise heart rate seems to enable the heart to pump the same amount of blood to the body's tissues at a lower energy cost for the heart. Another example of an adaptation is the increased muscle size that accompanies a strenuous weight-lifting program and enables the lifter to exert greater muscular force

than before training. Much of this increased strength persists for many months after the training program ends (54).

Factors that affect training response

The training is influenced by many factors such as intensity, duration, frequency and type of exercise (55, 78, 79). Each of these is important for physical adaptations. To achieve the training improvement, these major factors must be considered.

Exercise intensity

The intensity of training is the most important factor for improving aerobic capacity (55, 78, 79). It reflects both the caloric cost of the work and the specific energy systems activated. Intensity can be applied either on an absolute or relative basis.

Absolute intensity: An example of an absolute training intensity would be to have all individuals do the same work at the same rate, such as 200 watts on the bicycle ergometer or expend 300 kcal in a 30-minute exercise session. With everyone doing the same amount of work, a considerable exercise stress for one person might be below the training threshold intensity for another more highly conditioned person. For this reason, training is usually assigned based on the relative stress placed on a person's physiologic system (78).

Relative intensity: Relative intensity is usually assigned as some percentage of maximum function, for example, maximum oxygen consumption ($\dot{V}O_2max$), maximum heart rate (HRmax), or maximum working capacity. The general practice for

establishing aerobic training intensity is to either directly measure or estimate the person's $\dot{V}O_2max$ or maximum heart rate, and then assign a work schedule that corresponds to some percentage of these maximums (78).

Although establishing training intensity from measures of oxygen consumption is reasonably accurate, it is impractical without sophisticated equipment. An effective alternative is to use heart rate to classify exercise in terms of relative intensity and to establish a training protocol. This practice is based on the fact that percent $\dot{V}O_2max$ and HRmax are related in a predictable way, regardless of gender, fitness level or age. Selected values for percent $\dot{V}O_2max$ and corresponding percentages of HRmax obtained from several sources are presented in Table 1 (80-82).

Table 1. Relationship between percent HRmax and percent $\dot{V}O_2max$ (79).

Percent HRmax	Percent $\dot{V}O_2max$
50	28
60	40
70	58
80	70
90	83
100	100

The error in estimating percent $\dot{V}O_2max$ from percent HRmax, or vice versa, is about $\pm$ 8%. Because of this intrinsic relationship, it is only necessary to monitor heart rate to estimate percent $\dot{V}O_2max$ (78, 79).

As a general rule, aerobic capacity improves if exercise is of sufficient intensity to increase heart rate to about 70% of maximum. This is equivalent to about 58%

$\dot{V}O_2$max or, for college-aged men and women, to a heart rate of 130 to 140 beats per minute with leg exercise as in cycling, walking, and running. This intensity appears to be the minimal stimulus required to provide training improvements. An exercise at heart rate of 70% maximum represents moderate exercise that can be continued for a long time with little or no discomfort (78). Generally, the greater the relative training intensity above threshold, the greater is the training improvement (83, 84). However, this is only true within certain limits. Although there may be a minimal threshold intensity below which a training effect does not occur, there may also be a ceiling "threshold" above which there are no further gains. The lower and upper limits may depend on the participant's initial capacity and state of training. For people in relatively poor condition, the training threshold may be closer to 60% of HRmax that corresponds to about 45% $\dot{V}O_2$max. Individuals at higher fitness levels generally have a higher threshold levels (85). The ceiling for training intensity is unknown, although 85% $\dot{V}O_2$max (corresponding to 90% HRmax) is thought to be the upper limit. At present, no definitive research is available to either prove or disprove this notion (78, 79).

Duration of training

One of the most common inquiries concerning exercise participation deals with the duration of the daily workout. In general, performing less exhaustive, moderate-pace exercise for 20 to 30 minutes per session is a realistic recommendation for exercising in terms of intensity of effort and time commitment. However, most competitive endurance athletes spend from 2 to 3 hours or more per training session in activities geared to enhance the functional capacity of their physiologic systems (86).

For optimal improvement depends on the interaction of many factors including the total work accomplished, exercise intensity, training frequency, and initial fitness level. Whereas 3 to 5 minute periods of daily exercise produce training effects in some poorly conditioned people, 20 to 30 minute exercise sessions achieve more optimal results if the intensity is at least 70% HRmax. At higher-intensity training, significant improvements will occur during a 10-minute workout. Conversely, 60 minutes of continuous exercise may be required to produce a training effect when exercise intensity is below the threshold heart rate (78).

Frequency of training

Training frequency is another important factor in eliciting cardiovascular improvements (87). However, this factor may be considerably less important than either exercise intensity or duration (83, 84). Exercise at least 3 days a week for at least 6 weeks generally is necessary to bring about adaptive changes in the aerobic system (55). More frequent training is beneficial if the training is performed at a lower intensity. The extra investment of time needed to increase training frequency may not always be that profitable in inducing improvements in physiologic function (78, 79). Training fewer than 2 days per week generally does not produce meaningful changes in anaerobic or aerobic capacity or body composition (55).

Mode of exercise

If exercise intensity, duration, and frequency are held constant, training improvements are similar, regardless of training mode as long as the exercise involves relatively large muscle groups and improvement is evaluated in the exercise mode used

during the training (79). The magnitude of training changes may vary considerably depending on the mode of testing. Likewise, individuals that train by swimming show the greatest improvement when measured during an upper body exercise (88, 89). However, most mode of exercise, when used in a training program will lead to substantial and equal gains in fitness (79).

General physical fitness testing

The progress of training can be determined by the assessment of general physical fitness of individual. The recommended tests for the general physical fitness components have been grouped into the following areas:

1. **Muscular strength** is the capacity of a muscle to exert a maximal force against a resistance. Strength training results in some enlargement of the muscle fiber (hypertrophy) and a relative increase in one's ability to apply force. It is fundamental to all sports. A lack of reasonable strength obviously contributes to poor performance in sports (54).

Muscular strength can be measured using equipment such as dynamometer, cable tensiometers and force transducers and recorders or one-repetition maximum test such as bench press, standing press, leg press, half squats and arm curl (90).

2. **Muscular endurance** is the capacity of a muscle to exert a force repeatedly over a period of time. It is the ability to apply strength and sustain it. Three common tests of muscular endurance are the sit up, the pull up and the flexed-arm hang (86, 91).

3. **Flexibility** is the ability to move the body throughout its maximum range of motion and concerns the stretching of the muscles and tissues around skeletal joints.

Flexibility will normally be measured with either of the three tests: shoulder reach, trunk flexion (sit & reach) and trunk extension (86, 91).

4. **Cardiorespiratory endurance** is the most essential physical fitness component. Test conducted under stress for the evaluation of aerobic metabolism can be performed by subjecting the athlete to maximal or submaximal work. $\dot{V}O_2max$ is the most expressive parameter of aerobic capacity, and it can be estimated by two methods: (a) direct measurement using spiroergometric methods and (b) indirect measurement using submaximal exercise tests (86). The direct measurement of $\dot{V}O_2max$ requires expensive laboratory equipment, a considerable amount of time, and a high level of motivation on the part of the subject. Alternatively, submaximal exercise tests can be used to predict or estimate the $\dot{V}O_2max$ of the individual. It assumes a linear relationship between heart rate, oxygen uptake and work intensity. While this is true for light to moderate work loads, the relationship between oxygen uptake and work becomes curvilinear at heavier work loads (91).

5. **Body fat assessment** : In clinical settings, exercise science and health professionals routinely assess body fat (BF) levels of their subject using methods such as skin folds (SKF), bioelectrical impedance analysis (BIA), and near-infrared interactance (NIR) (92). Hydrodensitometry or hydrostatic weighing has been also considered by some experts as a gold standard method for estimating %BF. However, this method is not error-free since accurate assessment of body fat using this method requires correcting total body volume for residual lung volume. Katch and Katch (93) reported that a 600 ml difference in residual volume may affect the estimation of relative body fat (%BF) by as much as 8% BF. Thus, in field study, the most

acceptable method for measuring body fat composition is skinfold technique, due to its simplicity, low cost, and comparable reliability. In using skinfold calipers, 2-3 sites of skinfold measurements are normally performed. The sum of data from skinfold mesurement is used to calculate the percentage of body fat (%BF), the poorer the athletic performance (94).

Anaerobic and aerobic training

All three energy-transfer systems- the ATP-CP system, the glycolytic or lactic acid system, and the aerobic or oxygen system-operate at different times during exercise. Their relative contributions to the energy continuum directly relate to the duration and intensity of specific activity (86). Exercise is broadly classified, in terms of its duration and the predominant energy pathways, into two categories: anaerobic and aerobic exercise.

Anaerobic training

Anaerobic training is the training to improve the capacity to perform all-out exercise for brief periods of time (up to 60 seconds). Anaerobic exercise largely depends on ATP generated by the immediate and short-term anaerobic energy system. Sports such as football, weightlifting and various other brief sprint activities rely almost exclusively on energy derived from the ATP and CP that comprise the muscle's high energy phosphates. In training to enhance ATP-CP energy transfer capacity, the activities selected must engage the specific muscles at the movement speed and power output for which the athlete desires to improved anaerobic power. As the duration of all out effort extends beyond 10 second, dependence on anaerobic energy from the

intramuscular phosphates decreases while the magnitude of anaerobic energy generated in glycolysis increases. To improve the capacity for energy transfer by the short-term lactic acid energy system, training must overload this aspect of energy metabolism (78, 79).

Aerobic training

The aerobic, or oxygen, system releases energy for ATP production from the breakdown mainly of carbohydrate and fat, and sometimes of protein. Aerobic training program requires two important factors. Firstly, the training must provide a sufficient cardiovascular overload to stimulate increases in stroke volume and cardiac output. Secondly, this central circulatory overload should be accomplished by exercising the sport-specific muscle groups to enhance their local circulation and "metabolic machinery". This consideration essentially embodies the specificity principle as applied to aerobic training. Simply stated, runners should run, cyclists should bicycle, rowers should row, and swimmers should swim. In conclusion, the two major goals of aerobic training are 1. to develop the capacity of the central circulation to deliver oxygen, and 2. to enhance the capacity of the local musculature to supply and process oxygen (79).

Relatively brief bouts of repeated exercise (interval training), as well as continuous, long-duration efforts (continuous training), enhance aerobic capacity, provided the exercise is sufficiently intense to overload the aerobic system (55, 79).

Effects of exercise training on lipid peroxidation and muscle damage. Exercise has been reported to induce an increased production of oxygen derived free radicals (20). However, there is no evidence that moderate or even strenuous exercise

performed regularly for many years results in impaired function of skeletal muscle (34, 95). Alessio et al. (72) studying in untrained and treadmill-trained rats, suggested that endurance training could result in a reduction in MDA during moderate exercise by the activation of the scavenging enzyme catalase. Venditti et al. (1), studying in untrained and swim trained rats, found a smaller increase in lipid peroxidation and a greater increase in glutathione peroxidase in trained rats when compared to untrained. Kanter et al. (25) had presented data indicated that blood and liver CAT, SOD, and GPX in 9 and 21 weeks swim-trained mice were higher than sedentary ones. Therefore, it seemed possible that regularly performed exercise might induce an enhancement of the enzymatic defenses against free radical damage in muscle, which could protect against injury from repeated exercise. However, Higuchi et al. (31) reported no increase in CAT or cytoplasmic SOD activities in 3 month treadmill-trained rats while Laughlin et al. (26) reported that exercise training induced an increase in GPX, a decrease in CAT and no change in SOD levels in treadmill-trained rats.

For human, studies on influence of exercise training on lipid peroxidation and scavenging enzymes activities are more limited and findings are also contradictory. Toskulkao et al. (32) compared the effect of endurance exercise (1 hr by using cycle-ergometer) in sedentary, short and long distance runners. They reported that plasma MDA, CPK, and LDH were greater in untrained group while scavenging enzyme (SOD, CAT and GPX) activities were higher in either short or long distance runners. Most recently, Marzatico et al. (33) determined MDA and erythrocyte scavenging enzymes (SOD, CAT, GPX) in control subjects, marathon runners and sprint-trained athletes found that MDA was higher in both sprint-trained athletes and marathon

runners than in control subjects. In sprint-trained athletes, the enzymatic scavenging capacity showed a significantly higher SOD and GPX, while CAT was lower than in controls. In marathon runners only SOD was higher than in controls.

Up to now, it seems to be well accepted that regularly performed exercise or exercise training have positive effects on reducing oxidative stress of exercise both in animals and human. Its effect may involve an increase in antioxidant capacity of the body but which of the antioxidant enzymes, SOD, CAT, or GPX or all of them are important in scavenging ROS accompanying with exercise are still unclear.

CHAPTER III

MATERIALS AND METHODS

A. Subjects

Subjects were thirty-two school boy volunteers, aged between 10-14 years in Suphanburi province. All of them had normal medical histories (examined by the pediatrician). They were divided into 3 groups as follow.

Group 1: Sedentary or untrained group consisted of 7 boys from Kanasutsuksalai school, Suphanburi. They all had normal physical activities and actively involved in sports during their leisure time (1 hour/day), but none of them were in any regular exercise training program.

Group 2: Non-weight bearing exercise trained group consisted of 10 boys from Sports School of Suphanburi who were trained to be future swimmers.

Group 3: Weight bearing exercise trained group consisted of 15 boys from Sports School of Suphanburi who were trained to be future football players.

The subjects in exercise trained groups (group 2 and 3) were assigned to exercise for at least 3-4 hours per day for 5-6 days per week (or approximately 20 hours per week) in the Sports School of Suphanburi for one year.

The experimental protocols were clearly explained to the subjects (and their parents) and written consent was obtained from each subject prior to the experiment. The project was approved by the Committee on Human Rights Related to Human Experimentation, Mahidol University, Bangkok.

B. Chemicals

All chemicals and reagents used throughout the investigation were analytical grade. Reagents for determination of MDA, CPK, SOD, CAT, and GPX were purchased from Sigma Chemical Company, St. Louis, MO, USA. Hydrogen peroxide (30% w/v) and Acetic acid (100%) were purchased from Merck, Darmstadt, Germany. Reagents for determination of LDH were purchased from Human, Taunusstein, Germany.

C. Equipments

The following equipments were used to measure physical fitness and to analyze blood samples.

1. Physical fitness test
   - Electrically braked bicycle ergometer (AR-6213, AREX, Taipei)
   - Heart rate monitor (Polar sport tester, PE 3000 Haknaantic, Finland)
   - Aubratic oscillometric digital Blood pressure monitor (Model HEM704C, Omron corporation, Tokyo, Japan)
   - Grip dynamometer (Takei Co., Japan)
   - Back and leg dynamometer (Takei Co., Japan)
   - Skinfold calipers (Cambridge Scientific Industries, England)
   - Anthropometric set (Yamakoshi Seisakusho Co., Ltd., Tokyo, Japan)
   - Weight balance (Mikaki Scale Co., Ltd., Japan)

- Metronome (YAMAHA MN, Nippon Gakki Co., Ltd., Tokyo, Japan)
- Stop watch (Hanhart profile JW., German)

2. Blood analysis
   - Spectrophotometer (Spectronic Genesys 5, Milton Roy Co., USA)
   - Automatic refrigerated centrifuge (RT 6000D, Sorrval®, California, USA)
   - Radiometer (PHM 92, Radiometer Analytical S.A., France)
   - Electrical balance (Precisa 205 A SCS, Switzerland)

D. Protocol of experiment

The protocol was divided into two parts. Part I was for the general physical fitness testing which was normally conducted one week prior to the second part. Part II was for the investigation of the effect of endurance exercise on markers of tissue damage such as MDA, CPK and LDH and on scavenging enzymes such as SOD, CAT and GPX. Each subject was requested to fill a questionnaire concerning his personal and educational background, medical history, health status, athletic history, daily nutrition and activity prior to the experiment.

Part I: General physical fitness testing

1. Percentage of body fat and lean body mass

After measuring of body weight and body height of each subject, the estimation of body fat percentage (%BF) and lean body mass (LBM) were done by using skinfold caliper to measure the subcutaneous fat at 2 selected sites namely triceps and subscapular (96). The skinfold measurements were done on the dominant side of subject in relaxed standing position. The two selected sites were:

1. Triceps: Distance between lateral projection of acromial process and inferior margin of olecranon process was measured on lateral aspect of arm with elbow flexed at 90°. Midpoint was marked on lateral side of arm. Skinfold was lifted 1 cm above marked line on posterior aspect of arm.

2. Subscapular : Fold was along natural cleavage line of skin just inferior to inferior angle of scapula, with caliper applied 1 cm below fingers.

The following skinfold equations (97) were used for the calculation of %BF.

%BF = 1.35 (sum of triceps + subscapular SKFs)

- 0.012 x (sum of triceps + subscapular SKFs)^2 – 3.4 (for age 6-11 yr)

%BF = 1.35 (sum of triceps + subscapular SKFs)

- 0.012 x (sum of triceps + subscapular SKFs)^2 – 4.4 (for age 12-14 yr)

The following equation was used to calculate lean body mass (LBM)

LBM = Body weight [1-(%BF/100)]

2. Muscular strength

Strength of the grip-squeezing muscles and leg and back muscles were measured using dynamometers as hand-grip and back and leg dynamometer, respectively (79, 90). Both dynamometers are spring devices. They operate on the same principle of compression. An external force applied to the dynamometer compresses a steel spring and move a pointer. Knowing the force required to move the pointer for a particular distance, one can determine exactly how much external force has been applied to the dynamometer.

Grip-strength testing procedures

Typically, the handgrip size was adjusted to a position that was comfortable for the individual. The subject stood erect, arms at the sides. The dynamometer was held parallel to the side, with the dial facing away from the body. The individual squeezed the dynamometer as hard as possible without moving the arm. Three trials were administered for each hand with a 1-minute rest between trials.

Leg-strength testing procedures

Using the back and leg dynamometer, the subject stood on the platform with trunk erect. The knees were flexed to an angle of 130° to 140°. The handbar was held using a pronated grip and was positioned across the thighs by adjusting the length of the chain. Without using the back, the subject's knees were slowly but vigorously extended. The maximum indicator pointer remained at the peak force achieved. Three trials with a 1-minute rest interval were administered.

Back-strength testing procedures

Using the back and leg dynamometer, the subject stood on the platform with the knees fully extended and the head and trunk erect. The handbar is grasped using a pronated grip. The handbar was positioned across the thighs and, without leaning backward, the subject pulled it straight upward using the back muscles. The shoulders were rolled backward during the pull. Subjects should be reminded prior to lifting to flex the trunk minimally and to keep the head and trunk erect during the test. Three trials were administered with a 1-minute rest between the trials.

3. Muscular endurance

Sit-up (bent knee) was used to determine the endurance of the abdominal muscles (90, 91).

Sit-up testing procedures

The subject was asked to assume a supine position with hands interlocked behind the neck and feet held in place. The subject drew the back toward the buttocks until they were flat on the floor (knees bent). The angle of legs to thighs should be approximately 90°. Curled the back and raised the trunk until the lower back was perpendicular to the floor then gently returned to the start position. This procedure was repeated as many times as possible within the time limit. For this experiment, the score was the number of sit-up completed in one minute.

4. Flexibility

Trunk flexion (sit and reach) was used to measure the amount of trunk flexion and the ability to stretch the back muscles and back thigh muscles (hamstrings) (90, 91).

Trunk flexion testing procedure

The subject sat with legs fully extended and the bottom of feet flat against a board projecting from the wall and extended arms and hands forward as far as possible and held for a count of three. The distance (in cm) before or beyond the edge of the board that subject could reach was measured with a ruler. Distances before the edge (not able to reach toes) were expressed as negative scores; those beyond the edge were expressed as positive scores.

5. Maximal oxygen uptake ($\dot{V}O_2max$)

The $\dot{V}O_2max$ value quantitatively expressed a person's capacity for aerobic resynthesis of ATP. It is an important information on the capacity of the long-term energy system. The PWC test is one of the submaximal exercise tests that can be used to predict $\dot{V}O_2max$ (96). This test is either continuous or discontinuous and are based on the assumption that heart rate and oxygen uptake are linear functions of work. Thus, the heart rate response to submaximal work loads was used to predict $\dot{V}O_2max$ in this experiment.

PWC_{170} testing procedure

1. Allowed subject to sit on saddle of the cycle ergometer with a suitable position. The height of the seat should be adjusted for individual subject to allow the seat to pedal length at full stretch around 109°.

2. Sport tester was attached to the anterior chest wall of the subject and used to continuously monitor subject's heart rate during exercise.

3. Subject was performed rhythmical pedaling (with the help of metronome) at 50 rpm with an initial work load of 100 w. Three increment work loads were selected and adjusted.

For each work load, the heart rate was recorded every minute for 6 minutes. When the heart rate at the end of the first work load was less than 120 bpm, the second work load was increased by 50 w. Thereafter, the 25 w increment was used until a steady heart rate was reached. In this case, testing time for that work load should be continued to 7 or 8 minutes.

4. A steady heart rate was assumed when the difference between two consecutive readings was less than 5 beats per minute. The values of heart rate at 5 and 6 minutes (or at the last two minutes of each work load) was used to calculate the averaged steady heart rate. i.e., when heart rate at minute 5 and 6 were 144 and 148 bpm, respectively, the steady heart rate was $\frac{144+148}{2}$ = 146 bpm.

5. The averages of heart rate responses to each work load were plotted (Figure 6). A straight edge was used to connect the points and to extend the line through the HR_{170} line. The amount of work corresponding to the HR_{170} was recorded as the individual's PWC_{170}. The correlation of PWC_{170} with actual $\dot{V}O_2max$ has been reported to be 0.88 and the standard error of prediction was noted to be $\pm$9.4%.

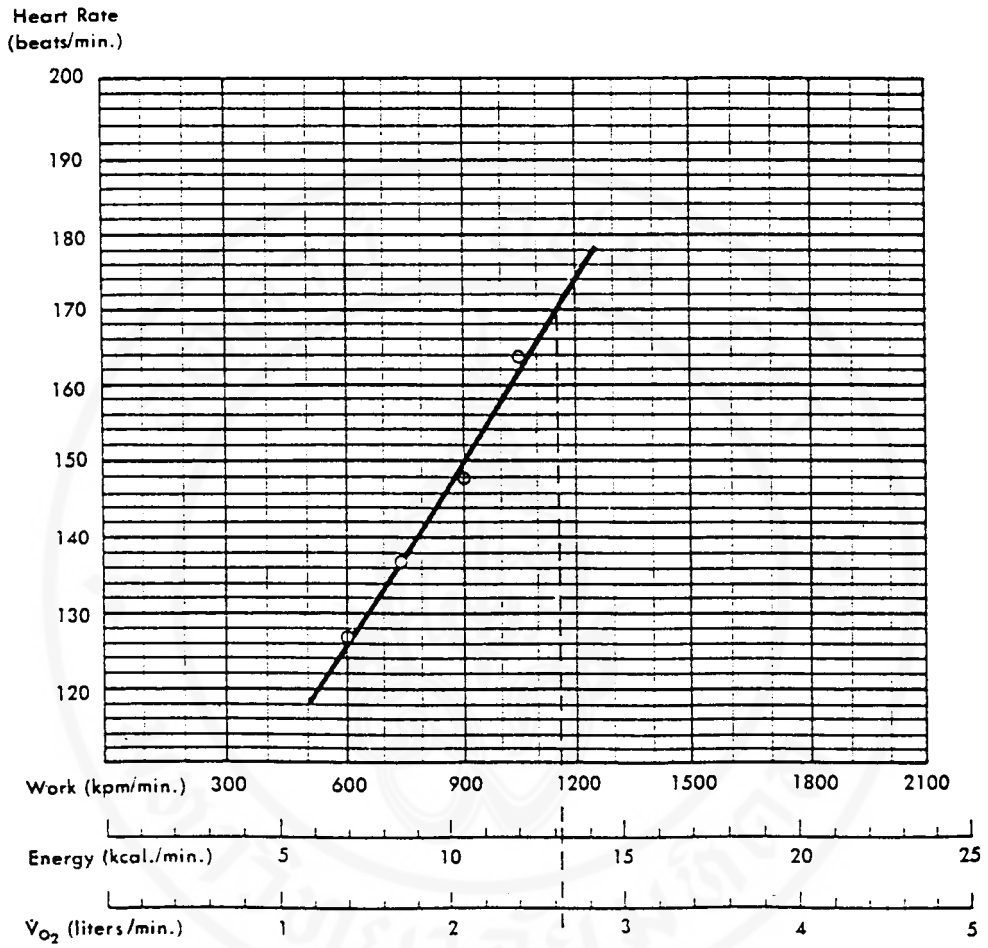
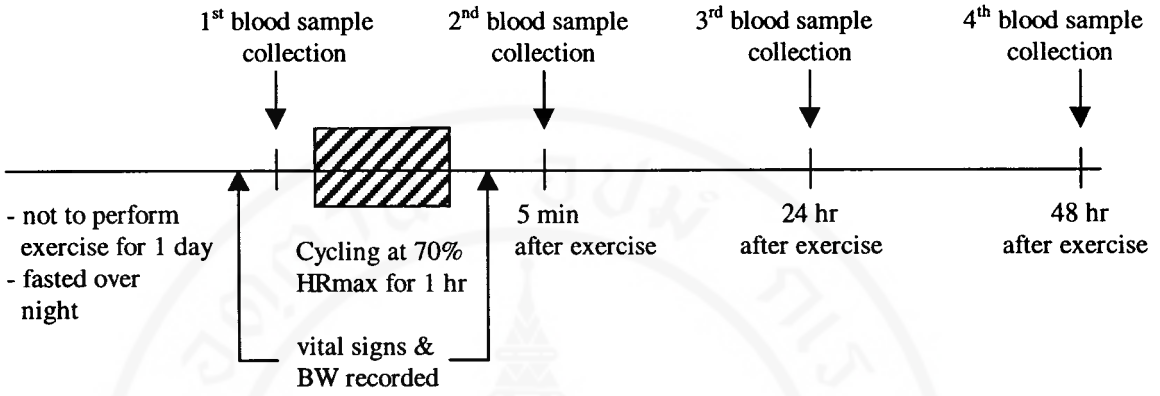


Figure 6. Prediction of PWC_{170} (for $\dot{V}O_2max$) from the heart rate responses to submaximal work loads.

Part II: Endurance exercise protocol

Each subject was requested not to perform any kind of physical exercise one day before the day of the experiment until after the 4^{th} blood sample collection. They fasted over night and rested for 15 minutes before starting the experiment. Vital signs and body weight were recorded for resting values. At the pre-exercise period, the first blood sample was collected from antecubital vein and kept at 4°C in a heparinized test tube. All blood samples were collected with subjects sat in an upright position to minimize postural effects on blood volume. Before starting the endurance exercise, the subjects were asked to warm up by stretching the muscle for 15 minutes. Then, each subject sat on saddle of the bicycle ergometer in comfortable position and cycling at 70% of maximum heart rate (calculate from 220-age of each subject) for 60 minutes duration. During this endurance exercise, the heart rate was monitored by sport tester and the work load of bicycle ergometer was adjusted to obtained the steady heart rate at 70% HRmax of each subject. At the end of exercise, the subjects were asked not to stop the exercise suddenly but gradually decreased intensity for cool down process. Vital signs and body weight were recorded again after exercise. Subsequently, the second, the third and the forth blood samples were collected at 5 minutes, 24 hours and 48 hours after exercise, respectively. The subjects were asked not to perform any kind of exercise until after the fourth blood sample collection.

Protocol of the experiment



First day
- recording of vital signs and body weight after 15 minutes-rest.
- 1st blood sample collection (blood kept in heparinized tube at 4°C).
- 5 minutes-warming up (by muscle stretching).
- Starting exercise using bicycle ergometer and continue for 60 minutes. Heart rate was monitored (using sport tester) and kept at 70% maximum heart rate (HRmax = 220-age) by adjusting the load of the bicycle.
- recording of vital signs and body weight after 5 minutes cooling down.
- 2nd blood sample collection (5 minutes after exercise).

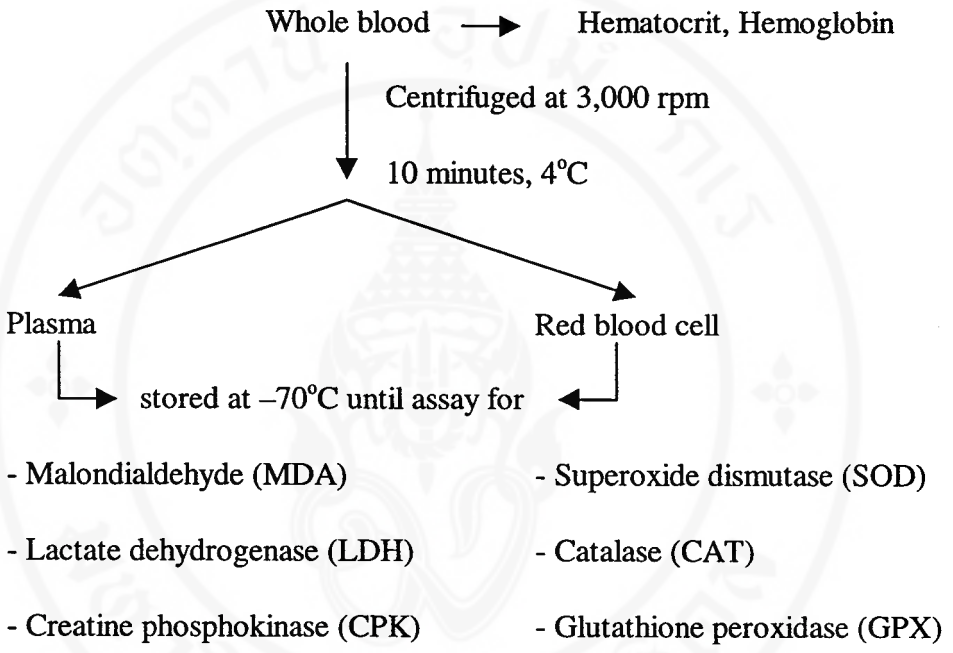
Second day
- 3rd blood sample collection (24 hours after exercise).

Third day
- 4th blood sample collection (48 hours after exercise).

Blood analysis

Hematocrit and hemoglobin were analyzed from whole blood by hematology section, Department of pathology, Jaoprayayommaraj Hospital. After centrifugation (at 3,000 rpm for 10 minutes under 4°C) malondialdehyde (MDA),

creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) levels in plasma were determined. Scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in red blood cells were determined.



E. Statistical analysis

All data were presented as means and standard errors of mean (SEM). Student's paired *t*-test was used to determine statistical significant difference between pre-exercise and post exercise values at various time intervals and also between the values at the beginning and at the end of one year in the same group of subjects. For comparison among groups, one way analysis of variance (one-way ANOVA) was used and the differences in pairs of means among groups were made by Student-Newman-Keuls test (SNK). If the statistical probability (p-value) was less than 0.05, the differences were considered to be statistical significant.

CHAPTER IV

RESULTS

1. Comparisons of general and physical fitness data among three groups of subjects at the beginning (month 0) of the experiment.

1.1 Age and anthropometric data

Age and anthropometric data in untrained (sedentary) and trained (swim and football) groups at the beginning (month 0) of the experiment were shown in Table 2. The average age of sedentary, swim, and football subjects were 12.6±0.2, 11.6±0.4, and 12.3±0.3 yr, respectively. The three groups had the averaged body weight of 42.2±4.2, 42.6±3.5, and 38.4±2.2 kg and the averaged body height of 156.5±3.1, 152.6±4.6, and 147.6±2.5 cm, respectively. There were no significant differences in the average age, body weight and body height among three groups of subjects. Lean body mass of these three groups of subjects were also relatively the same. They were 37.2±2.4, 37.1±3.3, and 33.3±1.8 kg in sedentary, swim-trained and football-trained subjects, respectively. However, the percent body fat of both swim-trained (13.2±12.6%) and football-trained (12.6±1.3%) subjects were significantly lower ($p < 0.05$) than that of the sedentary subjects (18.4±2.7%).

1.2 Physical fitness data

Physical fitness data including muscular strength, flexibility and predicted $\dot{V}O_2max$ of the subjects in all three groups were shown in Table 3.

Table 2. Age and anthropometric data in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) of the experiment.

Parameter	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)
Age (yr)	13.0 ± 0.3	12.2 ± 0.4	12.0 ± 0.3
Body weight (kg)	42.2 ± 4.2	42.6 ± 3.5	38.4 ± 2.2
Body height (cm)	156.5 ± 3.1	152.6 ± 4.6	147.7 ± 2.5
Lean body mass (kg)	37.2 ± 2.4	37.1 ± 3.3	33.3 ± 1.8
Body fat (%)	18.4 ± 2.7	13.3 ± 2.6*	12.6 ± 1.3*

Values are means±SEM.

*$p < 0.05$ Significant difference from sedentary subjects.

Table 3. Physical fitness data in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) of the experiment.

Parameter	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)
Right handgrip strength (kg/kgBW)	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
Left handgrip strength(kg/kgBW)	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
Leg strength (kg/kgBW)	1.9 ± 0.3	2.2 ± 0.2	2.0 ± 0.1
Back strength (kg/kgBW)	1.4 ± 0.1	1.8 ± 0.1*	1.8 ± 0.1*
Trunk flexion (cm)	12.3 ± 1.2	11.9 ± 1.5	8.7 ± 1.2
Sit up (time/min)	17.9 ± 1.4	24.8 ± 1.9**	21.9 ± 1.1
PWC_{170} (watt/kg BW)	2.3 ± 0.1	4.0 ± 0.2***	3.8 ± 0.2***

Values are means±SEM.

*p < 0.05,**p < 0.01,***p < 0.001 Significant differences from sedentary subjects.

Right and left handgrip strength

Right handgrip strengths of sedentary, swim, and football subjects were 0.6±0.01, 0.6±0.0, and 0.5±0.0 kg/kgBW, and their left handgrip strengths were 0.5±0.0, 0.6±0.0, and 0.5±0.0 kg/kgBW, respectively. No significant differences were found among three groups of subjects in right and left handgrip strengths.

Leg strength and back strength

Leg strengths of sedentary (1.9±0.3 kg/kgBW), swim (2.2±0.2 kg/kgBW) and football (2.0±0.1 kg/kgBW) subjects were not significantly different from each other. Back strength of the sedentary subjects was 1.4±0.1 kg/kgBW. This value was significantly lower than those of the swim and football subjects (1.8±0.1 and 1.8±0.1 kg/kg BW) with the p-value less than 0.05.

Trunk flexion

Trunk flexions of sedentary, swim, and football subjects were 12.3±1.2, 11.9±1.5, and 8.7±1.2 cm, respectively. No significant differences were found among them.

Sit up

The maximum numbers of sit up in swim (24.8±1.9 times/min) and football (21.9±1.1 times/min) subjects were found to be significantly higher ($p < 0.01$ and $p < 0.05$, respectively) than in sedentary subjects (17.7±1.4 times/min).

PWC_{170}

PWC_{170} (which is used as an indicator for aerobic capacity) in swim and football subjects (4.0±0.2, 3.8±0.2) were found to be significantly higher ($p < 0.001$) than in sedentary subjects (2.3±0.1 watt/kgBW).

However, there were no significant differences between swim and football subjects in all physical fitness parameters measured.

1.3 Hematological features and vital signs.

Table 4 shows hematological parameters [hemoglobin concentration (Hb) and hematocrit (Hct)] and vital signs [heart rate (HR), blood pressure (BP), and body temperature] of the subjects in all 3 groups. The concentrations of Hb and Hct in sedentary subjects were 13.7±03 g/dl and 39.1±0.8%, in swim subjects were 13.5±0.3 g/dl and 40.8±07% and in football subjects were 13.4±0.2 g/dl and 40.6±0.6%, respectively. There were no significant differences in Hb concentration and Hct among three groups of subjects.

Heart rates of the sedentary, swim, and football subjects were found to be in normal ranges of 79.6±3.5, 81.4±2.3, and 80.6±2.9 beat/min whereas body temperature, measured orally, were 36.7±0.2, 36.5±0.1 and 36.8±0.1°C, respectively. Diastolic blood pressures were 69.1±4.9 mmHg in sedentary, 62.0±2.0 mmHg in swim and 62.0±1.1 mmHg in football subjects. There were no significant differences among three groups in these parameters. However, significant difference was demonstrated in systolic blood pressure. Swim and football subjects had the systolic blood pressure of 101.0±1.6 and 96.0±1.6 mmHg, respectively, which were both significantly lower ($p < 0.01$) than 110.6±3.1 mmHg of sedentary subjects.

Table 4. Hematocrit, hemoglobin and vital signs at rest in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) of the experiment.

Parameter	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)
Hematocrit (%)	39.1 ± 0.8	40.8 ± 0.7	40.6 ± 0.8
Hemoglobin (g/dl)	13.7 ± 0.3	13.5 ± 0.3	13.4 ± 0.2
Heart rate (beat/min)	79.6 ± 3.5	81.4 ± 2.3	80.6 ± 2.9
Blood pressure (mmHg)			
- systolic	110.6 ± 3.1	101.0 ± 1.6**	96.0 ± 1.6***
- diastolic	69.1 ± 4.9	62.0 ± 2.0	62.0 ± 1.1
Body temperature (^{0}c)	36.7 ± 0.2	36.5 ± 0.1	36.8 ± 0.1

Values are means±SEM.

p <0.01,*p <0.001 Significant differences from sedentary subjects.

2. Comparisons of biochemical data among three groups of subjects, at the beginning (month 0) of the experiment (Table 5).

2.1 Lipid peroxidation product (plasma MDA level)

Resting plasma MDA level was 1.38±0.04 nmole/ml in sedentary subjects, while they were 1.85±0.16 nmole/ml in swim subjects and 2.38±0.19 nmole/ml in football subjects. The value of the sedentary group was found to be significantly lower than those of the swim and football groups with the P-values less than 0.05 and 0.001, respectively.

2.2 Index for muscle damage (plasma CPK and LDH)

Plasma LDH level at rest of the sedentary group (156.29±12.31 U/L) was significantly lower ($p < 0.01$) than those of the swim (251.37±14.01 U/L) and football (215±11.13 U/L) groups. There were no significant differences in resting plasma CPK activities among sedentary, swim and football subjects (70.39±13.33, 66.51±8.39 and 79.64±10.20 U/L, respectively).

2.3 Scavenging enzymes activities in red blood cells (SOD, CAT and GPX)

Resting SOD activities in sedentary, swim and football subjects were 3.21±0.02, 3.09±0.11 and 2.85±0.12 (x 10^3) U/gHb. CAT activities of these subjects were 5.11±0.35, 4.83±0.23 and 4.93±0.20 (x 10^4) U/gHb and GPX activities were 281.28±25.41, 290.16±16.95 and 311.96±21.99 U/gHb, respectively. There were no significant differences found in these resting antioxidant enzymes activities in all three groups of subjects.

Table 5. Plasma malondialdehyde (MDA), creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and erythrocyte scavenging enzymes (SOD, CAT and GPX) at rest in untrained (sedentary) and trained (swim and football) subjects at the begining (month 0) of the experiment.

Parameter	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)
MDA (nmole/ml)	1.38 ± 0.04	1.85 ± 0.16*	2.38 ± 0.19***
CPK (U/L)	70.39 ± 13.33	66.51 ± 8.39	79.64 ± 10.20
LDH (U/L)	156.29 ± 12.31	251.37 ± 14.01***	215.72 ± 11.13**
SOD ($x10^3$U/gHB)	3.21 ± 0.02	3.09 ± 0.11	2.85 ± 0.12
CAT ($x10^4$U/gHB)	5.11 ± 0.35	4.83 ± 0.23	4.93 ± 0.20
GPX (U/gHB)	281.28 ± 25.41	290.16 ± 16.95	311.96 ± 21.99

Values are means±SEM.

*$p < 0.05$,**$p < 0.01$, ***$p < 0.001$ Significant differences from sedentary subjects.

It can be noticed that, subjects of all 3 groups were in the same ranges of age, weight, and height. Their general physical fitness parameters measured were relatively at the same levels except that percentage of fat, numbers of maximum sit up, back strengths and PWC_{170} of the swim and football subjects were significantly higher than those of the sedentary. These results indicated that the swim and football subjects had somewhat greater physical fitness than the sedentary subjects from the beginning (month 0) of the experiment. It was also found that though the scavenging enzyme activities in the red blood cells and the plasma CPK of all 3 groups of subjects were relatively the same, but the swim and the football subjects had the higher plasma MDA and LDH levels than the sedentary subjects at the beginning (month 0) of the experiment.

3. Effects of endurance exercise on lipid peroxidation product, index of muscle damage and scavenging enzymes at the beginning (month 0) of the experiment.

Lipid peroxidation and muscle damage induced by 60 minutes endurance exercise in this study were determined by using plasma MDA, CPK, and LDH as indicators. The effects of 1hr-endurance exercise on these indicators were shown in Table 6 and Figure 7-9.

Malondialdehyde (MDA)

Plasma malondialdehyde (MDA) levels in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after 1 hr endurance exercise at the beginning (month 0) of the experiment were shown in Table 6 and Figure 7. Acute endurance exercise by 60 minutes cycling at 70% of maximum heart

Table 6. Plasma malondialdehyde (MDA), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) of the experiment.

Time	Malondialdehyde (nmole/ml)			Creatine phosphokinase activity (U/L)			Lactate dehydrogenase activity (U/L)		
	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)
Pre-exercise	1.38 ± 0.04	1.85 ± 0.16*	2.38 ± 0.19***	70.39 ± 13.33	66.51 ± 8.39	79.64 ± 10.20	156.29 ± 12.31	251.37 ± 14.01***	215.72 ± 11.13**
Post-exercise	t	tt	ttt					tt	
5 min	1.69 ± 0.13	2.62 ± 0.19***	2.94 ± 0.21***	73.87 ± 11.74	64.57 ± 10.52	88.20 ± 13.40	148.14 ± 20.01	206.69 ± 7.50**	224.10 ± 12.02**
	t	t						t	
24 hr	1.66 ± 0.08	2.30 ± 0.18	2.46 ± 0.28	76.39 ± 18.78	79.49 ± 11.23	94.74 ± 13.56	190.61 ± 17.54	213.55 ± 10.79	230.71 ± 16.48
			t					t	
48 hr	1.53 ± 0.07	1.72 ± 0.24	1.85 ± 0.18	65.46 ± 12.66	81.63 ± 20.75	97.88 ± 12.29	233.24 ± 24.39	211.91 ± 11.38	227.55 ± 16.61

Values are means±SEM.

*p < 0.05,**p < 0.01,***p < 0.001 Significant differences from sedentary subjects.

$^{t}p < 0.05$, $^{tt}p < 0.01$, $^{ttt}p < 0.001$ Significant differences from pre-exercise.

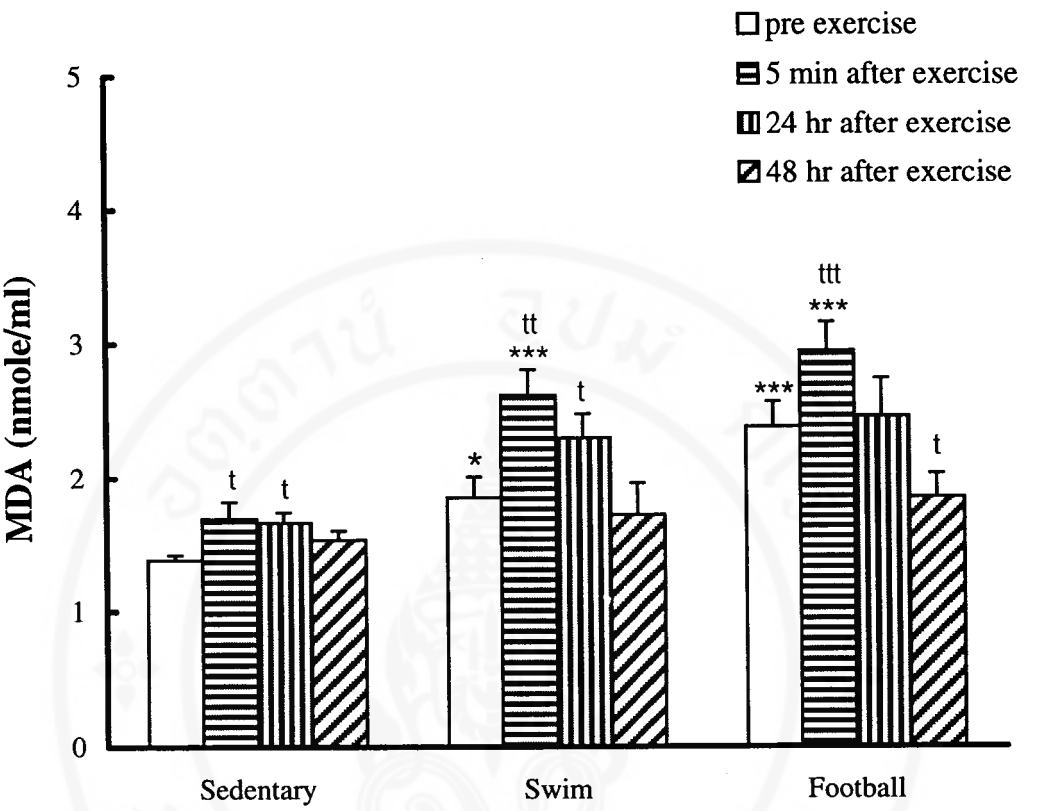


Figure 7. Plasma malondialdehyde (MDA) levels in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance execise at the beginning (month 0) of the experiment. Values are means±SEM.
*$p < 0.05$, ***$p < 0.001$ Significant differences from sedentary at the same time intervals.
$^{t}p < 0.05$, $^{tt}p < 0.01$, $^{ttt}p < 0.001$ Significant differences from pre-exercise of the same group.

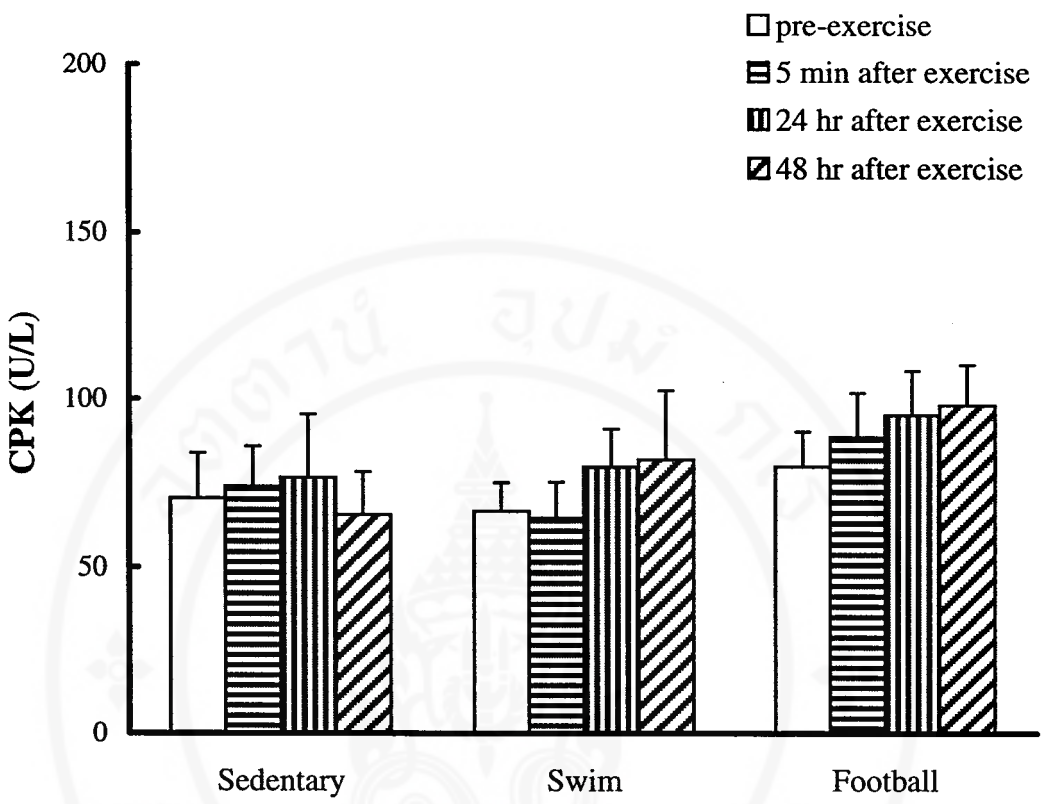


Figure 8. Plasma creatine phosphokinase (CPK) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month0) of the experiment. Values are means±SEM.

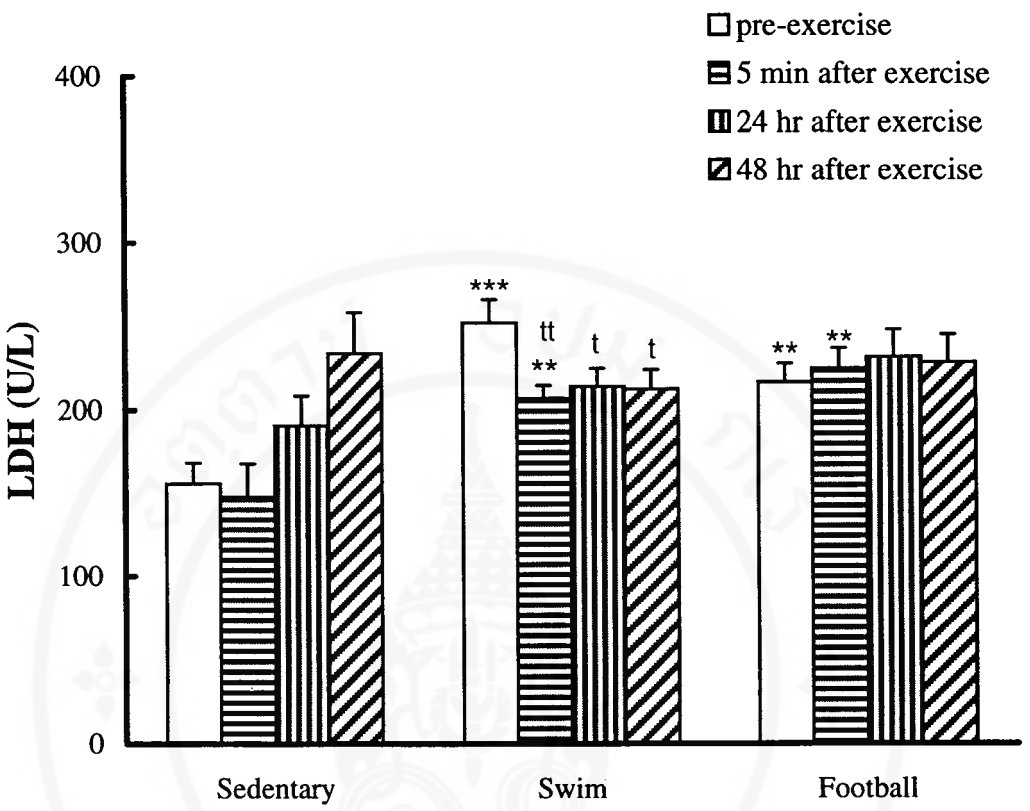


Figure 9. Plasma lactate dehydrogenase (LDH) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month0) of the experiment. Values are means±SEM.
**p < 0.01, ***p < 0.001 Significant differences from sedentary at the same time intervals.
$^{t}p < 0.05$, $^{tt}p < 0.01$ Significant differences from pre-exercise of the same group.

rate caused significant increases in plasma MDA in all three groups of subjects at 5 minutes after exercise when compared to pre-exercise values. In sedentary subjects, the pre-exercise value was 1.38±0.04 nmole/ml while the values at 5 min, 24 hr and 48 hr after exercise were 1.69±0.13, 1.66±0.08 and 1.53±0.07 nmole/ml, respectively. All the post-exercise values were significantly higher than the pre-exercise value at $p < 0.05$. MDA levels in swim subjects at 5 min and 24 hr after exercise were 2.62±0.19 and 2.30±0.18 nmole/ml which were higher than the pre-exercise value of 1.85±0.16 nmole/ml $p<0.001$ and $p<0.05$, respectively. At 48 hr after exercise the MDA level in swim subjects was not significantly different from the pre-exercise one. In football subjects, MDA levels was significantly increased from the pre-exercise value of 2.38±0.19 to 2.94±0.21 nmole/ml ($p < 0.001$) at 5 min after exercise. At 24 hr after exercise the value (2.46±0.28 nmole/ml) was not different from the pre-exercise one. At 48 hr, MDA level in football subjects was significantly decreased to 1.85±0.18 nmole/ml ($p < 0.05$). Comparing among groups at the same time intervals, pre-exercise lipid peroxide levels in swim and football subjects were significantly higher than in sedentary subjects. At 5 min after exercise, MDA levels in swim and in football subjects were still significantly higher than in sedentary subjects. However, there were no significant differences among three groups at 24 hr and 48 hr after exercise.

3.2 Creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

Table 6 and Figure 8 show plasma creatine phosphokinase activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after 1 hr of endurance exercise at the beginning (month 0) of the

experiment. There were no significant increases in plasma CPK activities in any group of subjects at 5 min, 24 hr and 48 hr after exercise comparing with pre-exercise levels. Furthermore, when compared at the same time intervals both before and after 1 hr-endurance exercise, there were also no significant differences in CPK activities among three groups of subjects.

Table 6 and Figure 9 showed plasma lactate dehydrogenase activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after 1 hr of endurance exercise at the beginning (month 0) of the experiment. There were no significant differences in plasma LDH levels before and at various time intervals after exercise in both sedentary and football subjects. In swim subjects, LDH activities at 5 min, 24 hr and 48 hr after exercise were all significantly lower than the pre-exercise one. When compared among three groups of subjects at various time intervals it was found that swim and football subjects had higher LDH activities than sedentary subjects both before and at 5 min after exercise.

3.3 Antioxidant enzyme activities in red blood cells.

Superoxide dismutase (SOD)

Superoxide dismutase activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after 1 hr-endurance exercise at the beginning (month 0) of the experiment were shown in Table 7 and Figure 10. SOD activities of sedentary subjects at pre-exercise, 5 min and 24 hr after exercise were not significantly different from each other. Only at 48 hr after exercise that SOD activity was significantly increased when compared with pre-exercise value. No significant differences in SOD activities were found in both swim and football

Table 7. Erythrocyte superoxide dismutase(SOD), catalase (CAT) and glutathione peroxidase (GPX) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) of the experiment.

Time	Superoxide dismutase activity ($x10^3$ U/gHb)			Catalase activity ($x10^4$ U/gHb)			Glutathione peroxidase activity (U/gHb)		
	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)	Sedentary (n = 7)	Swim (n = 10)	Football (n = 15)
Pre-exercise	3.21 ± 0.16	3.09 ± 0.11	2.85 ± 0.12	5.11 ± 0.35	4.83 ± 0.23	4.93 ± 0.20	281.28 ± 25.41	290.16 ± 16.95	311.96 ± 21.99
Post-exercise									t
5 min	3.60 ± 0.29	3.09 ± 0.17	2.87 ± 0.13*	4.72 ± 0.28	5.07 ± 0.28	5.37 ± 0.18	269.77 ± 23.11	288.05 ± 16.81	357.41 ± 18.30*
									t
24 hr	3.18 ± 0.18	3.05 ± 0.08	2.70 ± 0.12	4.57 ± 0.19	5.44 ± 0.27	5.49 ± 0.32	278.58 ± 27.55	264.68 ± 20.51	350.09 ± 26.34
	t								
48 hr	3.78 ± 0.14	3.14 ± 0.10**	3.05 ± 0.14**	4.66 ± 0.22	5.35 ± 0.26	4.74 ± 0.24	265.79 ± 20.98	302.12 ± 19.93	338.16 ± 24.38

Values are means±SEM.

*$p < 0.05$, **$p < 0.01$ Significant differences from sedentary subjects.

$^t p < 0.05$ Significant difference from pre-exercise.

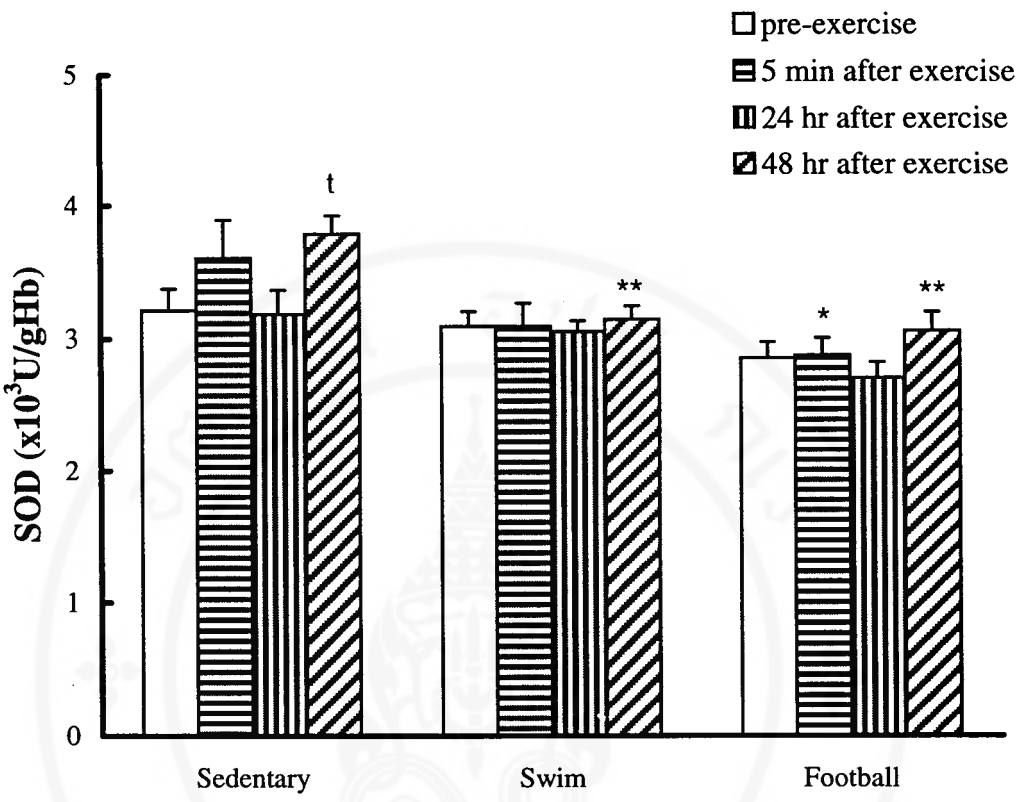


Figure 10. Superoxide dismutase (SOD) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month0) of the experiment. Values are means±SEM.
*$p < 0.05$, **$p < 0.01$ Significant differences from sedentary at the same time intervals.
$^{t}p < 0.05$ Significant difference from pre-exercise of the same group.

subjects before and at various time intervals after exercise. Comparisons among three groups of subjects at the same time intervals showed that at 5 min after exercise SOD activity in football subjects was significantly less than in sedentary subjects and at 48 hr after exercise, SOD activities of both swim and football subjects were significantly less than those of sedentary subjects. There were no significant differences in SOD activities among three groups at pre-exercise and at 24 hr after exercise.

Catalase (CAT)

Catalase activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after 1 hr-endurance exercise at the beginning (month 0) of the experiment were shown in Table 7 and Figure 11. It was found that 1 hr-endurance exercise didn't cause a significant change in catalase activities in any group of subjects. And there were also no significant differences in the values of CAT activities among three groups of subjects both before and at 5 min, 24 hr and 48 hr after exercise.

Glutathione peroxidase (GPX)

Glutathione peroxidase activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after 1 hr-endurance exercise at the beginning (0 month) of the experiment were shown in Table 7 and Figure 12. After endurance exercise, GPX activities in football subjects were significantly increased at 5 min, 24 hr and 48 hr from pre-exercise level. There were no significant differences between GPX activities of pre and post exercise in sedentary and swim subjects. Comparisons among groups at the same time interval also showed no significant differences in GPX activities both before and after exercise.

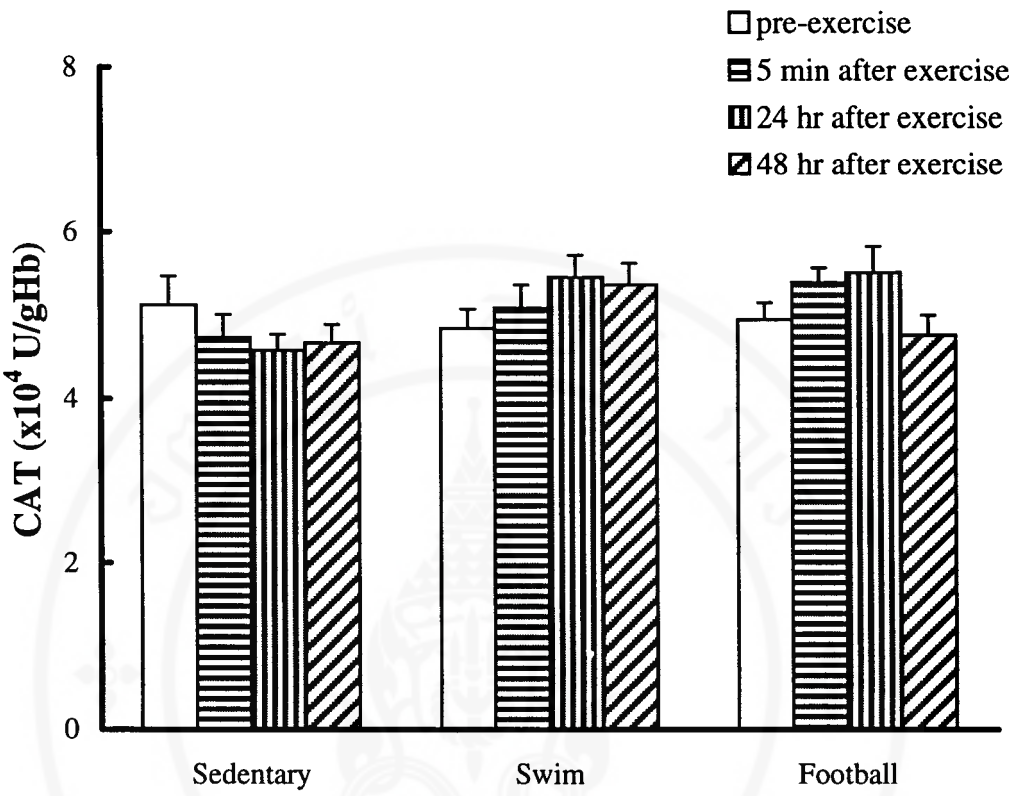


Figure 11. Catalase (CAT) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month0) of the experiment. Values are means±SEM.

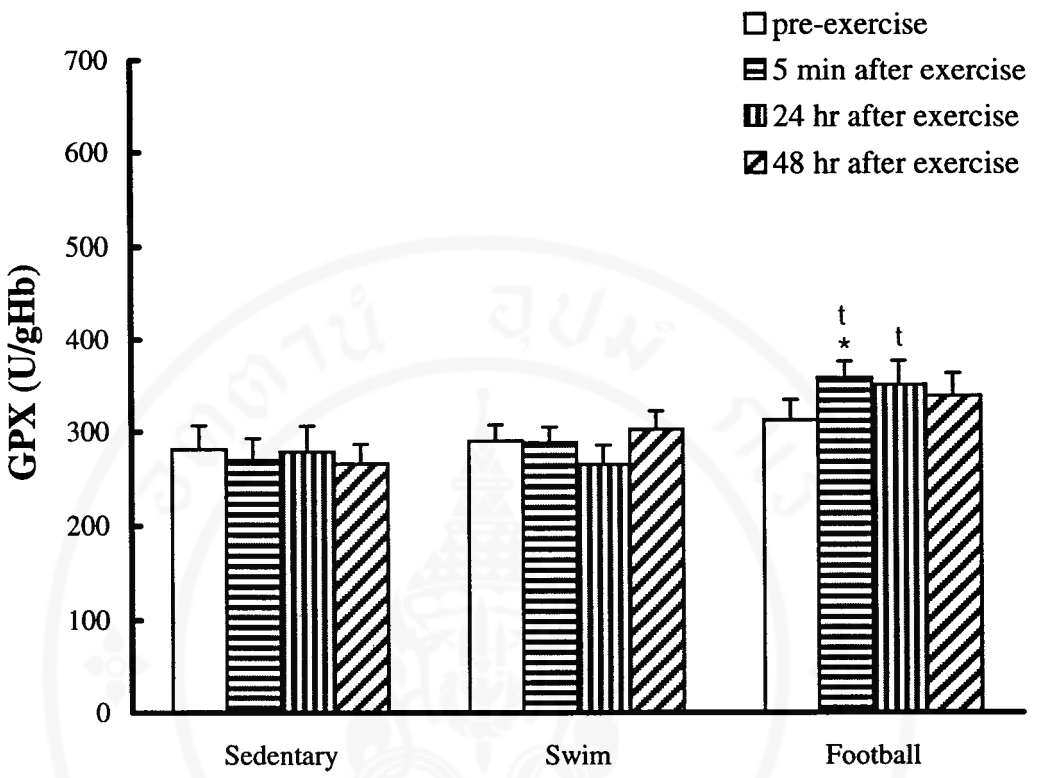


Figure 12. Glutathione peroxidase(GPX) activities in untrained(sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month0) of the experiment. Values are means±SEM.
*$p < 0.05$ Significant difference from sedentary at the same time intervals.
$^{t}p < 0.05$ Significant difference from pre-exercise of the same group.

In conclusion, at the beginning or month 0 of experiment, after 1 hr-endurance exercise by cycling at 70% HRmax, MDA which was used as an indicator for lipid peroxidation, increased in all 3 groups of subjects. CPK was unchanged in all 3 groups. LDH was also unchanged in sedentary and football groups but decreased in swim group after exercise. For scavenging enzymes activities, SOD was found to be increased at 48 hr after exercise only in sedentary subjects. CAT was unchanged in all 3 groups while GPX increased significantly in football group after exercise.

4. Comparisons of general and physical fitness data among three groups of subjects at the end (month 12) of the experiment.

4.1 Anthropometric data

Anthropometric data in untrained (sedentary) and trained (swim and football) groups at the beginning (month 0) and at the end (month 12) of the experiment were shown in Table 8. There were still no statistical significant differences in age, body weight, body height and lean body mass among three groups of subjects after 1 year. However, the percent body fat of both swim (13.0$\pm$0.6%) and football (13.6$\pm$0.9%) subjects were significantly lower than that of sedentary subjects (19.4$\pm$2.8%). When compared within each group, it was found that the percent body fat were not changed while body weight and lean body mass were significantly increased in all 3 groups of subjects at the end of one year.

4.2 Physical fitness data

Physical fitness data in untrained (sedentary) and trained (swim and football) groups at the beginning (month 0) and at the end (month 12) of the

Table 8. Anthropometric data in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) and the end (month 12) of the experiment.

Parameter	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Body weight (kg)	49.0 ± 3.8	54.0 ± 3.8 fff	42.9 ± 4.0	47.4 ± 4.5 ff	39.8 ± 2.9	42.0 ± 3.3 f
Body height (cm)	158.8 ± 2.6	162.4 ± 3.5	153.1 ± 4.6	157.5 ± 4.6 ff	149.3 ± 3.2	153.7 ± 3.1 fff
Lean body mass (kg)	39.1 ± 1.6	43.0 ± 1.6	37.8 ± 3.7	41.2 ± 3.8	34.3 ± 2.3	36.2 ± 2.7
Body fat (%)	19.0 ± 3.1	19.4 ± 2.8	12.2 ± 0.7*	13.0 ± 0.6*	13.2 ± 1.65*	13.6 ± 0.9*

Values are means±SEM.

*$p < 0.05$ Significant difference from sedentary subjects.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ Significant differences from the beginning (month 0) of the experiment.

Table 9. Physical fitness data in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) and the end (month 12) of the experiment.

Parameter	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Right handgrip strength (kg/kgBW)	0.6 ± 0.0	0.6 ± 0.0 f	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.6 ± 0.0 fff
Left handgrip strength (kg/kgBW)	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0 fff
Leg strength (kg/kgBW)	1.7 ± 0.2	2.3 ± 0.1	2.2 ± 0.2	2.8 ± 0.3 ff	2.1 ± 0.1	2.4 ± 0.2 fff
Back strength (kg/kgBW)	1.3 ± 0.1	1.6 ± 0.1	1.8 ± 0.1**	2.1 ± 0.1** ff	1.7 ± 0.1*	2.2 ± 0.1** ff
Trunk flexion (cm)	12.3 ± 1.5	14.2 ± 1.6	11.8 ± 1.9	16.2 ± 2.3 f	8.9 ± 1.6	11.1 ± 1.5 fff
Sit up (time/min)	18.3 ± 1.6	20.3 ± 1.3 ff	26.9 ± 1.6**	31.1 ± 1.3*** ff	22.1 ± 1.3	30.9 ± 1.2*** fff
PWC_{170} (watt/kg BW)	2.3 ± 0.1	3.4 ± 0.2	4.2 ± 0.3***	5.8 ± 5.0***	3.9 ± 0.3***	5.2 ± 0.2***

Values are means±SEM.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ Significant differences from sedentary subjects.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ Significant differences from the beginning (month 0) of the experiment.

experiment were shown in Table 9. There were no significant differences in right handgrip, left handgrip, leg strength and trunk flexion among 3 groups of subjects at the end of one year. However, back strength, number of sit up, and PWC_{170} in both swim and football trained groups were significantly higher than in the sedentary group at the end of the experiment. When compared within each group, it was found that football subjects, after one year training, had significantly higher right and left handgrip strength, leg strength, back strength, trunk flexion, number of sit up, and PWC_{170} (from 3.9 to 5.2 watt/kgBW, $p<0.001$). Swim subjects had higher back strength, trunk flexion, number of sit up, and PWC_{170} (from 4.2 to 5.8 watt/kgBW, $p<0.01$) while sedentary subjects had only increase in left handgrip strength and PWC_{170} (from 2.3 to 3.4 watt/kgBW, $p<0.01$).

4.3 Hematological features and vital signs.

Table 10 shows hematological features [hemoglobin concentration (Hb) and hematocrit (Hct)] and vital signs [heart rate (HR), blood pressure (BP), and body temperature] of the subjects in all three groups at the beginning (month 0) and at the end (month 12) of the experiment. There were no significant differences in Hb concentration and Hct among three groups of subjects. But when compared within each group between before and after one year, only sedentary subjects showed significant increased in Hct from 39.5 to 43.1% at $p<0.01$. After one year training, heart rate of swim (62.8 ± 2.4 beat/min) and football (62.7 ± 2.3 beat/min) subjects were dramatically and significantly lower ($p<0.001$) than that of sedentary subjects (78.0 ± 2.1 beat/min) at the end of one year. It was also found that one year training

Table 10. Hematocrit, hemoglobin and vital signs at rest in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) and the end (month 12) of the experiment .

Parameter	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Hematocrit (%)	39.5 ± 0.8	43.1 ± 1.2 ff	41.0 ± 0.8	40.9 ± 1.1	40.6 ± 0.8	40.4 ± 0.5
Hemoglobin (g/dl)	13.7 ± 0.4	14.3 ± 0.5	13.6 ± 0.3	13.8 ± 0.0 ff	13.8 ± 0.2	13.4 ± 0.2 fff
Heart rate (beat/min)	80.5 ± 2.8	78.0 ± 2.1	80.0 ± 2.5	62.8 ± 2.4***	85.5 ± 2.3	62.7 ± 2.3***
Blood pressure (mmHg)						
- systolic	111.5 ± 3.5	112.3 ± 3.9	101.3 ± 2.1**	112.1 ± 5.3	95.5 ± 2.1***	103.0 ± 4.8
- diastolic	70.3 ± 5.6	65.0 ± 2.5 f	62.5 ± 2.5	70.5 ± 3.7 f	62.7 ± 1.4	64.4 ± 4.6 f
Body temperature (°c)	36.7 ± 0.2	36.2 ± 0.2	36.6 ± 0.1	36.1 ± 0.1	36.8 ± 0.1	36.3 ± 0.1

Values are means±SEM.

**p < 0.01, ***p < 0.001 Significant differences from sedentary subjects.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ Significant differences from the beginning (month 0) of the experiment.

resulted in the great reduction of heart rates from 80.0 to 62.8 beat/min ($p<0.01$) and from 85.5 to 62.7 beat/min ($p<0.001$), in swim and football subjects, respectively.

For systolic/diastolic blood pressures and body temperatures, there were no significant differences in these parameters among three groups of subjects at the end of the experiment.

5. Comparisons of biochemical data among three groups of subjects, at the end (month 12) of the experiment.

Resting malondialdehyde (MDA), creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and scavenging enzymes (SOD, CAT, and GPX) in untrained (sedentary) and trained (swim and football) groups at the beginning (month 0) and at the end (month 12) of the experiment were shown in Table 11, Figure 13-14.

5.1 Lipid peroxidation product (plasma MDA level)

At the end of the experiment (month 12), resting plasma MDA level was 2.05 ± 0.16 nmole/ml in sedentary subjects, 2.37 ± 0.21 nmole/ml in swim subjects and 2.63 ± 0.26 nmole/ml in football subjects. There were no significant differences in MDA levels among three groups of subjects. When compared within each group between MDA levels at the beginning and at the end of one year, only sedentary subjects showed significant increase ($p<0.01$) in MDA while there were no changes in resting MDA levels in swim and football subjects after one year training (Figure 13a).

Table 11. Plasma malondialdehyde (MDA), creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and erythrocyte scavenging enzymes (SOD, CAT and GPX) at rest in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) and the end (month 12) of the experiment.

Parameter	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
MDA (nmole/ml)	1.36 ± 0.05	2.05 ± 0.16 ^{ff}	1.91 ± 0.19*	2.37 ± 0.21	2.11 ± 0.18*	2.63 ± 0.26
CPK (U/L)	74.47 ± 15.02	111.42 ± 22.90 ^{f}	69.98 ± 9.56	101.51 ± 21.61	71.69 ± 8.42	92.10 ± 21.47 ^{ff}
LDH (U/L)	151.05 ± 13.18	208.59 ± 9.93	255.04 ± 15.00***	244.36 ± 16.16	208.38 ± 11.90**	243.51 ± 14.22 ^{ff}
SOD (x10^{3}U/gHB)	3.35 ± 0.10	3.53 ± 0.20 ^{f}	3.09 ± 0.14	3.50 ± 0.20	2.83 ± 0.12	3.54 ± 0.17 ^{fff}
CAT (x10^{4}U/gHB)	5.14 ± 0.41	3.66 ± 0.09 ^{ff}	4.99 ± 0.23	4.49 ± 0.32	5.20 ± 0.21	3.54 ± 0.30 ^{fff}
GPX (U/gHB)	282.32 ± 30.04	446.85 ± 44.92	291.89 ± 17.19	356.73 ± 19.52	296.14 ± 21.13	433.35 ± 27.23

Values are means±SEM.

*p < 0.05, **p < 0.01, ***p < 0.001 Significant differences from sedentary subjects.

^{f}p < 0.05, ^{ff}p < 0.01, ^{fff}p < 0.001 Significant differences from the beginning (month 0) of the experiment.

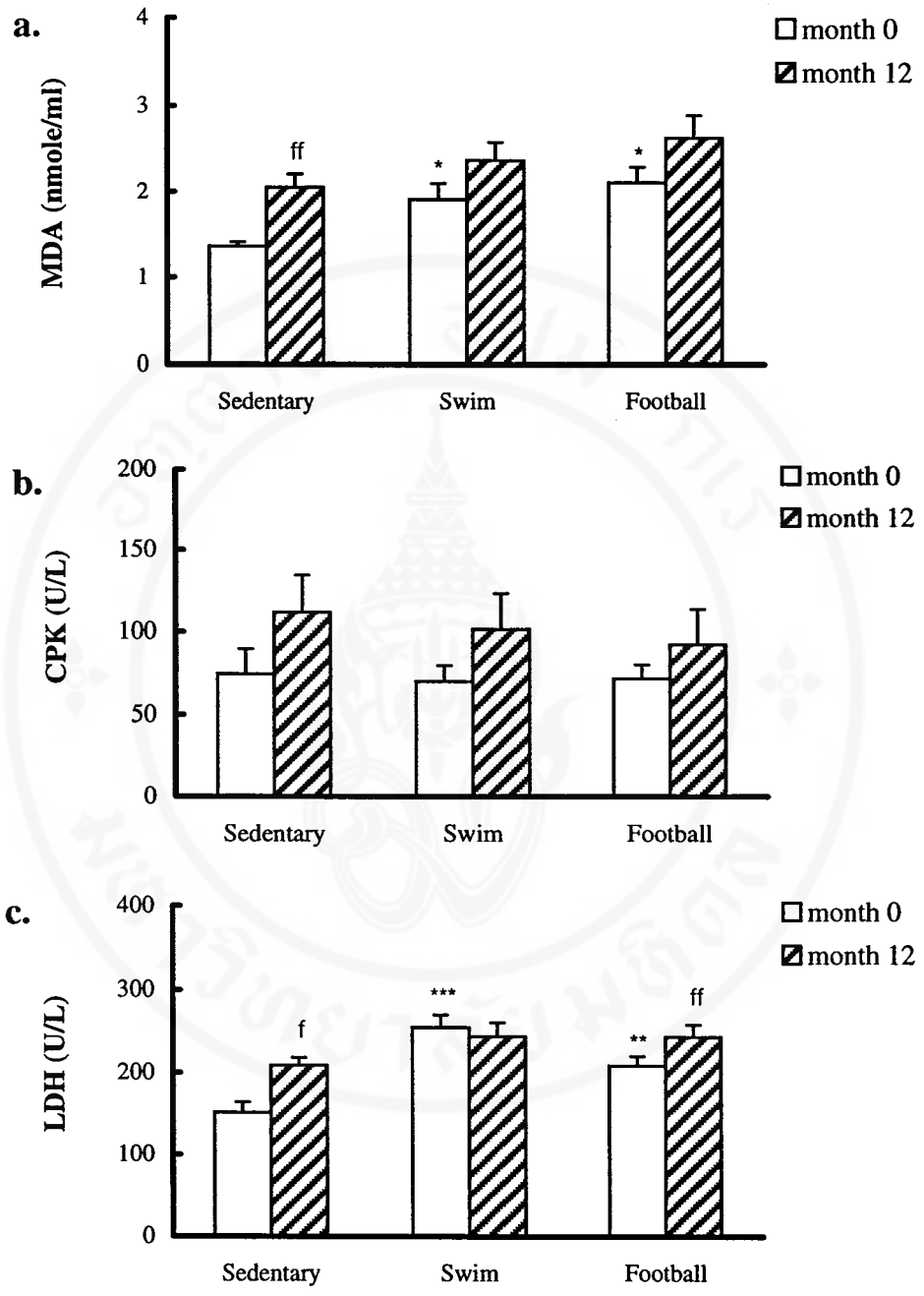


Figure 13. Changes in a. resting plasma malondialdehyde (MDA) levels, b. resting plasma creatine phosphokinase (CPK) activities and c. resting plasma lactate dehydrogenase (LDH) activities in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) and the end (month.12) of the experiment. Values are means$\pm$SEM.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ Significant differences from sedentary.

$^{f}p < 0.05$, $^{ff}p < 0.01$ Significant differences from the beginning (month 0) of the experiment.

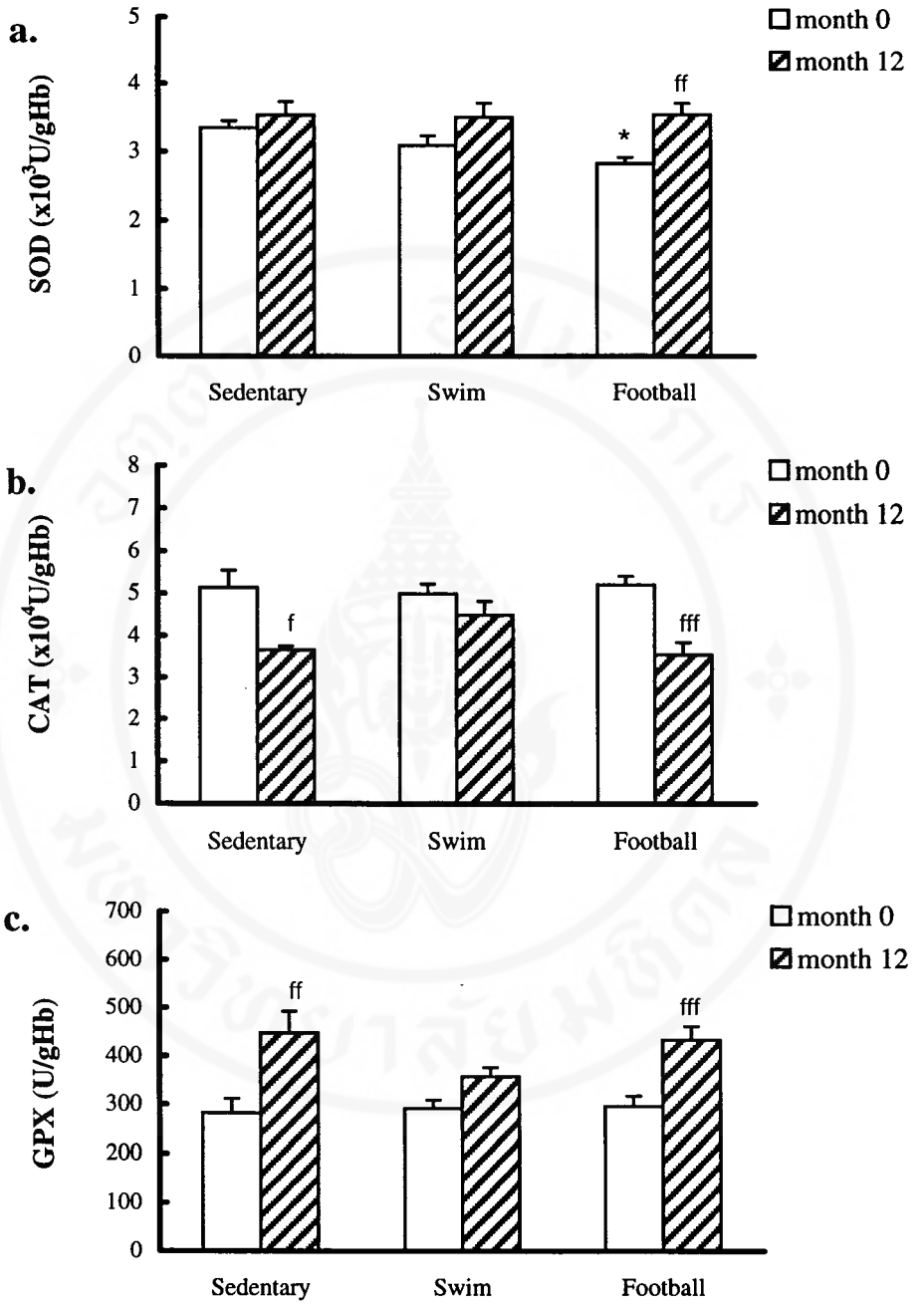


Figure 14. Changes in a. resting superoxide dismutase (SOD) activities, b. resting catalase (CAT) activities and c. resting glutathione peroxidase (GPX) activities in untrained (sedentary) and trained (swim and football) subjects at the beginning (month 0) and the end (month 12) of the experiment .Values are means±SEM.
*$p < 0.05$ Significant difference from sedentary.
$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$. Significant differences from the beginning (month 0) of the experiment.

5.2 Index for muscle damage (plasma CPK and LDH)

For resting plasma CPK levels, there were also no significant differences among three groups of subjects after one year. When compared within each group, CPK activities after one year were not significantly changed from the beginning, though tendency to be increased were shown in all three groups (Figure 13b).

Plasma LDH activities at rest of sedentary, swim, and football subjects at the end of one year were not significantly different from each other. However, significant increases were shown in LDH levels at the end of one year in sedentary and football subjects when compared with the corresponding values at the beginning of the experiment (Figure 13c).

5.3 Scavenging enzymes activities in red blood cells (SOD, CAT, and GPX)

Resting SOD activities in sedentary, swim and football subjects at the end (month 12) of the experiment were not significantly different from each other. When compared between the values at month 12 and month 0 of the experiment, there were no significant changes in resting SOD in sedentary and swim subjects, but there was a significant increase in SOD level in football subjects from $2.83 \pm 0.12 \times 10^3$ U/gHb at month 0 to $3.54 \pm 0.17 \times 10^3$ U/gHb at month 12 with $p<0.01$ (Figure 14a).

Resting CAT activities were also not significantly different among three groups of subjects at the end of one year. But CAT activities at month 12 of the experiment in sedentary and football subjects were found to be significantly lower than

the values at month 0 with $p<0.05$ and $p<0.001$, respectively. However, no reduction in CAT activity was found in swim subjects after one year training (Figure 14b).

Resting GPX activities were also found to be relatively comparable in sedentary and trained (swim and football) subjects at month 12 of the experiment. When compared within each group, they were significantly increased from 282.32±30.04 to 446.85±44.92 U/gHb ($p<0.01$) in sedentary subjects and from 296.14±21.13 to 433.35±27.23 U/gHb ($p<0.001$) in football subjects but only showed tendency to increase without statistical significance in swim subjects (Figure 14c).

6. Effects of endurance exercise on lipid peroxidation product, index of muscle damage and scavenging enzymes at the end (month 12) of the experiment.

6.1 Malondialdehyde (plasma MDA levels)

Malondialdehyde (MDA) levels in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after endurance exercise at the beginning (month 0) and at the end (month 12) of the experiment are shown in Table 12 and Figure 15.

After one year time for sedentary subjects and after one year training for swim and football subjects, 1 hr of endurance exercise by cycling at 70% HRmax caused a significant increase in plasma MDA level only in the sedentary subjects. About 40% increase in plasma MDA level (From 2.05±0.16 to 2.86±0.17 nmole/ml, $p<0.01$) was found at 5 minutes after exercise. At 24 hr and 48 hr after exercise, though plasma MDA of the sedentary subjects were still at the high levels of 2.51±0.3 and 2.76±0.34 nmole/ml, respectively, but when compared with the pre-exercise value of 2.05±0.16 nmole/ml, no statistical significant differences were found. for the trained

Table 12. Plasma malondialdehyde (MDA) in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) and the end (month 12) of the experiment.

Time	Lipid peroxide level (nmol/ml)					
	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Pre-exercise	1.36 ± 0.05	2.05 ± 0.16 ff	1.91 ± 0.19*	2.37 ± 0.21	2.11 ± 0.18*	2.63 ± 0.26
Post-exercise 5 min	1.60 ± 0.07 t	2.86 ± 0.17 tt,fff	2.73 ± 0.23* t	2.33 ± 0.29	2.67 ± 0.23* tt	2.66 ± 0.16
24 hr	1.60 ± 0.07 t	2.51 ± 0.30 f	2.46 ± 0.19 t	2.70 ± 0.30	2.14 ± 0.29	2.82 ± 0.32 f
48 hr	1.55 ± 0.08	2.76 ± 0.34	1.86 ± 0.29	2.55 ± 0.50	1.78 ± 0.21	2.60 ± 0.26

Values are means±SEM.

*$p < 0.05$ Significant difference from sedentary subjects.

$^{t}p < 0.05$, $^{tt}p < 0.01$ Significant differences from pre-exercise.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.01$ Significant differences from the beginning (month 0) of the experiment.

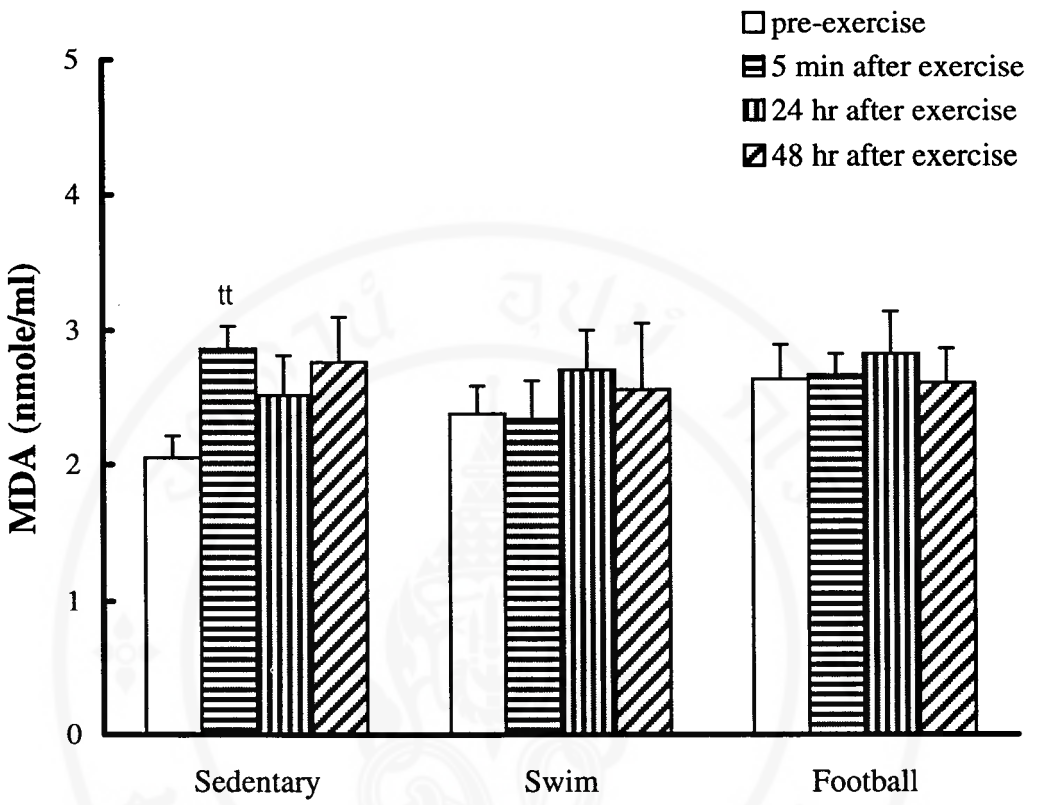


Figure 15. Plasma malondialdehyde (MDA) levels in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the end of one year (month12) of the experiment. Values are means±SEM.

$^{tt}p < 0.01$ Significant difference from pre-exercise of the same group.

subjects, there were no significant increases in plasma MDA levels after the same load of endurance exercise in both swim and football groups. When compared within the same group of subjects between the corresponding values at the beginning and at the end of one year., there were increases in MDA levels in sedentary subjects both before and after exercise at every time intervals measured. For swim and football groups, MDA levels after one year training (both before and after 1hr-endurance exercise) were quite comparable with those of the corresponding values at the beginning of the experiment except in football subjects at 48 hr after exercise that MDA level at month 12 was higher than at month 0 at $p<0.05$.

6.2 Index of muscle damage (CPK and LDH)

Creatine phosphokinase (CPK)

Plasma creatine phosphokinase (CPK) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) and at the end (month 12) of the experiment were shown in Table 13 and Figure 16. It was found that the endurance exercise caused no increase in plasma CPK activity in all three groups of subjects at 5 min, 24 hr, and 48 hr after exercise. In sedentary subjects, CPK activities were 111.42±22.90, 118.93±18.71, 95.33±13.82 and 112.10±32.48 U/L before and at 5 min, 24 hr, and 48 hr after exercise, respectively.

CPK activity were 101.51±21.61, 113.20±27.53, 96.05±17.63 and 103.82±13.85 U/L in swim subjects and 92.10±21.47, 96.74±21.92, 70.65±12.10 and 71.79±13.04 U/L in football subject before and at 5 min, 24 hr, and 48 hr after exercise, respectively. When compared the values of CPK levels at the beginning

Table 13. Plasma creatine phosphokinase(CPK) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) and the end (month 12) of the experiment.

Time	Creatine phosphokinase activity (U/L)					
	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Pre-exercise	74.47 ± 15.02	111.42 ± 22.90	69.98 ± 9.56	101.51 ± 21.61	71.69 ± 8.42	92.10 ± 21.47
Post-exercise 5 min	77.77 ± 13.10	118.93 ± 18.71	67.81 ± 12.46	113.20 ± 27.53	78.18 ± 13.05	96.74 ± 21.92
24 hr	81.91 ± 21.24	95.33 ± 13.82	77.45 ± 11.59	96.05 ± 17.63	86.60 ± 12.27	65.65 ± 12.10 [t]
48 hr	70.74 ± 13.66	112.10 ± 32.48	83.47 ± 25.30	103.82 ± 13.85	89.50 ± 15.15	71.79 ± 13.04

Values are means±SEM.

[t] $p < 0.05$ Significant difference from pre-exercise.

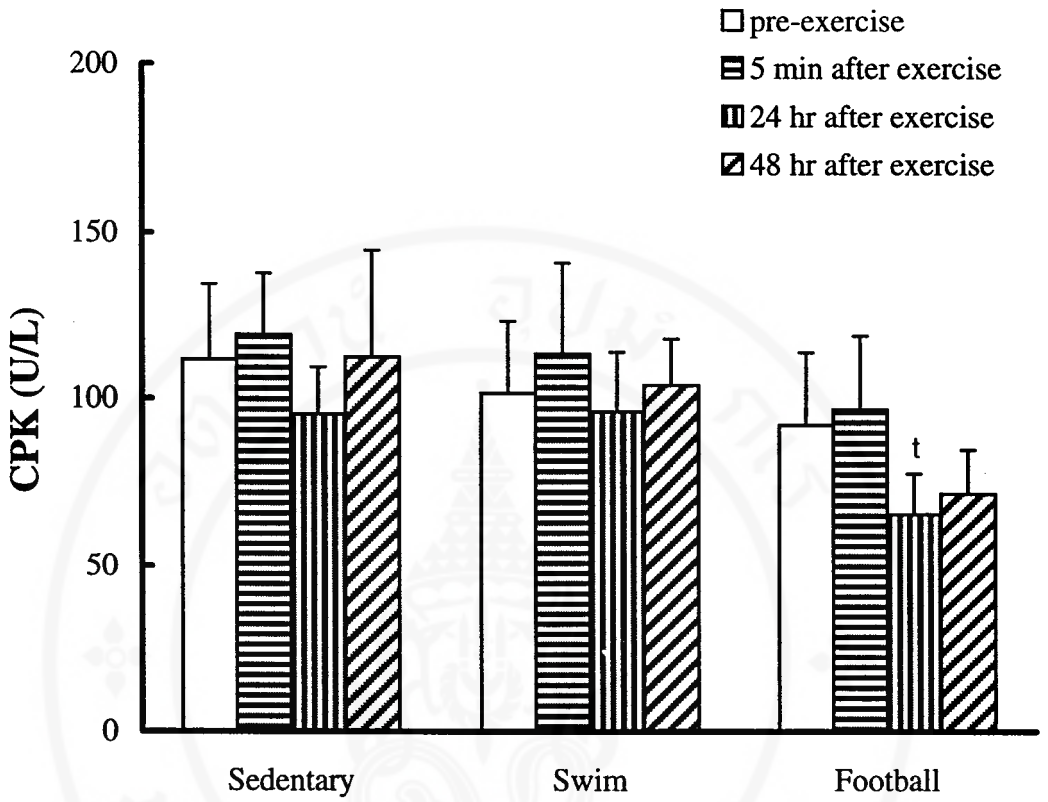


Figure 16. Plasma creatine phosphokinase (CPK) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the end of one year (month12) of the experiment. Values are means±SEM.

$^{t}p < 0.05$ Significant difference from pre-exercise of the same group.

(month 0) of the experiment with the corresponding values at the end (month 12) of the experiment, the results showed that there were trends for CPK levels to increase with one year-time in every groups of subjects at almost every time intervals but no statistical significance was found.

Lactate dehydrogenase (LDH)

Table 14 and figure 17 show plasma LDH activities in untrained (sedentary) and trained (swim and football) subjects before and at various time in tervals after exercise at the beginning (month 0) and at the end (month 12) of the experiment. In sedentary subjects, plasma LDH activity was significantly increased from resting level of 208.59±9.93 U/L to 228.88±13.453 U/L ($p<0.05$) and 222.80 ±10.68 U/L ($p<0.01$) at 5 min and 48 hr after 1 hr-endurance exercise, respectively. There was no significant difference between pre-exercise and 24 hr after exercise in sedentary subjects. In swim subjects, LDH activity were 242.60±13.52, 223.38±14.82 and 250.25±27.18 U/L at 5 min, 24 hr and 48 hr after exerise, respectively. There were no significant differences between these post-exercise values and the pre-exercise value of 244.36±16.16 U/L. In football subjects there were significant decreases in LDH levels at 24 hr (210.79±12.03 U/L) and 48 hr (220.01±11.43 U/L) after 1 hr-endurance exercise when compared with the resting level of 243.51±14.22 U/L.

6.3 Scavenging enzymes activities in red blood cells.

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after 1 hr-

Table 14. Plasma lactate dehydrogenase(LDH) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) and the end (month 12) of the experiment.

Time	Lactate dehydrogenase activity (U/L)					
	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month
Pre-exercise	151.05 ± 13.18	208.59 ± 9.93 ^{f}	255.04 ± 15***	244.36 ± 16.16	208.38 ± 11.9**	243.51 ±
Post-exercise 5 min	136.59 ± 19.33	228.88 ± 13.53 ^{t,fff}	210.35 ± 8.75** ^{t}	242.60 ± 13.52 ^{f}	223.79 ± 15.3**	249.02 ±
24 hr	175.39 ± 10.33	210.62 ± 12.45	217.53 ± 13.24	223.38 ± 14.82 ^{t}	230.27 ± 20.95	210.79 ±
48 hr	233.28 ± 28.86	222.80 ± 10.68 ^{tt}	215.32 ± 14.14	250.25 ± 27.18 ^{t}	218.06 ± 16.71	220.01 ±

Values are means±SEM.

**p < 0.01, ***p < 0.001 Significant differences from sedentary subjects.

^{t}p < 0.05, ^{tt}p < 0.01 Significant differences from pre-exercise.

^{f}p < 0.05, ^{ff}p < 0.01, ^{fff}p < 0.001 Significant differences from the beginning (month 0) of the experiment.

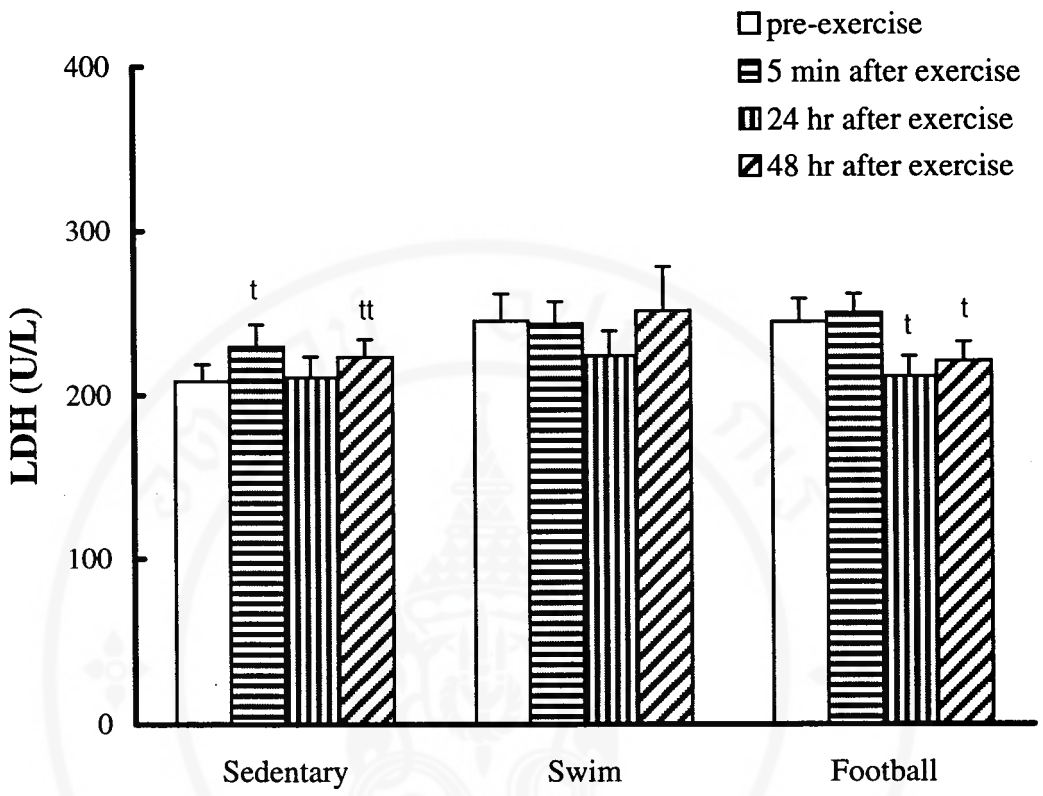


Figure 17. Plasma lactate dehydrogenase (LDH) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the end of one year (month12) of the experiment. Values are means±SEM. $^{t}p < 0.05$, $^{tt}p < 0.01$ Significant differences from pre-exercise of the same group.

Table 15. Superoxide dismutase(SOD) activities in untrained (sedentary)and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) and the end (month 12) of the experiment .

Time	Superoxide dismutase activity ($x10^3$ U/gHB)					
	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Pre-exercise	3.35 ± 0.10	3.53 ± 0.20	3.09 ± 0.14	3.50 ± 0.21	2.83 ± 0.09*	3.54 ± 0.17 ff
Post-exercise 5 min	3.79 ± 0.26	3.46 ± 0.17	3.14 ± 0.2*	3.39 ± 0.23	2.90 ± 0.15*	3.67 ± 0.14 t,fff
24 hr	3.23 ± 0.21	3.48 ± 0.20	3.04 ± 0.10	3.63 ± 0.31 f	2.72 ± 0.16 t	3.81 ± 0.12 t,ff
48 hr	3.82 ± 0.16	3.55 ± 0.28	3.14 ± 0.11**	3.71 ± 0.31	3.06 ± 0.16**	3.89 ± 0.18

Values are means±SEM.

*p < 0.05, **p < 0.01 Significant differences from sedentary subjects.

^{t}p < 0.05 Significant difference from pre-exercise.

^{f}p < 0.05, ^{ff}p < 0.01, ^{fff}p < 0.001 Significant differences from the beginning (month 0) of the experiment.

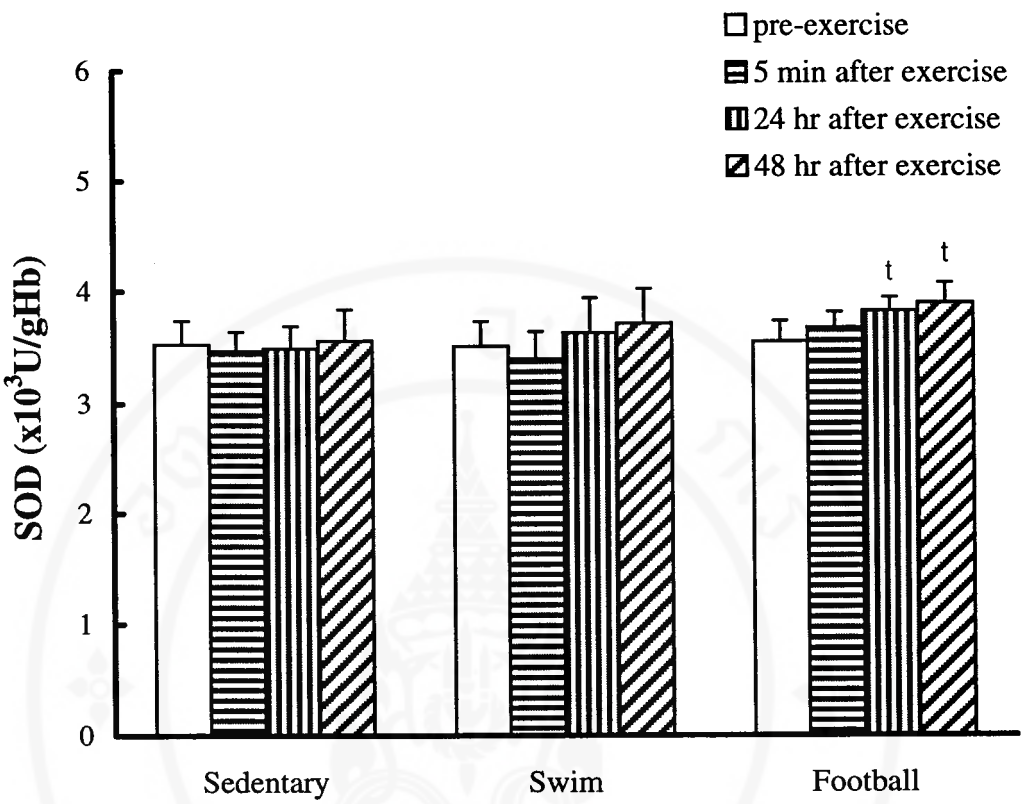


Figure 18. Superoxide dismutase (SOD) activities in untrained (sedentary) and trained(swim and football) subjects before and at various time intervals after one hour endurance exercise at the end of one year (month12) of the experiment. Values are means±SEM.

$^{t}p<0.05$ Significant difference from pre-exercise of the same group.

endurance exercise at the beginning (month 0) and at the end (month 12) of the experiment were shown in Table 15 and Figure 18.

In sedentary subjects, SOD activity before endurance exercise was 3.53±0.20 ($x10^3$) U/gHb and after exercise at 5 min, 24 hr and 48 hr were 3.46±0.17, 3.48±0.20 and 3.55±0.28 ($x10^3$) U/gHb, respectively. There were no significant differences found among these values. In swim subjects, they were 3.50±0.21, 3.39±0.23, 3.63±0.31 and 3.71±0.31 at rest, 5 min, 24 hr, and 48 hr after exercise, respectively. There were also no significant differences between pre-exercise and post-exercise values of SOD in swim group. The significant increases in SOD scavenging enzyme activities after 1hr-endurance exercise were found only in football subjects at 24 hr (3.81±0.12 ($x10^3$) U/gHb) and 48 hr (3.89±0.18 ($x10^3$) U/gHb) after exercise when compared with the pre-exercise value of 3.54±0.17 ($x10^3$) U/gHb. No significant differences in SOD activities at the same time intervals were found among three groups of subjects both before and after exercise.

Catalase (CAT)

Catalase (CAT) activities in untrained (sedentary) and trained (Swim and Football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) and at the end (month 12) of the experiment were shown in Table 16 and Figure 19. In sedentary subjects, CAT activities at 5 min, 24 hr, and 48 hr after exercise were 4.05±0.32, 4.02±0.26 and 3.19±0.18 ($x10^4$) U/gHB, respectively. They were not significantly different from the resting value of 3.66±0.09 ($x10^4$) U/gHb.

Table 16. Catalase(CAT) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time int after one hour endurance exercise at the beginning (month 0) and the end (month 12) of the experiment.

Time	Catalase activity ($x10^4$ U/gHB)					
	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month
Pre-exercise	5.14 ± 0.41	3.66 ± 0.09 ^{f}	4.99 ± 0.23	4.49 ± 0.32	5.20 ± 0.21	3.54 ±
Post-exercise 5 min	4.51 ± 0.22	4.05 ± 0.32 ^{ff}	5.17 ± 0.34	4.32 ± 0.26 ^{fff}	5.60 ± 0.18*	3.89 ±
24 hr	4.55 ± 0.22	4.02 ± 0.26	5.59 ± 0.29	4.07 ± 0.37 ^{ff}	5.46 ± 0.42	3.70 ±
48 hr	4.64 ± 0.26	3.19 ± 0.18	5.32 ± 0.34	3.92 ± 0.30	4.83 ± 0.28	3.82 ±

Values are means±SEM.

*$p < 0.05$ Significant difference from sedentary subjects.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ Significant differences from the beginning (month 0) of the experiment.

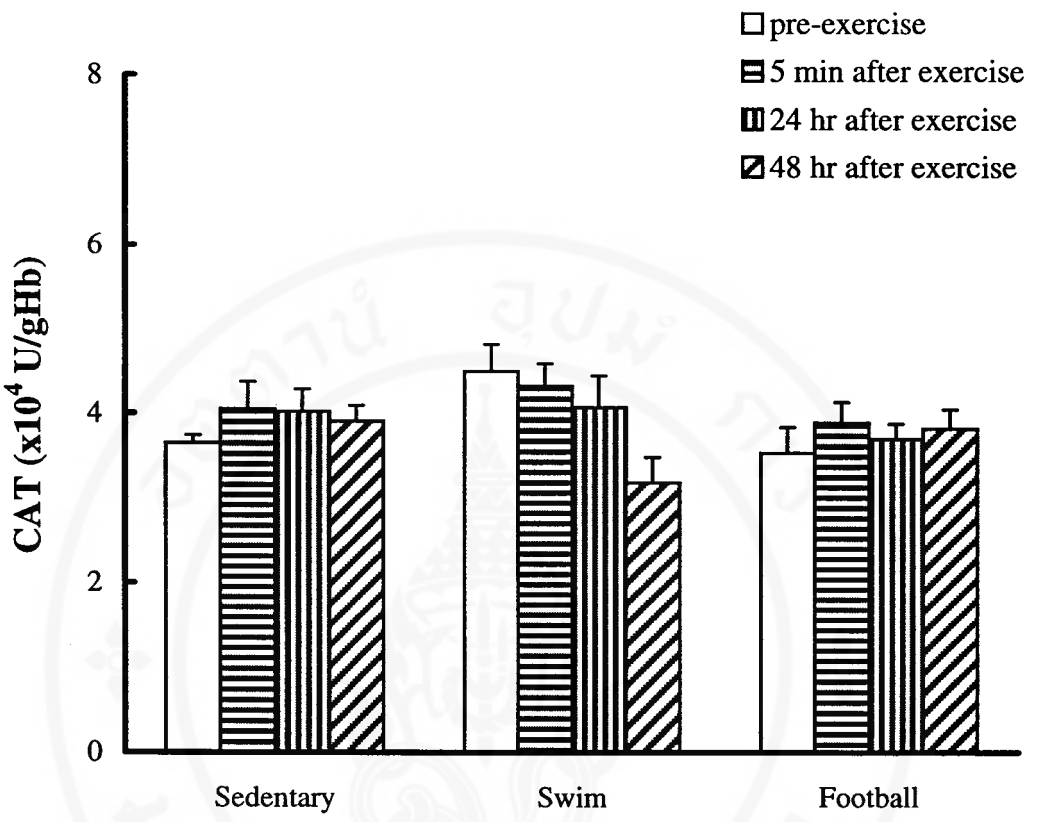


Figure 19. Catalase (CAT) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the end of one year (month12) of the experiment. Values are means±SEM.

CAT activities in the trained subjects after 1 hr-endurance exercise were found to be in the ranges of 3.70-3.89x10^4 U/gHb in football group and 3.92-4.32 x10^4 U/gHb in swim group which were considered not significantly different from their own pre-exercise values and also not significant different from the corresponding values of the sedentary subjects.

Glutathione peroxidase (GPX)

Glutathione peroxidase (GPX) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0) and at the end (month 12) of the experiment were shown in Table 17 and Figure 20.

There were significant increases in GPX activities after one hour endurance exercise in all 3 groups of subjects. In sedentary subjects, they increased from the resting value of 446.85±44.92 U/gHb to 501.99±46.14 and 491.96±44.67 U/gHb at swim, and 48 hr after exercise, respectively. In swim subjects, GPX activity increased from 356.73±19.52 at rest to 390.10±26.56 at 5 min after exercise. In football subjects, it increased from 433.35±27.23 to 467.25±28.19 at 24 hr after exercise. When compared between the values at month 12 and month 0, it was found that GPX activities increased significantly in every groups at almost every time intervals both before and after 1 hr-endurance exercise.

7. Changes in some resting parameters with age in sedentary group.

When compared the data of the sedentary subjects at the end (month 12) with those at the beginning (month 0) of the experiment, changes in some resting parameters with one year increase of age were found. The data were recruited from

Table 17. Glutathione peroxidase(GPX) activities in untrained (sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the beginning (month 0)and the end (month 12)of the experiment.

Time	Glutathione peroxidase activity (U/gHb)					
	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Pre-exercise	282.32 ± 30.04	446.85 ± 44.92 ff	291.89 ± 17.19	356.73 ± 19.52	296.14 ± 21.13	433.35 ± 27.23 fff
Post-exercise 5 min	276.53 ± 26.14	501.99 ± 46.14 t,fff	288.45 ± 17.46	390.10 ± 26.56 t,f	341.64 ± 17.03 t	421.20 ± 28.03 f
24 hr	283.97 ± 31.97	474.68 ± 50.37 ff	270.51 ± 23.94	357.42 ± 22.61 f	338.90 ± 28.02	467.25 ± 28.19 t,fff
48 hr	262.22 ± 24.46	491.96 ± 44.67 t,fff	302.94 ± 20.19	356.51 ± 29.51*	327.56 ± 23.53	431.09 ± 25.93 ff

Values are means±SEM.

*$p < 0.05$ Significant difference from sedentary subjects.

$^{t}p < 0.05$ Significant difference from pre-exercise.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ Significant differences from the beginning (month 0) of the experiment.

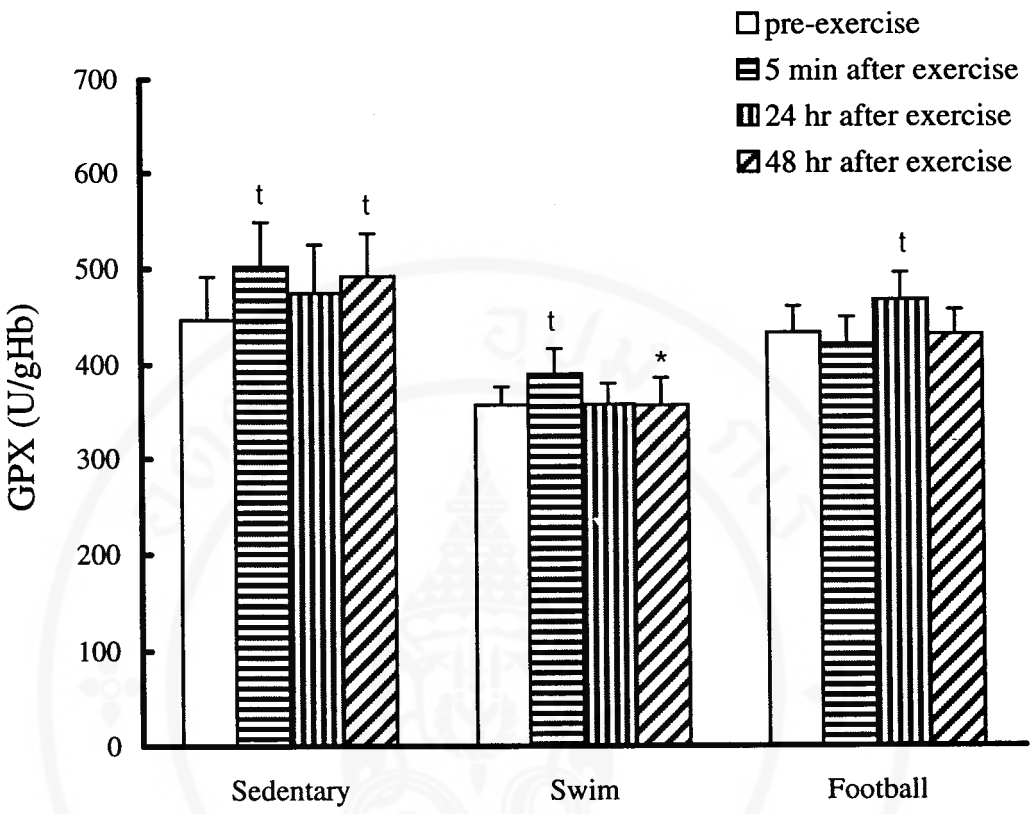


Figure 20. Glutathione peroxidase(GPX) activities in untrained(sedentary) and trained (swim and football) subjects before and at various time intervals after one hour endurance exercise at the end of one year (month12) of the experiment. Values are means±SEM.
*$p < 0.05$ Significant difference from sedentary at the same time intervals.
$^{t}p < 0.05$ Significant difference from pre-exercise of the same group.

Table 18. Changes in some resting parameters with age in sedentary group.

Parameter	Sedentary (n = 6)	
	month 0	month 12
Body weight (kg)	49.0 ± 3.8	54.0 ± 3.8 ^{fff}
Lean body mass (kg)	39.1 ± 1.6	43.0 ± 1.6 ^{fff}
Left handgrip strength (kg/kgBW)	0.5 ± 0.0	0.6 ± 0.0 ^{f}
PWC_{170} (watt/kgBW)	2.3 ± 0.1	3.4 ± 0.2 ^{ff}
Hct (%)	39.5 ± 0.8	43.1 ± 1.2 ^{ff}
Resting MDA (nmole/ml)	1.36 ± 0.05	2.05 ± 0.16 ^{f}
Resting LDH (U/L)	151.05 ± 13.18	208.59 ± 9.93 ^{f}
Resting CAT ($x10^4$ U/gHb)	5.14 ± 0.41	3.66 ± 0.09 ^{ff}
Resting GPX (U/gHb)	282.32 ± 30.04	446.85 ± 44.92

Values are means±SEM.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ Significant differences from the beginning (month 0) of the experiment.

Table 8-11 compared, and presented in Table 18. Besides the expected increase in body height, body weight and lean body mass with one year time, the sedentary subjects also showed the significant increase in PWC_{170} from 2.32 to 2.38 watt/kgBW ($p<0.05$), hematocrit from 39 to 43% ($p<0.05$), resting MDA from 1.38 to 2.05 nmole/ml ($p<0.05$), resting LDH from 136.29 to 208.59 U/L ($p<0.01$) and changes in the activities of some scavenging enzymes measured. Resting CAT activities was found to decrease significantly from 5.11 ± 0.35 ($x10^4$) U/gHb at the beginning (month 0) to 3.66 ± 0.09 U/gHb at the end (month 12) of the experiment with $p<0.001$. On the other hand, resting GPX activities in sedentary subjects was found to increase from 281.28 ± 25.41 to 446.85 ± 44.92 U/gHb at $p<0.05$.

8. Effect of one year exercise training (longitudinal study)

To see more clearly whether one year exercise training had any and how much effects on physical fitness, lipid peroxidation and muscle damage, and scavenging enzymes activities, all those data which demonstrated significant changes after one year training in swim and football subjects were recruited (from Table 9-11) and presented in Table 19. The corresponding data of untrained (sedentary) subjects are also presented in this table for comparing advantage. It was undoubtedly found that regular and systematic exercise training of more than 20 hours/week for one year resulted in great increases in physical fitness characteristic such as muscle strengths (handgrips, back and leg), muscular endurance (sit up) and trunk flexibility in both trained groups with mostly better improvement in football than in swim subjects. Aerobic capacity (predicted from PWC_{170}), were increased significantly ($p<0.001$) to a greater levels in both swim and football subjects when compared with sedentary subjects. The effect of

Table 19. Effects of one year exercise training on physical fitness, lipid peroxidation,muscle damage and scavenging enzymes activities.

Parameter	Sedentary (n = 6)		Swim (n = 8)		Football (n = 11)	
	month 0	month 12	month 0	month 12	month 0	month 12
Right handgrip strength (kg/kgBW)	0.6 ± 0.0	0.6 ± 0.0 f	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.6 ± 0.0 fff
Left handgrip strength (kg/kgBW)	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0 fff
Leg strength (kg/kgBW)	1.7 ± 0.2	2.3 ± 0.1	2.2 ± 0.2	2.8 ± 0.3 ff	2.1 ± 0.1	2.4 ± 0.2 fff
Back strength (kg/kgBW)	1.3 ± 0.1	1.6 ± 0.1	1.8 ± 0.1**	2.1 ± 0.1** ff	1.7 ± 0.1*	2.2 ± 0.1** ff
Trunk flexion (cm)	12.3 ± 1.5	14.2 ± 1.6	11.8 ± 1.9	16.2 ± 2.3 f	8.9 ± 1.6	11.1 ± 1.5 fff
Sit up (time/min)	18.3 ± 1.6	20.3 ± 1.3 ff	26.9 ± 1.6**	31.1 ± 1.3*** ff	22.1 ± 1.3	30.9 ± 1.2*** fff
PWC_{170} (watt/kgBW)	2.3 ± 0.1	3.4 ± 0.2 ff	4.2 ± 0.3***	5.8 ± 5.0***	3.9 ± 0.3***	5.2 ± 0.2***
Hematocrit (%)	39.5 ± 0.8	43.1 ± 1.2	41.0 ± 0.8	40.9 ± 1.1 ff	40.6 ± 0.8	40.4 ± 0.5 fff
Resting heart rate (beat/min)	80.5 ± 2.8	78.0 ± 2.1 ff	80.0 ± 2.5	62.8 ± 2.4***	85.5 ± 2.3	62.7 ± 2.3***
Resting MDA (nmole/ml)	1.36 ± 0.05	2.05 ± 0.16 f	1.91 ± 0.19*	2.37 ± 0.21	2.11 ± 0.18*	2.63 ± 0.26 ff
Resting LDH (U/L)	151.05 ± 13.18	208.59 ± 9.93	255.04 ± 15.00***	244.36 ± 16.16	208.38 ± 11.9**	243.51 ± 14.22 ff
Resting SOD ($x10^3$ U/gHb)	3.35 ± 0.10	3.53 ± 0.20 f	3.09 ± 0.14	3.50 ± 0.20	2.83 ± 0.12	3.54 ± 0.17 fff
Resting CAT ($x10^4$ U/gHb)	5.14 ± 0.41	3.66 ± 0.09 ff	4.99 ± 0.23	4.49 ± 0.30	5.20 ± 0.21	3.54 ± 0.30 fff
Resting GPX (U/gHb)	282.32 ± 30.04	446.85 ± 44.92	291.89 ± 17.19	356.73 ± 19.52	296.14 ± 21.13	433.35 ± 27.23

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ Significant differences from sedentary subjects.

$^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ Significant differences from the beginning (month 0) of the experiment.

long-term exercise training was also clearly demonstrated in the trained groups by the significant reduction in resting heart rate from 80.0 to 62.8 and 85.5 to 62.7 beats/min in swim trained and football trained subjects, respectively.

Fifty percent increase in resting MDA was found with advancing age in sedentary subjects, while there were no significant changes in resting MDA in swim and football trained subjects after one year. Moreover, the endurance exercise of one hour by cycling at 70% HRmax seemed to put less load to the swim and football subjects after one year training since there were no increase in plasma MDA after exercise in both trained groups while there was still 40% increase in MDA level in sedentary group after exercise (Table 12). For plasma LDH, there were no increases in this enzyme in swim and football trained groups but 10% increase in sedentary group at 5 min after exercise (Table 14).

For resting scavenging enzyme activities, SOD was increased with significance in football subjects and increased with no significance in swim subjects. CAT was greatly and significantly decreased in sedentary and football subjects while it was maintained in swim subjects. GPX activities showed tendency to increase in all three groups, but the significant differences were demonstrated only in sedentary and football subjects. After exercise, SOD was still found to be increased significantly in football trained subjects and the values at month 12 were higher than the corresponding values at month 0. In swim trained subjects, post exercise SOD at the end of one year was significantly higher than the corresponding value at the beginning (month 0) of the experiment only at 48 hr after exercise (Table 15). CAT activities

were not significantly changed while GPX activities were increased significantly after exercise in all three group of subjects (Table 16, 17).

CHAPTER V

DISCUSSION

During physical activity the rate of oxygen consumption in humans may increase up to 20 fold (10, 98). Consequently, during sustained exercise reactive oxygen species generation, such as $O_2^{\bullet}$ production, may increase compared with during rest (10, 99). Dillard et al. (100) and Lovlin et al. (101) reported that exercise caused an increase in lipid peroxidation breakdown products in rodents and human, respectively. Long-term and regular exercise training has been claimed to reduce the exercise-increased lipid peroxidation by inducing antioxidant or scavenging enzymatic activities. Most of the studies which reported the positive side of oxidative stress following regular exercise has been done in rodents (26, 29-31). For human, the evidence for the effect of long-term exercise training is scarce and controversial. Moreover, most of them come from the cross-sectional studies, comparing the data among different groups of subjects who have been trained for various durations (55). This study examine the effects of long-term regular exercise training on lipid peroxidation, muscle damage, and scavenging enzymes both at rest and after one bout of endurance exercise in young Thai subjects who were training to be swimmer and football athletes. To our knowledge, this is the first longitudinal study to examine differential effects of long-term exercise training up to one year, on both oxidative stress and antioxidant enzyme activities in human, before and after acute exercise.

Subjects

The trained subjects used in this study are the students from Sports School of Suphanburi. The main purpose of this school is to educate and to train children to be elite athletes at national or international levels. It is boarding school which admit students, who have some talents in certain types of sports, from primary to high school levels. Students stay and live together in the schools where they receive regular intensive sports training. So these students are under the same environmental condition, have the same nutrition and life style. Thus the students in sports schools are good models for the study of the influence of long-term intensive exercise training. The training program for all types of sports in this school takes approximately 20 hours per week which is much more than normal physical activities of general children of the same age range. Our subjects are all students in Mathayom Suksa one which is the first grade offered in this sports school so that all of them will be exposed to the regular intensive exercise training program of the school at the same time. The starting of the program was approximately at the beginning (month 0) of our experiment.

The sedentary subjects are the healthy students of Mathayom Suksa one from Kanasut Suksalai School of Suphanburi. The purpose of choosing sedentary subjects from the same province was to minimize the differences in environment, nutrition, and life style among subjects in the same and different groups.

Mode or type of exercise may be another factor to consider in lipid peroxidation reactions (3). In the present study, it was compared between weight bearing and non-weight bearing types of exercise. Weight bearing type is the exercise with the force of gravity acting on the body (102) and non-weight bearing type is the

opposite. In our study, the weight bearing exercise was represented by 'football' which the subjects in this group were trained for a unique set of skills such as running, ball handling, ability and power to kick for passing or shooting the ball (103). Non-weight bearing type was represented by swimming which the subjects in this group were trained for both sprint & endurance swimming skill. The daily training program for swim and football subjects were as follows:

6.30-7.30 a.m.	Basic training and running (the same for both groups)
15.30-18.00 p.m.	Specific training (specific for each group)

Intensity, duration, and mode of acute endurance exercise

Exercise of a sufficient intensity and duration has been shown to increase indicators of oxidative stress (3). In this study, a bout of acute ergometer cycling exercise at 70% of HR max for 60 minutes was chosen. This is equivalent to about 58% of the maximum aerobic capacity or $\dot{V}O_2max$ (Table 1). Although establishing exercise intensity from the measures of oxygen uptake is reasonably accurate, it is impractical without sophisticated equipment. An effective alternative is to use heart rate to classify exercise for relative intensity. This practice is possible because the percent $\dot{V}O_2max$ and percent HRmax are related in predictable way regardless of gender, fitness level, or age (78, 79). The error in estimating percent $\dot{V}O_2max$ from percent HRmax or vice verca, is about $\pm$8%. Since it is well accepted that aerobic capacity will be improved if exercise is of sufficient intensity to increase heart rate to about 70% of maximum, so it is likely that our exercise protocol was vigorous enough to encompass the oxidative stress encountered during exercise (10). According to the

study of Toskulkao and Klinsukon (32), the exercise program of 70% HRmax for 60 minutes was sufficient to produce the oxidative stress in adult.

Considering about the mode of exercise, cycling consists mainly of concentric contraction, in which the active muscle shortens (104). It was used as an exercise model in this study to avoid eccentric contractions that may cause inflammatory responses (105). Although inflammatory events cannot be completely ruled out, we assumed that most of the changes in plasma MDA, CPK, LDH and erythrocyte antioxidants, if found after endurance exercise in this study, were mainly the results of an elevation in metabolically induced oxygen radicals formation.

Comparisons of general and physical fitness data among three groups of subjects at the beginning (month 0) and at the end (month 12) of the experiment: the effects of long-term exercise training.

At the beginning of the experiment:

The subjects of all 3 groups were in the same ranges of age, body weight, lean body mass, and height. The football-trained subjects, though showed no statistical difference from the others, but from the observation and from the trends of the data shown in Table 2, they seemed to be smaller than the sedentary and the swim trained subjects. The significant differences were found in the percent body fat and some physical fitness characteristics between the trained and untrained subjects from the very beginning of the experiment. These significant differences in the percent body fat and physical fitness between trained and untrained groups and the non-significant difference in size of the football-trained group with the others might be due to the pre-selection process of the sports school to get the students with some talents in sports.

Most of the students of the sports school, used to be the young athletes of their previous schools, so they had been exposed to certain levels of training before entering the sports school and before being recruited into this experiment. However, those previous trainings were brief, not as systematic, intensive, and regular as of the sports school. So we still expected to see further changes in the trained groups at the end of the experiment.

At the end of the experiment

The subjects of all three groups showed comparable increases in body weight, lean body mass, and height after one year, indicating normal growth of subjects. The percent body fat of the trained subjects were still significantly lower than the sedentary subjects. The fat loss normally found with training could be due to the increased activities of enzymes involved in the activation, transport and break down or oxidation of fatty acid (106). The football-trained subjects, after one year training, had significantly increases in all physical fitness characteristics measured including PWC_{170} which represents aerobic capacity in this experiment. The swim-trained subjects had great increases in trunk flexibility, back strength, abdominal muscle endurance and aerobic capacity. The sedentary subjects had only increases in grip strength and aerobic capacity to the level that was still significantly lower than the trained groups. These results indicated that one year of regular exercise training program employed in Sports School of Suphanburi and in this experiment was effective in providing great increases in physical fitness characteristics, and the weight-bearing exercise training as football had more benefits in improving physical fitness than the non-weight bearing exercise training as swimming.

The increase in PWC_{170} in the trained subjects to the significantly higher values when compared with the untrained subjects found in this study was in accordance with the other studies which found that $\dot{V}O_2max$ in athletes was significantly higher than in sedentary subjects (107, 108).

The mechanisms by which the $\dot{V}O_2max$ could be increased with endurance training include a combination of increased size and number of mitochondria and oxidative enzymes in the active tissues (109, 110), as well as an increased blood volume, an increased cardiac output and a better perfusion of the active tissues (55). Long-term endurance training also causes muscular hypertrophy with an increased capillary density in skeletal muscle. This would enhance the removal of waste products from the working muscle (111, 112). Some investigators have suggested that since training increases the number and the size of muscle mitochondria (110, 113), with more mitochondria, the oxygen as well as the ADP and Pi required per mitochondrion will be less for a given submaximal exercise load after training as compared with before training. Because the levels of ADP and Pi control the rate of glycolysis (the higher the levels, the greater the rate of the glycolysis), their lower levels after training would cause the rate of lactic acid production to be slower. This may be one of the possible reasons to explain the greater muscle strength and endurance found in both trained groups in our study. The significant increase in PWC_{170} also found in sedentary subjects after one year in this study indicated that either the advancing age of one year or the normal physical activities of the sedentary subjects or both, can cause the increase in aerobic capacity in the untrained subjects to a certain level.

The heart rates of both trained groups of subjects after 1-year training were significantly lower than that of sedentary groups and significantly lower than the values before regular training. The resting bradycardia resulting from exercise training most likely involves two major components: (a) a decreased sensitivity of cardiac tissue to the catecholamines, which is also known to occur following training (55) and (b) an increase in parasympathetic (vagal) predominance on the cardiac pacemaker rate as a result of a decrease in sympathetic activity caused by exercise training (108, 114).

An important characteristic of the blood is the hematocrit, which is the percentage of the blood volume occupied by red blood cells. It is known that the hematocrit increases progressively through childhood and adolescence in boys (115). In our study, it was also found that the sedentary subjects showed increases in hematocrit after one year (from 39.5 to 43.1%, $p<0.01$). However, the hemoglobin concentration of the sedentary subjects only showed the tendency to increase with the increased hematocrit (from 13.7 to 14.3 g/dl), but no significant difference was obtained. In contrast to the sedentary subjects, the trained subjects of both groups did not show the increases in either Hct or Hb with advanced age. This might be due to the effect of exercise training. There have been numerous reports of sports anemia and various hypotheses have been proposed to explain the etiology of this condition (116-119). These include: dietary ion deficiency; gastrointestinal and urinary tract bleeding, haemolysis caused by repeated mechanical trauma; and osmotic and oxidative damage to RBCs during intensive exercise (118-119). However, considerable debate still exists as to whether the low values of hematocrit and hemoglobin concentrations observed in some athletes designate true anemia (RBC deficiency) since it was found

that iron supplementation has no beneficial effect on athletic performance (119). Thus, low RBC counts and hemoglobin concentration are more likely to be due to a training-induced increase in plasma volume which causes hemodilution (pseudoanaemia) (116, 120-121). It was proposed that endurance training can increase RBC mass but the rise in plasma volume is greater, so the observed decreased Hct is still found (119, 121). Therefore, our finding that Hct of the trained groups were not increased with age as in sedentary group was not unexpected.

Effect of one year exercise training on lipid peroxidation

The occurrence of lipid peroxidation can be detected by measuring its byproduct, malondialdehyde (MDA) (16, 17). The resting levels of MDA found in this study were in the range of 1.38-2.38 nmole/ml (Table 5). This range was comparable with those found by the other investigators which used the same TBAR method. At the beginning (month 0) of the experiment, resting MDA levels of the trained subjects were found to be significantly higher than that of the sedentary subjects, indicating the higher lipid peroxidation status which might reflect the higher physical activities in these groups of subjects. After one year of regular intensive training. The resting levels of plasma MDA in the trained groups were not significantly changed from the beginning, while that of the sedentary group increased significantly. From this result, it was likely to speculate that the occurrence of lipid peroxidation may increase with the advancing age. Humans are exposed to many toxic substance in daily life, so, with advancing age, the elevation of free radicals produced by these toxic substance may result in the increased lipid peroxidation (32). Since there were no further significant increases in MDA levels with age in the trained groups in this study, it might be

possible that training reduced the rate of peroxidation process. This idea was supported by several studies about adaptive responses of training. Salminen and Vihko (122) have suggested that endurance training may increase the resistance of skeletal muscle to injuries caused by lipid peroxidation according to their finding of a significant reduction of lipid peroxidation in muscle after three weeks of training. Jenkins et al. (123) reported that training enhanced muscle's ability to defend itself against hydroperoxide stimulated oxidant stress. These agreed with the finding of Alessio et al. (72) that endurance training resulted in a slowing down of the peroxidative reactions in tissues of rats. The mechanisms responsible for enhancing the body's defense against superoxide radicals and lipid peroxidation after endurance training are not clear. However, some mechanisms involved in the defense mechanisms that help to reduce some potentially harmful effect of superoxide radicals and lipid peroxidation have been proposed as (a) to enhance the degradation excretion of lipid peroxide (124) and (b) to increase the activity of antioxidant enzymes (25, 125-127).

Effect of one year exercise training on muscle damage

Plasma activities of specific muscle enzymes such as CPK and LDH were used as markers for muscle tissue damage in this study (22, 23). The resting values of plasma CPK and LDH found in this study were 70-80 U/L and 156-251 U/L, respectively, which were closed to the normal ranges of 15-110 U/L for CPK and 120-240 U/L for LDH, respectively. In the untrained-sedentary subjects, it was found that the increase of resting plasma MDA after one year was accompanied by the significant increases of resting plasma LDH (38%) and the non significant increases of resting

plasma CPK. This finding indicated that a certain degree of tissue damage was occurred following lipid peroxidation in sedentary subjects with one year advancing age. In the swim trained group there were no significant increases in both resting CPK and LDH levels indicating no increase in tissue damage after one year training. For football subjects, there was no increase in CPK but small (17%), significant increase in LDH. The small increase in resting LDH without the accompanying increase in MDA in football subjects indicating that the increased tissue damage marker found in this case might not be the result of increased oxidative stress after training but probably due to tissue trauma since football is considered as one of the contact sports. We may concluded that with the advancing age, there might be an increase in tissue damage as a result of increasing level of lipid peroxidation, and long-term regular exercise training might help reducing this damage possibly through the reduction of lipid peroxidation. It was also proposed that, with long-term exposure to daily training, fragile or susceptible fibers would be eliminated, and the viscoelastic properties of the muscle-connective tissue complex were stabilized (128).

Effect of one year exercise training on scavenging enzymes

The membranes of red blood cells are rich in polyunsatturated fatty acids and are continually exposed to high oxygen concentration (129, 130). So, red blood cells are highly susceptable to the damage caused by reactive oxygen free radicals. However, they are very well endowed with antioxidant protection system. Antioxidant enzymes provide the first line of defense against reactive oxygen species by converting them to more reduced species (5). The three major antioxidant enzymes in our body are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX).

In the present study, resting SOD activities tended to be increased in sedentary and swim subjects but significantly increased in football subjects after one year. This result was supported by the study of Brites et al. (131), who found that soccor players under regular training show an improved plasma antioxidant status, an increase in plasma superoxide dismutase activity, in comparison to sedentary controls. Earlier studies in both rodents (25, 132) and human (133, 134) also reported that SOD activity was increased by exercise training. Lukaski et al. (133) indicated that the increase in dietary copper was a functional adaptation of copper metabolism to aerobic training. In opposition, studies by Ji et al. (30), Laughlin et al. (26), Higuchi et al. (31) and Alessio and Goldfarb (72) found no evidence of exercise training induced upregulation of SOD activities in rodents. Ohno et al. (34) and Hellsten et al. (105) found no change in SOD activity after physical training in humans. It might be suggested that these disparate results are related to differences in training intensity, duration, or mode of exercise (26).

This study presented that CAT activities were significantly decreased in sedentary and football subjects but not significantly decreased in swim subjects at the end of 1-year when compared with at the beginning of the experiment. In contrast, GPX activities were increased in sedentary and football subjects but not significantly increased in swim subjects. These observations suggested that the antioxidative defense system is in a dynamic equilibrium, so that a decrease in one of the factors will to a certain extent be compensated through increased capacity of the other antioxidants (28). Catalase, a widely distributed enzyme responsible for catalyzing the conversion of H_2O_2 to H_2O and O_2, may function as the primary pathway for elimination of excess

H_2O_2 associated with oxidative stress, owing to the extremely high turnover rate of this reaction (29). Catalase, however, has not been shown to be consistently inducable by training. Jenkins (135) indicated, in a recent review of free radical biochemistry and exercise, that animal studies investigating the effects of training on CAT activity in skeletal muscle have also produced conflicting results. CAT activities in four of the muscle samples of the exercise trained rats were lower than control. These results are in agreement with our results and those of Alessio and Goldfarb (72), who recently reported that CAT activity was 30-40% lower in the red and white portions of vastus lateralis muscle of the trained rats. Higuchi et al., (31) reported that training did not produce any change in soleus CAT activity. Thus, available data indicated that exercise training either does not change CAT activity in skeletal muscle or produces decreases in CAT activities (26). Therefore, increases in GPX activity may represent an adaptive function to assist the cell in H_2O_2 removal (29). Moreover, activity of CAT, being present primarily in peroxisomes, also decreased during aging while GPX which is found in mitochodrial areas was found to have a positive correlation with age (136). With regard to erythrocyte GPX, its activity rises until 18 years, remains stable until 65 years then slightly decreases afterward (137). These reports supported our study that CAT activity was decreased and GPX activity was increased with the advanced age. Under physiological conditions the mitochondrial respiratory chain represents a major site of free radicals production (137). Therefore, the increase of mitochondrial antioxidant enzyme activity during aging might be an important mechanism compensating for the decreased cytosolic antioxidant enzyme activity and protecting mitochondria from oxidative damage.

Some studies reported that there were positive correlations between $\dot{V}O_2max$ and total SOD activity (134) and also between $\dot{V}O_2max$ and GPX activity in human (17). The high capacities to consume oxygen and consequently produce a large amount of ROS were accompanied by high SOD and GPX activities with both served to protect the organism from lipid peroxidation and cell membrane damage (17). The increases in SOD and GPX activities in relation to the increase in $\dot{V}O_2max$ were also found in this study.

Effects of one hour endurance exercise on lipid peroxidation, index of muscle damage and antioxidant enzymes at the beginning (month 0) and at the end (month 12) of the experiment.

Exercise training, acute exercise, and lipid peroxidation (MDA)

Strong evidence supports the hypothesis that the mechanisms of muscle damage are based on the role of free radicals during exercise (2-5, 8, 133). Several studies indicated that lipid peroxidation increased in skeletal muscles (2, 72), cardiac muscles (53) and in liver (2, 72) of untrained rats after acute exercise. In agreement with the above reports, we found that one hour endurance exercise caused an increase in plasma lipid peroxidation product (MDA) in untrained subjects both at the beginning and at the end of the experiment (Table 16, Figure 15). The magnitude of the increase in MDA after exercise at the end of one year was found to be significantly higher than those at the beginning of the experiment at every time intervals measured (5 min, 24 hr, and 48 hr). This result indicated that with advanced age, exercise of the same intensity and duration caused a greater effect on lipid peroxidation in untrained subjects. In contrast, both trained groups of subjects had increases in MDA levels

after one hour endurance exercise only at the beginning of the experiment, there were no significant increases in MDA after exercise at the end of one year training. It indicated that training status may also influence on the tolerance to oxidative stress caused by exercise (32). The findings in this study agreed with those of Viinikka et al. (139) and Alessio et al. (72). Viinikka et al. did not find any significant changes in serum MDA levels in well-trained runners during and after exhaustive exercise compared to pre-exercise. Alessio et al. demonstrated no increase in muscle MDA levels of the trained rats after moderate exercise, while an increase of MDA was caused by the same intensity of exercise in the sedentary group. The most contradictory results were on the reported peak of MDA (62, 140, 141). Parts of the reasons for the contradictory findings could be the use of different types, duration and intensity of exercise (28, 63) and different times of sampling after exercise (28). Therefore, in this study we chose to measure MDA at 3 different time courses after exercise (5 min, 24 hr, and 48 hr).

In this study, a bout of moderate exercise was prescribed according to the previous study from our laboratory (32). Subjects were cycling for 60 minutes at the resistance that produced 70% HRmax and the lipid peroxide levels in plasma were investigated. The plasma lipid peroxide level was found to increase to maximal level at 5 minutes after exercise (Table 16, Figure 15). The time to reach peak was different from the study of Toskulkao and Klinsukon (32) which reported that plasma lipid peroxide formation in untrained and trained subjects (short and long distance runners) reached maximal levels at 24 hours after exercise. However, a number of human studies of exercise induced lipid peroxidation in which plasma MDA were determined

reported an immediate increase in plasma MDA after exercise (9, 33, 101, 141). Our study supported the finding of Sen et al. (141) who reported that 30 minutes cycling with increased workload by 20 W/min produced an increase in plasma MDA at 2 minutes after exercise and plasma MDA was no longer seen at 24 hour, indicating a complete recovery. Endogenous malondialdehyde, a principle component of TBARS, undergoes rapid oxidative metabolism either in its primary form or after formation of an adduct with various macromolecules, eg., lysine, guanine and phospholipid base (141, 142). Therefore, our observation that an exercise-associated increase in plasma MDA level was not detectable in the 24 hour recovery sample was not unexpected. This might also explained why Ortenblad (28) which measured muscle and blood MDA only at 24 hour post exercise did not find exercise induced MDA.

The small degree of increase in plasma MDA levels found in our study might be explained by the fact that these compounds are cleared from plasma rapidly by several potential mechanisms, including excretion, catabolism, or redistribution to body tissues (143). The study of Jenkins provides evidence that MDA was cleared into the urine after exhausting exercise and that MDA diffusing from an exercising muscle might be rapidly cleared by the liver as the exercise was underway (116).

Exercise training, acute exercise and index of muscle damage (CPK, LDH)

The increase in plasma enzyme activities after physical exercise has been well established in various studies (22, 62, 64, 66, 75). Although the vast majority of studies conducted on the relationship between exercise and subsequent CPK levels disclosed elevations in CPK, there were experiments that failed to make such

observation (10, 145-147). Our studies also found that significant increase in CPK activities did not occur after 60 minutes bicycle exercise. The difference in findings may due in part to the difference in modes of muscle contraction (73). The increase in CPK response might be significant with eccentric rather than concentric or isometric contractions (148). Byrnes et al. (149) suggested that eccentric exercise results in more CPK with a longer time delay than isometric or concentric exercise. The cycling exercise in our study was considered to be of concentric type, so the resulting CPK rise was lower in magnitude and shorter in time course.

Higher CPK level was found to be associated with lipid peroxidation and the disruption of the muscle cell membrane (144). The elevation in plasma MDA could result from the increased lipid peroxidation in several tissues, but active muscle is likely to be the major source of oxidative damage products (9). In our study, significant rise in MDA was also found in sedentary subjects after exercise but the rise in MDA and CPK activities were not correlated. Discorrelation between plasma MDA and CPK was also seen in the study of Duthie (150) and Child (9). Both investigators studied in half marathon run and found that plasma CPK activities increased without associated changes in plasma indices of lipid peroxidation (MDA). In contrast, another previous report found a significant correlation between these damage indices in human after 80-km run (62). The difference might be due to the influence of different exercise durations on the release of creatine phosphokinase. With increasing exercise duration, however, peroxidative damage may have been more important in elevating the serum activity of myocellular enzymes (9).

After one year training, LDH activities were not increased in swim subjects and even decreased in football subjects after exercise. It is likely that training caused positive adaptation in reducing LDH activities induced by exercise. This finding supported the study of Thorstensson et al. (75) who indicated that aerobic training resulted in a decrease in total LDH activity in human skeletal muscle. Moreover Apple et al. (155) found that LDH activities in skeletal muscles of men and women runners were significantly decreased after 9 week training when response to a marathon race.

Exercise training, acute exercise, and scavenging enzymes (SOD, CAT, GPX)

In the present study, we found that there were no changes in SOD activities after exercise in the sedentary subjects both at the beginning and at the end of one year. This finding was supported by the study of Alessio and Goldfarb (72) that acute exercise (20 min treadmill running) caused no change in total SOD activity in rats. Ohno et al. studied the effect of 30 min cycling at 75% $\dot{V}O_2$max on erythrocyte scavenging enzyme responses and found that SOD (Cu, Zn) activity of the untrained subjects after exercise was not changed (152). From these reports, it appears that acute exercise has no definite effect on SOD activity in Humans (36).

In contrast, in the trained subjects we found that an hour endurance exercise gave rise to the significant increase in SOD activities, both at the beginning and at the end of the experiment. After one year training, the post exercise values of SOD in both swim- and football-trained subjects were significantly higher than the corresponding values before training, especially at 48 hr after exercise. These results indicated the role of long-term exercise training on inducing SOD activities both before

and after acute exercise. This idea was supported by the study of Mena et al. (153) which reported that SOD activities in RBC of professional cyclists were increased only after 20 days bicycle race but not in 6 days or 8 hours bicycle races. It seems likely that the increased red blood cell SOD activity in the professional cyclists who covered 2,800 km in 20 days, was due to adaptation to aerobic endurance training, although the exercise period was not long (153).

In this study, long-term exercise training seems to have no effects on exercised induced changes in CAT and GPX scavenging enzymes since there were no changes in responses of both enzymes at the beginning and at the end of the experiment in all 3 groups of subjects.

In the present study, the increases in GPX activities were found while there were no changes in CAT activities in every groups of subjects after acute endurance exercise. These findings suggested that GPX is either more capable of eliminating H_2O_2 when compared with CAT (154) or GPX is the major enzyme involved in removing H_2O_2 and affords a major role in defending the tissues against peroxidative damage (30) after exercise. Although, the literature regarding GPX appears to be more consistent in favor of the exercise-inducibility of this enzyme (26, 30, 155). A study by Salminen and Vikko (122) reported no changes in GPX activities in both red and white muscles of exercise-trained mice. The explanation for the discrepant results cited above may be due, in part, to differences between exercise protocol employed by the different investigators (155).

CHAPTER VI

CONCLUSION

In the present study, the effects of long-term regular exercise training on lipid peroxidation byproduct (MDA), muscle damage indicators (LDH, CPK), and scavenging enzymes (SOD, CAT, GPX) activities were studied in human both at rest and after one hour endurance exercise by ergometer cycling at 70% maximum heart rate. The newly admitted students of Sports School of Suphanburi which were subsequently trained for one year to be swimmer and football athletes were used as the trained-subjects and students of the same age range (10-14 yr) from the other school of Suphanburi were used as the untrained (sedentary) subjects.

From the present results, conclusions could be drawn as follow;

1. One year of regular exercise training program employed in this study (20 hr/week) was effective in providing great increases in physical fitness characterics to the trained subjects.

2. The occurrence of lipid peroxidation increased with advancing age in the sedentary subjects but not in the trained subjects, indicating the beneficial effect of long-term exercise training in reducing age-related increased lipid peroxidation.

3. One hour endurance exercise by cycling at 70% maximum heart rate was sufficient to cause increases in lipid peroxidation and tissue damage in the untrained subjects.

4. With advancing age, the same intensity and duration of an acute endurance exercise caused higher degree of increases in lipid peroxidation and tissue damage in the untrained subjects.

5. There were no increases in lipid peroxidaton and tissue damage in the trained subjects after endurance exercise, indicating the beneficial effects of long-term exercise training in reducing the exercise-induced lipid peroxidation and tissue damage.

6. Resting and exercise induced scavenging enzymes activities in the swim-trained, the football-trained, and the sedentary subjects were different.

7. At the end of one year: there were significant decreases in resting CAT activities and significant increases in GPX activities in the sedentary and the football-trained subjects while there were no changes in CAT and GPX activities in the swim-trained subjects. Resting SOD activities were found to be increased significantly only in the football-trained subjects, and increased with no statistical significance in the swim-trained subjects.

8. After acute endurance exercise, SOD activities were found to be significantly increased in the football-trained subjects. The levels of post-exercise SOD at the end of one year were significantly higher than those at the beginning of the experiment at every time intervals after exercise in football-trained subjects, at 48 hr after exercise in the swim-trained subjects, and were not different at all in the sedentary subjects.

9. The level of post-exercise CAT was decreased and the level of post-exercise GPX was increased in both trained and untrained subjects after one year.

10. The beneficial effects of long-term exercise training in reducing age related and exercise induced increases in lipid peroxidation and tissue damage were possibly due to the increased SOD scavenging enzymes found in the trained subjects.

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APPENDIX I

PREPARATION OF BLOOD

Seven ml of venous blood from each subject was drawn into heparinized tubes and centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was collected for lipid peroxide content (MDA), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) analyses. The red cells, after buffy coat removing, were washed 3 times with cold (4°C) 5 mM phosphate buffer saline. The pack red cells were then assayed for superoxide dismutase, catalase and glutathione peroxidase.

APPENDIX II

LIPID PEROXIDE CONTENT

(Ohgawa et al., 1979)

PRINCIPLES

Oxidation of polyunsaturated fatty acids leads to numerous peroxidic and aldehydic compound, in particular the volatile low molecular weight aldehyde, malondialdehyde (MDA). The chemical composition of the end products of peroxidation will depend on the fatty acid composition of the lipid substrate used and upon what metal ions are present. Thus copper and iron ions give different end-product distributions as measured by the thiobarbituric acid (TBA) test. This is one of the most commonly used methods for detecting and measuring lipid peroxidation. The lipid material is simply heated with TBA at low pH, and the formation of a pink chromogen is measured at or close to 532 nm. The chromogen is formed by reaction of one molecule of malondialdehyde (MDA) with two molecules of TBA.

REAGENTS

1. 8.1% Sodium dodecyl sulfate (SDS)

Dissolve SDS 8.1 g in distilled water and allow to stand overnight at room temperature until it is dissolved. Then make up to 100 ml.

Do not shake because this solution will produce a lot of bubbles and store in refrigerator.

2. 20% of acetic acid solution (pH 3.5)

Pipette 26.61 ml of 37% HCl into volumetric flask (1,000 ml) and make up to 1,000 ml with distilled water. 0.27 M HCl is then obtained. Add 20 ml pure acetic acid in 80 ml 0.27 m HCl. Adjust the solution to pH 3.5 with 1 N NaOH.

3. 0.8% Thiobarbituric acid (TBA)

Weigh TBA 0.8 g. Then add distilled water, heat and stirr until it is dissolved. Make this solution up to 100 ml and mix.

4. 1,1,3,3-Tetramethoxypropane (TMP) or malondialdehyde bis (dimethyl acetal) solution is used as an external standard. The level of lipid peroxide is expressed as nmole of MDA.

Pipette 16.4 µl stock TMP and make up to 100 ml with distilled water. Then pipette 0.04, 0.08, 0.12, 0.16, 0.20 ml of this stock TMP solution and add distilled water to 10 ml in each concentration. These will give the following concentration of standard TMP: 4, 8, 12, 16, 20 nmole/ml. Store the stock solution in refrigerator.

PROCEDURE

1. Pipette the following solutions into a series of glass tubes with screw capped:

Solution	Blank (ml)	Standard (ml)	Unknown (ml)
Sample	-	-	0.5
8.1% SDS	0.2	0.2	0.2
20% Acetic acid (pH 3.5)	1.5	1.5	1.5
0.8% TBA	1.5	1.5	1.5
TMP stock standard	-	0.5	-
Distilled water	0.8	0.3	0.3

2. Heated the tubes in the water-bath at 95°C for 60 min.

3. After cooling with top water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) are added and shaken vigorously (at least 1 min).

4. After centrifugation at 4,000 rpm for 10 min, the organic layer is taken and its absorbance at 532 nm is measured.

5. The content of lipid peroxide is expressed in terms of nmole MDA/ml.

CALIBRATION CURVE

1. Prepare a series of tube containing TMP stock standard in water in the following concentrations: 2.0 nmole/0.5 ml, 4.0 nmole/0.5 ml, 60 nmole/0.5 ml, 8.0 nmole/0.5 ml and 10.0 nmole/0.5 ml.

2. Perform the procedure as in step 2.

3. Determine the absorbance at 532 nm. Then plot the optical density versus nmole of MDA/ml.

REFERENCE

Ohgawa H, Ohishi N, Yaki K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.

APPENDIX III

CREATINE PHOSPHOKINASE

(Sigma Chemical, St. Louis, Missouri, USA)

PRINCIPLES

The enzymatic reactions involved in the assay are as follows:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G-6-P}$$

$$\text{G-6-P} + \text{NAD} \xrightarrow{\text{G-6-PDH}} \text{6-PG} + \text{NADH}$$

CK catalyzes the reaction between creatine phosphate and adenosine diphosphate (ADP), forming creatine and adenosine triphosphate (ATP). The ATP formed is utilized to phosphorylate glucose, producing glucose-6-phosphate (G-6-P) in the presence of hexokinase (HK). Subsequently, G-6-P is oxidized to 6-phosphogluconate (6-PG) in the presence of nicotinamide adenine dinucleotide (NAD). This reaction is catalyzed by glucose-6-phosphate dehydrogenase (G-6-PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH increasing the absorbance at 340 nm. The rate of change in absorbance is directly proportional to CK activity.

REAGENTS

1. Enzyme substrate mixture
2. Buffer solution

REAGENT PREPARATION

Add deionized water into a vial containing enzyme substrate mixture and mix gently by inversion then store the solution at 2°-6°C.

PROCEDURE

1. To cuvette labelled test, add 1.0 ml CPK reagent and bring to incubated for 3 min.
2. Add 0.02 ml sample and mix by inversion.
3. Place cuvette in constant temperature cuvette compartment and incubated for 3 min.
4. Read and record Absorbance (A) of test at 340 nm versus deionized water as reference. This was Initial A.
5. Continue incubation at 30°C and record absorbances at 30 sec intervals for a period of 120 sec was Final A.
6. Calculate Δ A per min by subtracting Initial A from Final A and dividing by 2.

CALCULATION

Determine CPK activity as follows:

$$\text{CPK (U/L)} = \frac{\Delta \text{ A per min} \times \text{TV} \times 1{,}000}{6.22 \times \text{LP} \times \text{SV}}$$

where:

Δ A per min = Change in absorbance per min at 340 nm.

TV = Total volume (ml)

SV = Sample volume

6.22 = Millimolar absorptivity of NADH at 340 nm

LP = Lightpath

1,000 = Conversion of units per ml to units per litre

REFERENCES

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APPENDIX IV

LACTATE DEHYDROGENASE

(Human Chemical, Taunusstein, Germany)

PRINCIPLES

The enzymatic reactions involved in the assay are as follows:

$$\text{Pyruvate} + \text{NADH} \xrightleftharpoons{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

Lactate dehydrogenase reversibly catalyzes the conversion of lactate to pyruvate, with the simultaneous reduction of NAD to NADH. Since NADH absorbs at 340 nm and NAD does not, the activity of LDH is assayed by following the increase in absorbance at 340 nm using lactate and NAD as substrates.

REAGENTS

1. Buffer solution

- Tris buffer (pH = 7.4)	50	mmol/l
- Pyruvate	1.2	mmol/l
- EDTA	5.0	mmol/l

2. Starting reagent

- NADH	0.15	mmol/l

REAGENT PREPARATION

Pipette 2 ml from reagent 2 into reagent 1 (10 ml) and mix thoroughly by inversion. Store the reagent mixture at 2-8°C and kept light protected.

PROCEDURE

1. To curette labelled test, add 0.02 ml sample in 1.0 ml LDH reagent mixture and mix by inversion.
2. Read and record Absorbance (A) of test at 340 nm, 30°C versus deionized water as reference. This was Initial A.
3. Continue incubation at 30°C and record absorbance at 60 sec intervals for a period of 180 sec was Final A.
4. Calculate Δ A per min by subtracting Initial A from Final A and dividing by 3.

CALCULATION

Determine LDP activity as follows:

$$\text{LDH (U/L)} = \Delta \text{ A per min} \times 8095$$

where:

Δ A per min = Change in absorbance per min at 340 nm.

8095 = Value calculated from $\dfrac{\Delta \text{ A per min} \times TV \times 1{,}000}{6.22 \times LP \times SV}$

REFERENCE

Will I and Trendelenbury C. Joint study to establish reference values for clinical chemical parameter in childhood. J Clin Chem Biochem 1982;20(4) 235-242.

APPENDIX V

SUPEROXIDE DISMUTASE

(Modified from the method of Winterbourn, 1975)

PRINCIPLES

Superoxide dismutase catalyzes the breakdown of the superoxide free radical ($O_2^{\bullet -}$) according to the reaction:

$$2O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

The assay system is based on the ability of the enzyme SOD to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radical which is generated by its reaction of photoreduced riboflavin and oxygen.

REAGENTS

1. 0.1 M EDTA (ethylene diaminetetraacetic acid)

   3.72 g of EDTA is dissolved in 100 ml of distilled water.

2. 1.5 mg KCN in 100 ml

   1.5 mg of KCN is dissolved in 100 ml of distilled water.

3. 1.5 mM NBT (nitroblue tetrazolium)

   Dissolve 123 mg of NBT in 100 ml of distilled water.

4. 0.12 mM Riboflavin

   Dissolve 14.5 mg of Riboflavin in 100 ml of distilled water.

5. 0.067 M Phosphate buffer pH 7.8

Mix 0.067 M of K_2HPO_4 in 0.067 M KH_2PO_4 to make phosphate buffer pH.7.8.

PROCEDURE

1. About 0.4 ml of red blood cell was hemolysed by adding approximately 1.5 volumes of cold water (4°C).

2. The hemoglobin concentration of this hemolysate was adjusted to about 10 g/dl by cold water (4°C).

3. A chloroform-ethanol extract was prepared by adding 0.5 ml of hemolysate to 3.5 ml of cold water (4°C), followed by 1.0 ml of ethanol, then 0.6 ml of chloroform. Solution should be mixed at addition, and finally shaken for 1 min.

4. Sample tubes were centrifuged for 10 min, 3,000 rpm at 4°C. The clear top layer was collected for enzyme assay.

5. For each sample to be assayed, the tubes were set up containing 0, 10, 20, 40, 60, 80, 200 and 500 µl of SOD extract from red cells. The reagents were added into these tube as following:

0.2 ml of 0.1 m EDTA.

0.2 ml of 1.5 mg of KCN in 100 ml.

0.1 ml of 1.5 mM nitroblue tetrazolium (NBT)

0.05 ml of 0.12 mM riboflavin (lastly added)

0.067 M phosphate buffer pH 7.8 was added to give a total volume of 3 ml.

6. The tube which contained no extract was a controls for each run. All tubes were then illuminated with a light box for 12 min at room temperature (25°C).

7. Optical density was measured at 560 nm.

CALCULATION

Results were expressed as unit of superoxide dismutase per gram of hemoglobin and 1 unit is defined for a particular system as that the amount of enzyme causing half the maximum inhibition of NBT reduction. The percent inhibition of NBT reduction versus the amount of red cell extract was plotted on linear graph paper. The volume of extract (µl) required to inhibit the reduction of NBT by 50% was used in the following equation.

$$E = \frac{100{,}000}{\mu l \text{ of SOD extract}}$$

where:

E = the enzyme activity expressed as 50% inhibition in unit/g HB.

As percentage inhibition could be calculated from this formula :

$$\% \text{ Inhibition} = \frac{\text{O.D. of control} - \text{O.D. of experiment} \times 100}{\text{O.D. of control}}$$

REFERENCE

Winterbourn CC, Hawkings RE, Brain M, Canell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1975; 85: 337-341.

APPENDIX VI

CATALASE

PRINCIPLES

Catalase function is decomposition of H_2O_2 to H_2O and O_2 which can be measured by the decrease in O.D. at 240 nm. The difference in O.D. per unit time is the catalase activity.

$$2H_2O_2 \xrightarrow{\text{Catalase}} H_2O_2 + O_2$$

REAGENTS

1. 50 mM phosphate buffer pH 7.0

   a. Dissolve 6.81 g KH_2PO_4 in distilled water and make up to 1,000 ml.

   b. Dissolve 8.90 g $Na_2HPO_4{:}2H_2O$ in distilled water and make up to 1,000 ml.

   Mix the solutions a and b in proportion of 1:1.55

2. 30 mM hydrogen peroxide

   Dilute 0.34 ml 30% hydrogen peroxide with phosphate buffer (solution 1) to 100 ml.

PROCEDURE

1. Prepared a stock hemolysate containing 5 gHb/100 ml by the addition of cold (4°C) distilled water.

2. Prepared a 1:3,000 dilution of this hemolysate with phosphate buffer immediately before the determination.

3. The following reagents were added to cuvette.

Reagents	Blank (ml)	Sample (ml)
phosphate buffer	1.0	-
sample (hemolysate)	2.0	2.0
H_2O_2 solution	-	1.0

4. The decrease of O.D. of the system was measured against that of the blank at 240 nm.

CALCULATION

$$A = \frac{\Delta \text{ O.D. x Vc x 100 x Dilution factor}}{0.071 \text{ x } V_H \text{ x Hb}}$$

where :

A = enzyme activity in units per gram of hemoglobin (U/gHb)

0.071 = molar extinction of H_2O_2.

Vc = the cuvette volume

V_H = the volume of hemolysate in the reaction system (ml)

Hb = the concentration of hemoglobin (g/dl)

REFERENCE

Beutler E. Red Cell Metabolism. A manual of Biochemical methods. Grune and Stratton, Inc, New York. 1971; 30-36.

Bergmeyer Ulrich Hans. Methods of enzymatic analysis. Academic Press, Inc., 1974; V2: 673-684.

APPENDIX VII

GLUTATHIONE PEROXIDASE

PRINCIPLES

Glutathione peroxidase catalyzes the reaction of hydroperoxides and hydrogenperoxide with glutathione as the reductant.

$$2GSH + ROOH \xrightarrow{GPX} ROH + GSSG + H_2O$$

$$2GSH + H_2O_2 \xrightarrow{GPX} 2H_2O + O_2$$

The simplest method is a spectrophotometric assay in which the reduction of GSSG is coupled to the oxidation of NADPH through glutathione reductase.

$$GSSH + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} 2GSH + NADP^+$$

t-Butyl hydroperoxide is the most suitable substrate for the assay of the enzyme. The oxidation of NADPH was measured at 340 nm.

REAGENTS AND PROCEDURE

1. The following reagents were added to cuvette with a critical volume of less than 1 ml.

Reagents	Blank (µl)	Sample (µl)
1M Tris - HCl, EDTA 5 mM, pH 8	100	100
0.1 M GSH	20	20
Glutathione reductase 10 U/ml	100	100
1 mM NADPH	100	100
1: 20 hemolysate	10	10
H_2O	670	660

2. Mix throughly and preincubate at 37°C for 10 min.

3. Add 7mM t-Butyl hydroperoxide 10 µl in system solution.

4. The decrease of O.D. of the system was measured against that of the blank at 340 nm.

CALCULATION

$$A = \frac{\Delta \text{ O.D. x Vc x 100 x Dilution factor}}{6.22 \text{ x } V_H \text{ x Hb}}$$

where :

A = enzyme activity in units per gram of hemoglobin (U/gHb)

6.22 = molar extinction of NADPH.

Vc = the cuvette volume.

V_H = the volume of hemolysate in the reaction system (ml).

Hb = the concentration of hemoglobin (g/dl).

REFERENCE

Beutler E. Red Cell Metabolism. A manual of Biochemical Methods. Grune and Stratton, Inc, New York. 1971; 30-36.

Beutler E. Red Cell Metabolism. A manual of Biochemical Methods. Grune and Stratton, Inc, New York. 1975; 71-73.

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